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Micromethods in Molecular Biology

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Rp376

The purpose of this book is to introduce scientists to the use of various highly sensitive micromethods and their application to the broad field of molecular biology. The methods are described in great detail, so that any experimenter can adapt them to his own field of interest. New, unpublished methods and results from the authors' laboratories are included. In essence, this is a "cook book," giving all the normally unpublished information which is necessary for the successful application of micromethods.

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Cover picture

A statue which has been reassembled
from fragments found in a shipwreck
near the Greek island of Antikythera
in the Aegean Sea. The technique
used to fit the fragments together
correctly is described on page 47.



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Nature accepts three types of communications:

- Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.

- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).

- 'Matters arising' permits occasional short discussion of papers that have previously appeared in *Nature*. A limit of 350 words is placed on contributions in this category.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. $\exp(a)$ is preferred to e^a if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

(i) If it is necessary to refer to several references by the same author at once, only one reference number need be given.

(ii) The last page as well as the first of any reference should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

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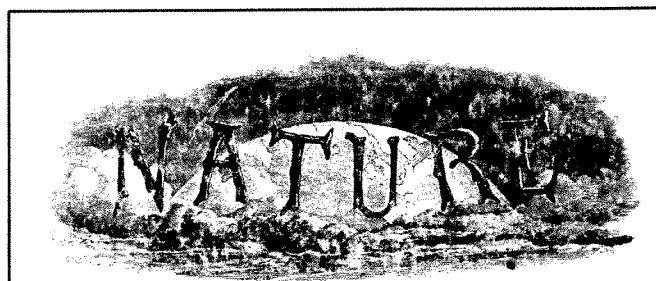
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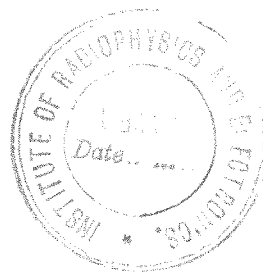
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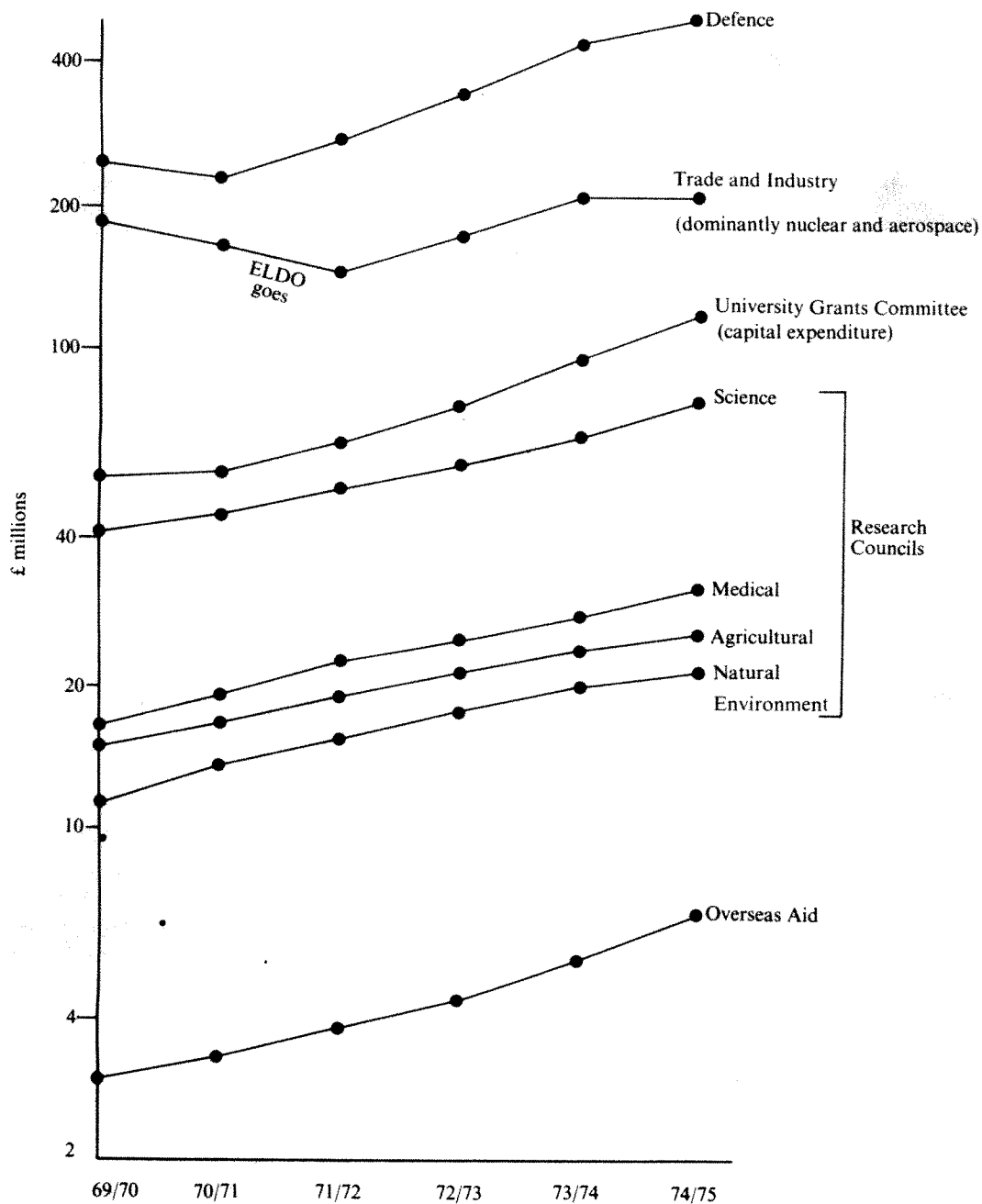
100 years ago

WE take the following from the *Academy*:—"Some of the American papers state that Prof. Huxley is likely to be the successor of Prof. Agassiz, at Harvard. We hope there is no truth in this. Are the English Universities so rich in really eminent professors, and so poor in money, that they can or must allow Prof. Huxley to go to America in order to find leisure for work? It would require nothing but the will for either Oxford or Cambridge to offer Huxley two or three thousand a year, without anybody suffering for it. There are hundreds of non-resident Fellows, doing no good to the University, doing harm to themselves in resting on their oars, when they ought to be pulling with all their might. Why not give five or ten such Fellowships to men like Huxley, and make the Universities again what they were in the middle ages, the very centres of intellectual force and light in the country? The Universities are so rich that they could beggar the whole world. Will they allow themselves to be beggared by Harvard?"

From *Nature*, 10, 195, July 9, 1874.



How Britain spends its research and development money



Sources

Advisory Board for the Research Councils, First Report, Appendix V, Table 1, June 1974.

Memorandum by the Chief Secretary to the Treasury, 1974/75.

Adjustments have been made to 'de-Rothschild' research council expenditures for the past two years.

Chilean scientists remaining in the country are labouring under very difficult circumstances which are mainly economic. Funds for research are very limited, and it is very difficult for these people to carry out experiments using modern techniques.

From Carlos Eyzaguirre, Chairman of the Department of Physiology, University of Utah College of Medicine, Salt Lake City, and Visiting Professor, Department of Neurobiology, Catholic University of Chile, Santiago.

I READ with interest a letter (*Nature*, March 29) signed by H. M. Gerschenfeld and others, regarding the situation of the academic community in Chile. It is intriguing and provoking since it shows a rather desperate situation: instances of police brutality, wholesale dismissals of faculty and students and a total disregard for academic freedoms and human rights on the part of the military government. The letter is inaccurate and the charges very serious. Thus, a reply is in order.

I started my academic career in Chile and went permanently to the United States about 17 years ago. Since then, I have returned to Chile many times for academic purposes and have continuously maintained close contacts with Chilean academics. At present I am a Visiting Professor of Neurobiology at the Catholic University and have been in the country since April. Thus, I feel better qualified to judge the present situation than the gentlemen signing the letter to *Nature*.

Dr Allende's Government started with high hopes for the underprivileged who are numerous in this country. Unfortunately, slowly and steadily things deteriorated to the point of chaos and anarchy. The universities were not immune to this decaying process: funds were drastically cut to the point where research came to a standstill. CONICYT (equivalent to the National Research Council), which actively supported research during the previous Frei administration, became a planning agency which developed a number of programmes more often than not politically motivated. The Academy of Sciences of the Instituto de Chile was left practically without funds. There was total lack of student discipline to the point where it was extremely difficult to conduct normal academic activities. In addition, there were frequent armed confrontations on the streets, incredible government corruption and, near the end, people had to queue for hours to get a few

essentials such as bread, soap, fuel, cigarettes. In the middle of this lovely picture the military struck with vigour on September 11, 1973. Interestingly enough they were shot at by supposedly defenceless civilians. In the melee many people were killed (including soldiers and policemen) and mopping up operations continued for a while. In context, the military operation in Chile was not a palace coup but a civil war which, fortunately, lasted only a few days. Casualties were many, but they could have been much more numerous if the military had been less decisive.

No one denies that there have been mistakes, cases of police harshness and unfair firing of people from their jobs. Some of these unpleasant practices have, however, been corrected; the country is again at work with the consequent increase in productivity, there is more discipline and normal activities have resumed. The people have co-operated, not necessarily because of fear, but the coup had the acceptance of the majority of the people. In a country like this, with a profound democratic tradition, nobody can govern without popular acquiescence. The democratic process has not been restored yet. Congress and the political parties are in recess and the press is still quite cautious. There is, however, hope for the future and the military have promised full democracy as soon as possible, whenever that may be. Many people believe that these are not vacuous statements since the military in Chile have been and are civic minded. Proof of this is that they have supervised all elections since 1938, when under President Pedro Aguirre (head of a Popular Front coalition) they got that authority. This practice continued under Dr Allende and there is consensus that elections in Chile have been generally fair.

Why did the military strike? They did so to restore the country to a more normal way of life, to eliminate the unconstitutional excesses of the previous government and the political, moral and economic chaos present in Chile at that time.

It was not an easy task, from a public relations point of view, since Dr Allende enjoyed great popularity in foreign countries. He was pictured as a democrat trying to reach socialism by peaceful and constitutional means,

a David fighting the United States Goliath and opening new ways of government to improve the lot of the poor; in short, Sir Galahad under a poncho. Most of his countrymen saw him in a different light: a scheming, corrupt and manouevering politician trying to outflank the wishes of Congress and of the Courts and bribe the Armed Forces, and very incompetent in the business of running the country. Moreover, they resented the fact that Dr Castro and the Soviet Union had too much to say about national affairs. Some of these complaints were unfair since there was political and economic pressure from the United States and he had to turn somewhere for help. The fact remains, however, that he could not manage the affairs of the nation and Chile would have faced certain economic, moral and political ruin had he remained in power.

Soon after the military coup, the heads (rectors) of the different universities were fired by the government. They were replaced by retired senior armed forces officers. Also, an army general was made head of CONICYT. These appointments seem, on the surface, to go against traditional university procedures, at least in Chile where most university presidents have been academicians. In other countries such as the United States, university presidents vary from academic to scientific administrators (such as Dr Fletcher at the University of Utah and now head of NASA) or retired politicians or generals (Eisenhower at Columbia). Thus, the appointments of Admiral Swett as Rector of the Catholic University or General Ruiz at the University of Chile would not be too far from practices that are not unknown in the United States. The difference is that both Admiral Swett and General Ruiz were appointed very soon after the military takeover in Chile. Interestingly enough, Admiral Swett's appointment was confirmed by Cardinal Silva, head of the Catholic Church and Chancellor of the Catholic University.

The new university rectors faced several problems: (1) an unruly student body heavily loaded with Marxist activists. Some of them were not students in the traditional sense of being properly registered, attending classes, taking examinations and so on; (2) a

peculiar faculty, some members being almost exclusively interested in the propagation of Marxism and organising revolutionary and guerrilla activities. Some did not even have a BA degree. This situation became intolerable for some faculty members who left the country and sought employment abroad; (3) chaotic finances which prevented normal university activities and the payment of adequate personnel salaries. One wonders if any university in a civilised country would have tolerated that situation for long.

The rectors had to act and they did the following: (1) students at some universities had to re-register. Students who did not qualify as such in the traditional sense (taking a certain number of courses for credit, passing qualifying examinations and so on) were dismissed. This weeded out the purely political 'students', but is this so terribly unfair? At the Catholic University, however, all of 11,500 students remained and no one was required to re-register. (2) The faculty went through the same screening procedure. Those who did not qualify for lack of degrees or for being exclusively political activists were dismissed. This procedure would have been applied in any university under normal circumstances. Left-leaning faculty members who carried out their duties of teaching and research during Allende's government were not asked to resign. Many of them are still in Chile. Some have left because of their inability to come to terms with the present government. This is their prerogative and their exit from the country has not been impeded.

Wholesale executions of faculty or scientists because of political views have not occurred. Very few fell either immediately after or during the coup because, allegedly, they were caught either shooting at soldiers, carrying arms or actively participating in the organisation of revolutionary activities. For the past several months, however, there has been no indication that such a thing has occurred again. Some academicians, deeply involved in extremist activities were detained for investigation. Most of them have been freed and many have returned to jobs in the same or different universities. Others have either not returned to the country or have sought asylum. There



Rector Ruiz

are charges against some of these people and if they return they will face legal prosecution. But prosecution is one thing and persecution another. Some academicians have had trouble with the police because of denunciations by neighbours or colleagues. These people have been investigated and, if cleared, they have been employed again in the same or similar jobs.

The question is then, what constitutes an offence leading to academic dismissal that would prevent further academic employment? I am not privy to the deliberations of the rectors, but it is my opinion that such an offence would be a deliberate effort on the part of an individual to topple the present government by either propaganda or more violent means. In other countries these activities are not looked on with favour by the governments involved. Thus, it is asking rather too much of the present Chilean government to tolerate these activities, particularly since the military came to power only a short time ago and after a rather messy civil war. This deals directly with a statement in the letter to *Nature* regarding the Catholic University. The statement is inaccurate. Only 3.25% of faculty members were removed after the coup because they did not do teaching and research but engaged exclusively in different forms of political activity. To assume that the rest (96.75%) were all right-wing, God-fearing, church-going and thoroughly nice fellows would be naive in the extreme. In fact, many of them have views that would not please the present government. But Admiral Swett has been a careful, intelligent and conscientious administrator who

has strived very hard to maintain and improve the present academic structure. He has the confidence and respect of both faculty and students.

With regard to the Chilean scientists remaining in the country the following must be said. These people are labouring under very difficult circumstances which are mainly economic. They have very low salaries (which are in line with those of others), mainly because of the difficult economic conditions of the country caused by the previous government; recovery is still very incomplete. For the same reason, funds for research are very limited, and it is very difficult for these people to carry out experiments using modern techniques. Furthermore, subscriptions to journals in libraries are running far behind, also for lack of funds, which makes the situation doubly difficult. Nevertheless, Chilean scientists are trying very hard to maintain a research establishment that is in bad shape although it was flourishing four or five years ago. But there is hope. CONICYT is operating again, fulfilling the purpose for which it was originally created during the Frei administration. Also, the government has rescued the Academy of Sciences from financial disaster. More important is the fact that government authorities are listening and are sympathetic to the plight of the local scientists in terms of improving salaries and making available more funds for research and so on.

Inaccurate letters of the type published in *Nature*, although well meaning, do not help those still in Chile. They create a distorted image of the country that may prevent foreign help which is badly needed in keeping what is left after the debacle of the past three years. Scientists who remain in Chile are not fascists but people sincerely devoted in pursuing their academic careers and training future researchers. In fact, some have received tempting offers from abroad and have refused to leave the country. They are far more courageous than those who left to practice 'gauchisme de salon'. The latter and their supporters should be more accurate in their statements, as one would expect from scientists, since these shot-gun approaches only hinder the struggle of dedicated scientists working in a difficult environment. ●

international news

THE Central Policy Review Staff (CPRS), the Think Tank, surfaced this week to publish a report on Energy Conservation (HMSO, £1). It has already been estimated that Britain should eventually be able to save up to 10% of its forecast energy consumption and the report details how this may be most usefully achieved. With its eyes firmly on the possible, it eschews long term once-and-for-all solutions such as nuclear fusion and the efficient use of solar energy, and concentrates on the small scale short to medium term answers. The electric car is a firm favourite; and better insulation to reduce heat loss, smaller cars, greater use of diesel rather than petrol engines and a reliance on the self-regulating effect of more expensive fuel all figure as feasible ways of conserving our energy over the next 25 years.

The most important conclusion for British research is a negative one, that Britain cannot conceivably follow up fully all the possible answers. The report does, however, pick out some fields in which practical research should be stepped up. For the rest, we should concentrate on monitoring the work of other countries, mainly though not exclusively through the Energy Technology Support Unit at Harwell. One of the favourite topics for further research in Britain is the electric car. For this to be feasible it is assumed that the electricity to recharge the batteries will by then be generated mainly by nuclear power.

Three ways of powering the electric vehicle are considered: hybrid systems

Get cracking and count the kilowatts

comprising a battery and a small internal combustion engine, fuel cells, and electric batteries. Of these, electric batteries are considered to be the most attractive. Further work is certainly justified, the report thinks, on the sodium-sulphur battery, to establish its technical viability and costing.

The only strong contender to replace fossil fuels in electricity generation is nuclear fission. Solar power and nuclear fusion, along with windmills, tidal power and magnetohydrodynamics are dismissed as too long term. But wave power is given the go ahead and the technical and economic appraisal now in progress at the National Physical Laboratory is welcomed.

One major recommendation, which has been increasingly advocated by the architectural profession, is an improvement of insulation standards in new domestic housing, and encouragement of owners of existing housing to bring their insulation up to scratch. The insulation in many British homes would not be acceptable to a modern pig farmer for his pig houses, says the CPRS, and there is therefore great scope for improvement in this direction. The Health and Safety at Work

Bill, now going through Parliament, will in fact enable the insulation standards in new housing to be raised. A massive programme to educate the public is envisaged, and this will probably concentrate on the house owner's pocket.

No policy can hope to reduce energy consumption drastically in the very near future. Any measures that the government do put in hand will be unlikely to show results until after the 1980s, especially as the emphasis of the report is on voluntary and self-regulating measures. No energy police for instance. The report has no revolutionary suggestions, but points out that relatively small individual savings, as a result of, for instance, better insulation, more efficient electricity generating equipment and the use of smaller motor cars, should eventually be able to save the target 10% of energy consumption, provided that they are acted on now as a matter of urgency.

The message from the top is, therefore, that people must be made conscious of the full cost of energy and the opportunities for conservation. This should encourage them to save their own money and is preferred to the strong-arm approach. One idea for industry is that companies should be encouraged to start 'energy auditing' and even publish the results in their annual reports. The psychology of economic self interest and the dislike of public disapproval are obviously considered to be more powerful in the long run than a corps of Energy Inspectors or Temperature Police.



BRITISH Secretary of State for Energy, Eric Varley, has appointed Dr Walter Marshall (left) as Chief Scientist in the Department of Energy. Initial reactions of relief that a key post has been filled after months in caretaker hands have been tempered somewhat by the details of the new man's brief, which says specifically that Dr Marshall shall not hand out any advice on overall nuclear policy or on UK Atomic Energy Authority (UKAEA) matters unless he is specifically requested to do so by the Department of Energy with the prior agreement of the UKAEA.

Since Dr Marshall has been a member of the UKAEA for two years, and

Director of the Atomic Research Establishment at Harwell since 1968, it seems on the face of things that the government is missing the point of the operation by directing his attention to energy issues excluding those which he is uniquely equipped by his experience to judge.

However, the choice of reactor for the next stage of Britain's nuclear programme has probably been made already, and whatever the department may say about Dr. Marshall's brief avoiding 'conflicts of interest', it's highly unlikely that he wasn't among the people consulted by Mr Varley, however informally, before the choice was made.

WHEN President Nixon abolished the Office of Science and Technology last year, thereby ending 15 years of scientific presence in the White House, his action was greeted with a predictable flood of complaints from the scientific community and rumblings of discontent have continued ever since. Now the National Academy of Sciences, the most prestigious scientific body in the United States, has taken to print to deplore the banishment of scientists from the corridors of power and to urge that they be restored through the establishment of a Council for Science and Technology in the White House.

The idea is neither new nor particularly startling, but the fact that it has come from the academy has accorded it considerable attention, particularly on Capitol Hill. The proposal is, essentially, for a three-member council to be established to help in dividing the budgetary pie for science and technology, to provide independent analyses of military research and development programmes and to have a strong input into foreign and domestic science policymaking.

When Nixon scrapped the Office of Science and Technology (OST) by executive fiat in January last year, he also designated Dr H. Guyford Stever, Director of the National Science Foundation, as science adviser—a position previously occupied by the Director of OST. Stever, who is head of one of the smaller scientific agencies in the federal landscape, was, however, given no authority to advise on military research and development—which accounts for more than half of the federal science budget—because “the Department of Defense has strong capabilities for assessing weapons needs”. And he was also told to report to the President through Dr George Shultz, who was then Nixon’s adviser on economic affairs. •

Although Stever has generally been credited with doing a commendable job, given the limit to his powers, the fact that the science advisory apparatus was shifted from the White House to a relatively obscure part of the federal bureaucracy rankled considerably among members of the scientific establishment. And it did not help matters much when Nixon, seeking advice on energy research and development, turned not to Stever but to Dr Dixy Lee Ray, chairman of the Atomic Energy Commission. Since that was perhaps the most important exercise in science policymaking last year, the fact that Stever was seemingly overlooked when the task was assigned suggested that Nixon was not paying much attention to the science policy machinery that he had established.

Central to the complaints that have

Scientists want a foot in the White House

by Colin Norman, Washington

been raised about the new arrangements for science policy is that although Stever may be an able man, and although he has established an office to provide staffwork on policy questions, the fact that he is not in the White House puts him in a weak position to orchestrate the federal government’s scientific activities, which are scattered over numerous different agencies and departments. The White House is the place where interagency disputes are settled, where coordination of programmes takes place and, most important, where final decisions on the Administration’s budget are taken. Thus, the argument goes, for scientific advice to be effective it must come from a White House office.

That, in short, is the reasoning behind the academy’s proposal for a Council for Science and Technology. The proposal, which was released during Congressional hearings last week recommends the following features for the Council for Science and Technology:

- It would consist of at least three people, appointed by the President; it would have a small staff, and it would be able to call upon consultants and panels of outside scientists for advice.
- The council would establish strong links with the three chief policymaking organisations in the White House—the Domestic Council, which provides coordination and policy guidance for a range of domestic programmes, the National Security Council, which does the same for defence matters, and the Office of Management and Budget, which holds the purse strings for all government departments and agencies. The academy suggests that the chairman of the Council for Science and Technology should sit on the Domestic Council, since that body must deal with “a substantial number (of policies and problems) which involve components of science and technology”. Arrangements with the National Security Council would be more informal, but participation by the proposed Council for Science and Technology in defence policymaking would at least rectify one of the major deficiencies in the present science policy system—lack of independent scrutiny of Defense Department programmes. As for the Office of Management and Budget, that body establishes priorities among federal programmes and agencies through the annual budget. Thus, the academy argues, it is essential that it be provided

with strong input from the scientific community in its deliberations over the federal government’s \$20,000 million science budget.

- Finally, the academy recommends that the proposed council should deliver an annual report on major developments in science and technology. In fact, OST did attempt to produce such a report during its last year of life, but the effort was eventually dropped partly because it engendered considerable opposition from some sectors of the government.

Nowhere in its report does the academy say outright that the present system is not working properly—indeed, at one point, the report says that “we view with admiration the efforts of the Director of the National Science Foundation”—but implicit in its argument is that the arrangement just cannot cope with many of the problems with which it has to deal, simply because it is one step removed from the centre of power. But a fundamental question is whether any science advisory apparatus can be made to work in an Administration which has not so far shown much enthusiasm for science advice. The OST arrangement, for example, even though it was at the centre of power, lost considerable influence during the later stages of its life, partly because PSAC made some public recommendations which were diametrically opposed to Administration policy—a prime example being a report opposed to development of the SST which was made public when the Administration was fighting Congress to get approval for the SST programme.

Nevertheless the Academy’s proposals met with a warm reception on Capitol Hill, for last week Senators Frank Moss and Warren Magnuson proposed a bill which would create a Council of Advisers on Science and Technology in the White House. Hearings will be held on the measure on July 11 by the Senate Committee on Aeronautical and Space Sciences and the Senate Committee (which Moss and Magnuson, respectively, chair) and there is a good chance that the Senate will endorse the proposal by the end of the year. But the House of Representatives is unlikely to move that quickly. The Committee on Science and Astronautics is now in the middle of a protracted study of the national science policy machinery, and although the Academy’s recommendations went down well with the committee, it is unlikely that it will be ready to suggest changes in the machinery in time for the House to act this year. But there is every chance that Congress will act next year to reestablish a White House science policy office, in which case no President would be able to get rid of it without congressional approval.

Cooperation in coal research

by John Wilson

BRITAIN and the United States are to share the results of almost all their research into the mining and use of coal. This was decided formally on June 26, when an agreement was signed simultaneously in London by Sir Derek Ezra, Chairman of the National Coal Board (NCB) and in Washington by the Honorable Rogers C. B. Morton, United States Secretary of the Interior. Over the live telephone link from Washington Mr Morton said that it was "not only feasible but imperative" that the two countries exchange information now. For his part, Sir Derek described the agreement as "about the most comprehensive list that could have been laid down". He emphasised, however, that the agreement should not be seen as being exclusive, and mentioned commitments to other countries.

The full text has not yet been released but fifteen major areas of research are specifically named. These are the gasification, hydrogenation and solvent extraction of the coal; long wall mining techniques (needed in deep mines); automatic cutting machine control; coal preparation techniques; automatic measurement of coal characteristics; effluent control; minestone disposal; noise and dust control; safety research in the workings (degasification, subsidence studies, roof supports, and prevention of spontaneous combustion); air pollution control (treatment of flue gases); open cast mining techniques (including restoration of the site); advanced power systems and fluid bed combustion.

How cooperation on many of these topics should best be established is not yet finalised but further discussions are expected to take place at an international meeting on coal research which is to be held in London in October.

The agreement runs initially for three years and is extended automatically for periods of two years unless one of the parties withdraws. Each side must call for the information it wants and there are provisions in the agreement for the safeguard of patents and the exchange of personnel.

With Sir Derek Ezra in London was Dr William Gouse, Director of the United States Office of Coal Research and Development and Acting Director of the Office of Coal Research. Saying that coal "was something that the United States had discovered last fall" he described its importance to the United States as an energy source of its own from which clean fuel could be prepared. But he said that there was a shortage of people with the appropriate research background to exploit

these resources—a weakness in mining technology being the weak link.

As part of President Nixon's drive to make the United States independent of external energy sources by 1980, the Department of the Interior plans to spend about \$3,500 million on mining and coal utilisation research over the next five years. And it hopes that this sum will almost be matched by private industry.

Britain will spend a lot less. In plans which Sir Derek Ezra says have been "well received" by the Secretary of State for Energy, Mr Eric Varley, the NCB is calling for \$100 million (£40 million) to be made available over the same period.

But this disparity in expenditure should not be taken as a measure of each country's contribution to the research agreement. Sir Derek Ezra says that "this agreement will be very much a two-way process. We are not just latching on to a great American effort, we have much to give in return". The NCB's knowledge of the basic processes in coal utilisation methods is sound, he continues, and the United States has the resources to set up the necessary pilot schemes.

The United States is particularly interested in the fluid bed combustion of coal—a technique in which the bed of coal and ash inside a furnace is given the properties of a liquid by the upward passage of air. It hopes to use this method to generate electricity and is spending \$30 million next year on the building of a pilot plant. Under the agreement just signed, the results from this and other similar pilot projects will be available to Britain.

The United States has only recently discovered that it will have to dig deep for its coal. This is not only to obtain sufficient quantities of high grade coal but also to avoid the wrath of the environment lobby which is violently opposed to strip mining.

Noting that Britain has by far the biggest mining industry in Western Europe, Sir Derek suggests that the NCB can assist the United States with the long-wall mining technique that it must now use—perhaps by the export of specialised mining equipment. The NCB also has considerable experience of the health problems posed by noise and dust in deep mines (indeed over the past four years Britain has allocated £4.5 million to medical and engineering research connected with dust control and pneumoconiosis) so there are many areas of both mining and coal utilisation research where Britain's expertise will benefit "Project Independence". But perhaps the most important aspect of the new agreement is that it will allow ideas generated on either side of the Atlantic to be shared between both parties.

Copper exploration proposed for Exmoor

by Roger Woodham

BRITISH Kynoch Metals Ltd. has applied for planning permission to explore for copper on the borders of the Exmoor National Park in Devon. The company, jointly owned by Imperial Metal Industries and British Insulated Callender's Cables, is thinking in terms of scout drilling of ten holes less than 350 feet deep to start with, but would ultimately seek permission to re-establish a mining operation in the area if things turn out well.

The first thing to be said is that open-cast mining is out of the question because of the nature of the mineralisation. The metal ores in the region are in lensoid bodies that are relatively narrow and lie *en echelon*. There is not the large extent of relatively poor grade ore (less than 1%) that makes open-cast mining attractive.

British Kynoch is hoping to prospect on "a few square miles" of the Stucley estate at Heasley Mill in the North Molton area of Devon. This part of the estate includes several old workings, in particular the Bamfylde Mine which was used intermittently between the early eighteenth century and 1884. Its output was never very great by today's standards, however—between 1860 and 1881 it produced 5,000 tons of copper ore, the grade of which may have been 17% or so locally but was probably more like 5-7% overall. The mine goes down 900 feet and the main vein is some 2,000 feet long.

Mr P. F. A. Loffler, Managing Director of British Kynoch, said last week that geochemical and geophysical surveys had provided "some encouragement". These methods are, however, no substitute for drilling and examining a core because copper can find its way into the soil from artificial sources and the detection by geophysical methods of conducting material at depth may indicate no more than the presence of iron ore or graphite, hardly a prize for any mining concern.

Mr Loffler said that as the area is well wooded and made up of valleys and combs, the operations could easily be screened. His company has declared that the "visual impact of equipment, plant and offices would be minimal" in the event that mining was started again and that no local smelting is contemplated.

Local reaction is hard to gauge at present because nobody has had time to investigate fully the implications of the planning application. There is, however, general relief that open-cast operations are not being considered for the site.

THE unofficial Moscow seminar on "Collective Phenomena and the applications of physics to other fields of science", scheduled for July 1-5, 1974 now must pass into history as "the seminar that never was". With the arrest on Friday June 28 of Sinologist Vitali Rubin, it would seem that all leading members of the organising committee were in custody at the time of going to press. According to a cousin of Professor Mark Azbel, the arrested organisers are held outside Moscow, in detention centres which have previously been used for the temporary removal of dissidents from circulation. The centres mentioned are at Volokolansk and Serpukhov—the second showing a certain irony on the part of the authorities. Those intending participants not in custody are under virtual house arrest with police cars parked outside.

Seminar that never was

On hearing of the arrests, Professor Edward Stern, one of the three international secretaries of the seminar, began an intensive campaign in Washington to effect the scientists' release. A delegation from the intending participants met Senators Jackson, Javits and Ribicoff, who undertook to send a telegram to Dr Kissinger, asking for their release. They also met Senator Hartke of Indiana who claims to be on good terms with Brezhnev, and who promises to intercede personally on their behalf. Acting Secretary of State Sisco also showed considerable interest in the plight of the scientists, and undertook to bring it to Dr Kissinger's

attention. Eleven Nobel Laureates endorsed the principle, which has been conveyed to Mr Nixon in Moscow, that the right of scientists to emigrate without harassment should be written into any bilateral agreement between the United States and USSR.

Meanwhile, it has been learned that Mrs Nina Voronel was informed on Saturday June 29 that the group will be released after the scheduled seminar dates have safely passed. What their subsequent fate will be remains uncertain. One of them, Corresponding-Academician Venyamin Levich, has, however, now been granted permission to emigrate "at the end of 1975", while his sons Venyamin and Aleksandr can leave at the end of this year. If this is an omen of events to come, the ill-fated Seminar will not have been convened in vain.

It is ten years since the June 1964 elections to the Soviet Academy of Sciences which marked the beginning of the end of Lysenkoism and the re-introduction of Mendelian genetics in the Soviet Union. Although the Russians are usually eager to mark any significant anniversary, this particular one is not liable to receive the usual acclamation of celebratory meetings and publications. Nevertheless, a recent decree of the Central Committee of the Communist Party of the USSR and the Council of Ministers of the USSR does, in oblique fashion, form a kind of epitaph to the Lysenko period, by indicating the harm done to the development of Soviet science by almost a quarter of a century of opting out of world trends in research in genetics and molecular biology.

The Decree, published on May 21, 1974, deals with "the question of measures to accelerate the development of molecular biology and molecular genetics and to use their achievements in the national economy". It begins with the face-saving observation that "in recent years, on the basis of the wide use in biology of the achievements of chemistry, physics and mathematics, it has become possible to investigate the molecular mechanisms of the most important processes determining the existence and development of living matter". (This would logically imply that the earlier decision, in 1948, to stop all genetic research and, on the orders of the MGB—now the KGB—to destroy all *Drosophila* held in laboratories by drowning them in boiling water, was quite 'correct'; the necessary achievements in other fields had not yet been reached.)

Now, however, "fundamental discoveries in this branch of the natural sciences" have been made (it is not said by whom), which "have great

Epitaph to Lysenkoism

from Vera Rich

theoretical and practical significance for the development of agriculture, medicine and a number of branches of industry". Nevertheless, it is found that "the general level and scale of research on molecular biology and molecular genetics in our country is still insufficient. There are only a few highly qualified specialists making ready in this field. There are serious deficiencies in the organisation of the production of special scientific instruments and high class apparatus, the necessary range of chemical reagents, materials and biological preparations."

Accordingly, since "the Central Committee of the Communist Party of the Soviet Union and the Council of Ministers of the USSR consider that the achievement in the shortest possible time of the foremost level of development of molecular biology, molecular genetics and other branches of natural science immediately connected with the study of the physico-chemical principles of life phenomena" constitutes "one of the most important problems of Soviet science at the present time", the appropriate steps are to be taken. The Academy of Sciences, the State Committee for Science and Technology, the State Planning Committee and the various ministries and departments are charged with "ensuring the necessary rate of development of these sciences and a wide use of their achievements in agriculture, medicine and industry", with "strengthening the basic trends of fundamental research", and also with drawing up a concrete programme of research and design for 1974-80.

This last clause is extremely signi-

ficant—the new policy becomes effective immediately, without waiting, as would normally be expected, for the beginning of the next five-year plan in 1976. Since the logistics of implementing such mid-plan changes of policy in the framework of the rigid quinquennial budgeting of financial and manpower resources are considerable, it seems clear that what is involved is not only a change of planning policy, which could have waited another 18 months, but something close to panic measures.

The practical details contained in the Decree are sparse, as always. We learn, however, that a special Inter-departmental Scientific and Technical Council has been formed to coordinate research in this field, that means have been assured for the training of specialists, scientists and instructors, that new scientific research establishments and training colleges are to be opened and existing ones expanded, and also that "research bases" are to be constructed. The production of the required instruments, reagents and other necessities is to be "considerably expanded".

The Decree ends with the conventional expression of the confidence of the Central Committee and Council of Ministers that all persons and organisations concerned will carry out their appointed tasks in this new expansion of Soviet science in the fields of molecular biology and molecular genetics. In this atmosphere of all shoulders to the wheel, it seems unfortunate, for the Soviet authorities, that they can no longer call on the help and assistance of the chronicler of the Lysenkoist "pseudoscience", who did so much towards its overthrow—Zhores A. Medvedev, who, a year ago, they saw fit to deprive of his passport.

In India the explosion is seen very differently

from Narender K. Sehgal, Jullundur

The news on May 18 of India's nuclear explosion sent a wave of elation through the country. People were simply thrilled, despite the difficult economic situation. Even Indians abroad could not remain unaffected by the event. The President of the Indian Students' Association at the University of Illinois in Chicago wrote in a letter to a New Delhi daily newspaper: "... If anything, the reaction of the average American has been one of incredulity as to how such a country could pull off something like this. ... The Indian community by and large welcomed the blast as a veritable success for Indian scientists and engineers". Back home, to show their appreciation, the Federation of Jullundur Engineering Associations passed a resolution announcing a token cash award of Rs2100 for Shri H.N. Sethna, Chairman of the Atomic Energy Commission (AEC), for "successful explosion of a nuclear device".

The Indian scientific community, like other sections of the population, hailed the underground nuclear experiment as an important development in the country's atomic energy programme, signalling attainment of a certain degree of sophistication in nuclear technology. Dr S. M. Sircar, Director of the Bose Institute in Calcutta, said that the Atomic Energy Commission had done a good job. Dr D. Bose, Director of the Indian Association for Cultivation of Science, said it was a good step forward in making use of atomic energy for purposes other than war. Dr S. P. Ghosh, Head of the Department of Nuclear Chemistry at Patna University in Bihar, said India's success in the field of nuclear technology represented peaceful research activities for geological exploration. He added that in the peaceful use of nuclear energy there was also a possibility of constructing atomic weapons and this explosion shows that "Indian technologists are capable of doing so."

Following the explosion, atomic energy in general and the country's programme in this area in particular became hot topics for discussions, talks and seminars. Dr H. S. Hans, Head of the Department of Physics at the Panjab University in Chandigarh, hailed the nuclear experiment in a radio talk from Jullundur and explained various aspects of India's atomic energy programme. Speaking on "Nuclear Explosion" at the India International Centre in New Delhi Dr B. K. Nayar, Executive Secretary of the Indian National Science Academy,



said the success achieved by India in the nuclear explosion must be a matter of both envy and dismay to many. He felt that so far as application of nuclear energy for peaceful purposes in India was concerned, a better experiment might not be easily envisaged. He stressed that a crater-forming explosion could be utilised successfully in creating channels, dams and storage for underground gases and radioactive materials released by nuclear tests.

Reactions to the explosion from abroad have been on expected lines. The Chairman of the French Atomic Energy Commission has congratulated his Indian counterpart. According to scientists, India had merely staged a neat and skilful demonstration of what she had been believed capable of for some time. Even so, there has been criticism from some foreign quarters, mostly in the form of unofficial press comments.

Although there is undoubtedly an understanding here about stands taken by non-nuclear countries, protestations from those possessing stockpiles of nuclear weapons have failed to impress anyone. Doubts have been cast on India's peaceful intentions in regard to the explosion, pointing out rather irrelevantly that even Russia and the United States have had little success in using nuclear explosions for peaceful purposes. Maybe India will be able to show the way.

By and large scientists here seem satisfied with the countrywide favourable response evoked by the nuclear experiment. There is concern, however, especially among those connected in any way with the atomic energy programme, at the patently false impression (created by press and other comments from abroad) that the entire Indian effort in the field of atomic energy had been directed to-

Shot in the arm

ward detonating a nuclear device. Nothing could be further from the truth. India's atomic energy programme comprises activities ranging over a wide variety of fields: radio isotopes, medicine, food and agriculture, electronics, lasers, minerals, metallurgy and so on—and of course power generation, which has been one of AEC's major concerns.

In a television interview on May 31, of the AEC, the Chairman, Shri Sethna, pointed out that the commission was spending seven times more on nuclear research in agriculture, medicine and cancer studies than what was spent on conducting the test which, according to him, cost the AEC Rs 30 lakhs (lakh = 100,000).

To Indian science the explosion has come as a timely shot in the arm. This modest achievement (and perhaps others that may follow) could provide just the fillip needed to bring Indian science and technology into their own.

Indian scientists feel that India's mastery over nuclear explosion technology for peaceful purposes is bound to result in important advantages: India's nuclear neighbour will feel appropriately restrained and chances of confrontation between the two will diminish; Indian views on disarmament, particularly with respect to nuclear weapons, will carry a lot more weight now; the idea and concept behind the nuclear Non-Proliferation Treaty will have to undergo adequate changes to make it more just for the non-nuclear-weapon countries; and India may now be in a far better position to press for initiation of discussion on dismantling of all existing nuclear weapons and on a worldwide ban on all types of nuclear activity for war purposes.

"THE choice before mankind is a very simple one: nuclear disarmament or oblivion. In the absence of disarmament, more and more nuclear weapon powers will emerge. A limited nuclear war will eventually break out which may well escalate to a strategic nuclear exchange between the superpowers and extinguish our civilisation. The nuclear paradox is Man's total inability to cope with the obvious, even when it is a matter of life and death for the human race." With these words Dr Frank Barnaby, Director of the Stockholm International Peace Research Institute (SIPRI), justified the pessimistic outlook of the Institute's *Yearbook 1974* recently published in Stockholm. Like its four predecessors, this yearbook is about armaments and disarmament. After surveying the state of the armed world and the developments affecting it during 1973, the institute restates its commitment to general and complete disarmament as the only possibility for survival.

SIPRI readers have by now learned to expect the authoritative and sobering picture which emerges from this yearbook as from the institute's many other publications. It comes as no surprise to read that "... by the end of 1973 there was still little evidence that the degree of disorder in international affairs was decreasing". The bright spots of the year fall into place as acts of political necessity which have done nothing to stop the proliferation of conventional weapons or the continuing pressures towards technological refinement of both conventional and nuclear arms.

In 1973 the world spent \$207,000 million on arms, of which \$20,000 million was for military research and development. Since 1968 world military expenditure has been constant at about this level; therefore the latest figure represents a slightly lower proportion of world GNP now than it did then.

SIPRI pulls it all together

The unchanged figure however, disguises a disturbing trend: "The share of world military expenditure absorbed by the United States, the Soviet Union, France and the United Kingdom, taken together, has declined from 82% in 1955 to 70% in 1973. This does not reflect a reduction in the military capability of these four countries but rather indicates the magnitude of the increase in militarisation elsewhere." India's recent nuclear explosion illustrates this only too well.

The peculiar value of the SIPRI year books is that they relate all the happenings of a year, putting the events hailed as political milestones in perspective beside continuing world trends. Amidst the triumphant conclusion of the Israeli-Syrian disengagement agreement and the optimistic forecasts of its effect on future oil deliveries, for example, it is easy to overlook what is happening in other parts of the Middle East. The Persian Gulf is a critically important area for the shipment of oil. In recent years, the countries bordering the Gulf have, according to the yearbook, dramatically increased their military expenditures. Iran and Saudi Arabia have led the way. Over the decade 1963-1973 the average annual rate of increase of military expenditure in these countries was 23%. During 1973, Iran had outstanding orders for about 800 Chieftain main battle tanks, 250 Scorpion light tanks, more than 200 F-4E/F-5E fighter aircraft and nearly 500 helicopters, including about 200 Sea Cobra gunships armed with the latest US anti-tank missiles. The prospect of the Gulf countries being armed to the teeth and about to wield such influence

over oil shipments may well make us rethink the current relief over developments in another part of the Middle East.

Current nuclear events make the yearbook's discussion of nuclear policies particularly interesting. The statement on January 10 by the United States Secretary of Defense that the United States would in future pursue a counterforce strategy is interpreted by SIPRI as a justification for continuing the development of weapons for whose future deployment the doctrine of 'mutual assured destruction' provided no rationale. Of particular concern is the fact that enormous resources are being devoted to antisubmarine warfare technology, which is already sufficiently advanced to allow the destruction of a portion of the enemy's strategic nuclear submarine force. On the tactical level, it has been suggested that accurately delivered low yield nuclear weapons should replace the higher yield nuclear weapons now deployed, particularly in Europe. SIPRI objects to this proposal because "it is of paramount importance that an absolute 'firebreak' should be maintained between nuclear and conventional war".

It is surprising that SIPRI emphasises its basic commitment to effective disarmament as the only sure way of lessening threats all over the world, in spite of the facts that the yearbook is aimed largely at governments and that the institute is well aware that 'disarmament' is a dirty word in many government circles. The official intolerance of disarmament detracts from the practical value of the Yearbook's solution, for history shows that political will is essential for negotiated progress in any direction. In going against the stream, SIPRI is not merely being politically naive. It is attesting to the fact that it sees no middle way between disarmament and annihilation.

THE US House of Representatives voted last week to kill the Large Space Telescope (LST)—one of the most important astronomy programmes being planned for the 1980s—by deleting funding for the project from NASA's budget. If the Senate follows suit, NASA will have to revamp the project and come up with a cheaper option, but according to congressional sources, it is likely that the Senate Appropriations Committee will restore at least some of the money to keep LST alive.

NASA had asked for some \$6.2 million for the 1974-75 fiscal year for planning and design of the LST, before moving into the development phase. But the Appropriations Committee recommended that the money should

LST in danger

be denied this year and that "A less expensive and less ambitious project be considered as an alternative." The recommendation was approved by the House itself last week when it voted on NASA's budget.

A 120-inch optical telescope, the LST would be about 100 times more powerful than the largest ground-based instruments now in operation. It would be launched in the early 1980s—probably in 1981—by the space shuttle and it would be periodically serviced and upgraded during its 15-year lifetime. It has been enthusiastically endorsed by the Space Science Board of the

National Academy of Sciences but the House Appropriations Committee was evidently concerned at the large estimated costs of the project—between \$200 and 300 million.

Now that it has been approved by the House, NASA's budget must be considered by the Senate. The Senate Appropriations Committee is likely to report out a bill later this month and if the full Senate does eventually restore funding for the LST, the matter would have to be resolved by a House-Senate conference committee. Congressional sources predicted that the funds would be approved by the Senate and agreed to by the conference committee, but the outcome at this stage is far from certain.

news and views

Single mutation with two effects in tRNA

SINCE suppression was first discovered by the isolation of mutations that allowed some transfer RNAs to respond to nonsense codons instead of to their usual nucleotide triplets, the effects which mutational alteration may have upon tRNA have proved to be one of the most useful probes for investigating its function. The classical suppression of ochre (UAA) and amber (UAG) nonsense codons proved to be due to substitutions in the anticodons of tRNAs for glutamine, leucine or tyrosine, all possessing codons related to the nonsense triplets by a single base change. Missense suppression can result from a similar phenomenon, when a tRNA suffers a change in its anticodon which causes it to recognise a codon that usually represents some other amino acid. Another form of suppression involves frameshift mutations, in which a mutant tRNA restores the correct reading frame of the message, apparently acting at the codons GGG or CCC; whereas tRNA for glycine, for example, usually recognises GGG, a mutant seems able to respond to GGGG.

All these mutations are dominant; the presence of mutant tRNA achieves suppression. But what happens in the haploid bacterium at codons to which the mutant tRNAs would formerly have responded? When dominant suppressors can be isolated, presumably more than one tRNA must recognise their wild type codons, so that the capacity to translate these triplets remains even after mutation of one of the tRNAs. This explains why only some of the amino acids possessing codons related by single changes to the nonsense triplets can generate suppressors—presumably the others are recognised by only a single, essential tRNA whose mutation is lethal.

But the objection to mutation in essential tRNAs is overcome if the cell is made diploid for these genes: as Soll and Berg (*Proc. natn. Acad. Sci. U.S.A.*, **63**, 392; 1969; *Nature*, **223**, 1340; 1969) showed, in this case further suppressors can be isolated and they are recessive-lethals—if the bacterium is returned to a haploid condition, the suppressor tRNA is lethal, because there is no tRNA able to respond to its former codons. One of the two recessive-lethal suppressors that they isolated, *su7⁺*, has the surprising property of inserting glutamine at amber codons, surprising because dominant glutamine suppressors have previously been isolated and recessive-lethals were expected to implicate new amino acids in suppression. The unusual properties of the glutamine lethal-recessive suppressor are the subject of studies reported in the *Journal of Molecular Biology* by Soll (**86**, 233; 1974) and Yaniv, Folk, Berg and Soll (*ibid.*, 245).

Until very recently, all suppression—nonsense, missense or frameshift—mediated by tRNA seemed to be due to changes in the sequence of the anticodon that change the coding response of the transfer molecule. But Hirsh (*J. molec. Biol.*, **58**, 439; 1971) found that a UGA suppressor results from a mutation at position 24 of tryptophan tRNA, not located in the anticodon but allowing the molecule to respond to UGA as well as to its usual codon, UGG. And mutations affecting not the codons recognised by tRNA but instead the amino acid with which it is charged were recently isolated by Smith and Celis (*Nature new Biol.*, **243**,

66; 1973) and Celis *et al.* (*Nature new Biol.*, **244**, 261; 1973; see also *Nature*, **249**, 690; 1974). By examining the ability of cells possessing the *su3⁺* gene, which inserts tyrosine at amber codons, to suppress nonsense mutations at which tyrosine is not acceptable, they isolated mutants of *su3⁺* which continue to recognise the UAG amber codon, but insert glutamine instead of tyrosine. All five mutations constituted single base substitutions in the amino acid acceptor arm of the tRNA.

That the insertion of glutamine by the *su7⁺* suppressors results not from mutation of a glutamine tRNA but instead from a change in the charging specificity of another tRNA is the conclusion supported by the studies of Soll and Yaniv *et al.* Soll reports that the *su7* locus in *Escherichia coli* is identical to a locus in *Salmonella typhimurium* at which Miller and Roth (*J. molec. Biol.*, **59**, 63; 1971) previously isolated recessive-lethal suppressors of UAG and UGA codons that seem to be allelic. By obtaining a $\phi 80$ phage carrying the *su7⁻* gene, Soll showed that the *E. coli* locus also can suffer single step mutations to yield *su7⁺*_{UAG} or *su7⁺*_{UGA} suppressors. That both suppressors result from mutation of a single tRNA gene carried on the phage is suggested by the subsequent conversions possible from the

Is Jupiter lord of the Solar System?

THE idea that Jupiter and the Sun might be considered more sensibly as a binary system than in the respective roles of humble planet and dominating star is not particularly new. But on page 35 of this issue of *Nature*, Drobyshevski takes the idea an intriguing step further; according to his calculations, the dominant partner in the early stage of the development of the binary was Jupiter, and not the Sun.

Jupiter is certainly more like a small starlike body which has insufficient mass to trigger nuclear burning than like the other planets of the Solar System. According to Drobyshevski, one can be more specific and identify Jupiter with the core of the protosun. The process by which the original primary in the evolving binary system has become very much the secondary will be familiar to students of stellar evolution. At one time, it was something of a puzzle that the lower mass components in many binary star systems are found to be more highly evolved than their more massive companions, since a more massive star should evolve more rapidly. But this has now been explained in terms of mass exchange between the components of binary systems.

That is essentially how Drobyshevski explains the evolution of the early Solar System, with details of the process also offering a reasonable explanation of the formation of the other planets. And as a final bonus, the slow rotation of the Sun also emerges from the calculations when the angular momentum of the material streaming between the original binary companions is considered.

JOHN GRIBBIN

$su7^{+}_{UAG}$ and $su7^{+}_{UGA}$ alleles. The $su7^{+}_{UAG}$ tRNA can be mutated to yield an ochre suppressor recognising UAA and UAG. The $su7^{+}_{UGA}$ tRNA can be mutated to yield a UAG suppressor, although this results in loss of UGA suppression. These conversions can all be explained by single base mutations if the $su7^{-}$ tRNA represents the single species in the cell coding for tryptophan that responds to UGG. This can generate a UGA suppressor by mutation at position 24 in the dihydrouridine loop; and a change in the anticodon would generate a recessive-lethal UAG suppressor. Except for the initial generation of $su7^{+}_{UGA}$ from $su7^{-}$ tRNA^{Trp}_{UGG}, all the conversions observed can be achieved by single changes in the anticodon.

Sequencing studies confirmed that the amber and ochre $su7^{+}$ suppressors are derived by single base changes in the anticodon of the tryptophan tRNA. Yaniv *et al.* found that a change from CCA in the wild type anticodon to CUA in the mutant $su7^{+}_{UAG}$ is the sole difference between the transfer RNAs. The $su7^{+}_{UAA/G}$ suppressor generated by mutation of $su7^{+}_{UAG}$ represents a further single base substitution, having an anticodon of UUA. But although derived from tryptophan tRNA, the $su7^{+}_{UAG}$ and $su7^{+}_{UAA/G}$ suppressors both insert glutamine at the nonsense triplets to which they respond. This means that the mutation from C to U at the middle position of the anticodon must have two effects: it changes the coding response so that the tRNA recognises UAG instead of UGG; and it alters the charging specificity so that the transfer molecule is recognised by glutamine-tRNA synthetase instead of tryptophan-tRNA synthetase. The second C to U change, generating the ochre $su7$ suppressor, must change the coding specificity to allow recognition of UAA as well as UAG, but leaves unimpaired the new ability of the molecule to be charged with glutamine instead of tryptophan.

Comparing the sequences of the two glutamine tRNAs (which recognise CAG and CAA and differ in seven nucleotides) with the $su3^{+}$ tyrosine suppressor shows few similarities. All five of these mutations are located in the amino acid acceptor stem of the molecule. Four disrupt one of the first two base pairs, and may therefore be effective because they induce a conformation change; the other replaces an adenine not involved in base pairing with a guanine, and presumably this base must be recognised directly, or must influence the conformation of the molecule by an interaction with some other region in the tertiary structure. Each individual mutation may establish some similarity with glutamine tRNA and Inokuchi, Celis and Smith (*J. molec. Biol.*, **85**, 187; 1974) recently showed by constructing a double mutant that two together may enhance charging with

glutamine compared with the single mutants.

In view of the implication of the amino acid acceptor stem as a site for recognition by gln-tRNA synthetase, it may perhaps be significant that the only similarities between glutamine tRNA and $su7$ tryptophan tRNA lie in the base pairs of this region and of the anticodon stem, although of course recent research has pointed to the conclusion that base pairs as such, not their individual bases, may be important in recognition of tRNA. It is not at all obvious why a single change in the $su7^{-}$ tRNA anticodon allows the molecule to be recognised by gln-tRNA synthetase. Perhaps the isolation of further mutants, and determination of the relative extents to which each substitution allows false recognition of tRNA, may allow some definition of how synthetases recognise transfer molecules; but at present this remains unclear.

BENJAMIN LEWIN

Saiga finds safety in numbers

from D. Michael Stoddart

THE story of the saiga (*Saiga tatarica*) is one which could easily have had an unhappy ending. Intense piratical hunting and severe 'djuts' (glazed snow coverings of great persistence) brought the world's total of this antelope during the first quarter of this century to just a few hundred. In the 1930s the game authorities in the Soviet Union totally prohibited hunting and took steps to reduce the herds of nomadic cattle that overgrazed the saiga's range. Nature lent a kindly hand by offering a few winters of relative mildness without serious djuts. By 1954 there were 1 million head of saiga and in 1973 more than 2 million in the Soviet Union. Cropping started in 1955 and during the next 17 years nearly 3 million head were harvested and these produced 58,000 tons of meat. Current studies in Kazakhstan and other areas in which saiga overwinter are being made to find ways of lessening the impact of serious djuts.

An important feature of the ecology of the saiga is the high reproductive

The saiga antelope, chosen as the emblem of the First International Theriological Congress and depicted on one of a special set of postage stamps issued to mark the event.



rate. Old females regularly give birth to twins, sometimes triplets, and young females breed first at 7 months. The rate of embryo resorption is low but noticeably higher after winters with severe djuts when the crust of glazed snow diminishes available food and the animals enter spring in an emaciated state. In concluding his remarks to the First International Theriological Congress held in Moscow from June 6–12, A. A. Sludsky drew attention to the importance of this natural resource to the more isolated regions of the Soviet Union where rail and road transportation links are poor.

A less rosy outlook was presented by I. I. Barabash-Nikiforov and L. V. Shaposhnikov for the Russian desman (*Desmana moschata*), a large, aquatic shrew-like mammal. Like its Pyrenean cousin, the desman is a Tertiary relict. Everywhere it is becoming much less abundant. Today it is to be found only on parts of the River Ob' in West Siberia. Various reasons are thought to have brought the desman to the brink of extinction. Foremost among these is the use by fishermen of creels and trap nets in which many desmans drown. The draining programme of flood plains and the building of levées, the destruction by cattle of riverside vegetation and the interspecific competition with muskrats for bank space are other influential factors. It seems as if the economic and human factors will prevail and even spread to the few safe desman pockets where the creatures can find safe harbourage. Unless special reserves, like the Kljazma Reserve which was abandoned in 1951, are created and recreated soon, the desman will fast become another species only to be found in zoos.

An experimental farm for moose (*Alces alces*) at Kostroma, some 200 miles north-east of Moscow, is leading the way to a new line of domestic meat-bearing species. Bottle feeding of moose calves, group rearing and feeding are giving promising results. Moose cows breed in captivity when they are 16 months old. Their calves grow at a rate of about 300 g per day. On the farm they keep up this rate throughout the year, though wild calves do not usually increase in weight at all during the winter. E. M. Dzshurovich and A. P. Mikhailov are confident that a significant new branch of livestock hus-

bandry is just around the corner.

The congress was not concerned solely with mammals from the Soviet Union, nor just with ecology and production biology. About 1,000 zoologists from many countries of the world took part in a range of seminars and symposia which covered topics such as zoogeography, evolution and taxonomy, endocrinology and gestation, growth physiology, ethology, orientation and signalling, and the problems to be overcome in conserving bears and marine mammals. The USSR Academy of Science (see *Nature*, **249**, 502; 1974) organised the meeting in its 250th anniversary year. The congress has significantly fostered a closer spirit of interaction and cooperation between Soviet and Western zoologists than has hitherto been the case.

Antibody manipulation by malaria parasite

from F. E. G. Cox
Parasitology Correspondent

WITH more than one thousand million potential malaria victims in the world it is not surprising that considerable attention has been paid to possible methods of vaccination against this disease. All the attempts so far, however, have been relatively unsuccessful. All have involved killing or attenuating malaria parasites and using large amounts of antigen. Even then, the results have been variable and protection has been limited. Many workers believe that vaccination against malaria is not possible because of the existence of antigenic variation and cell mediated responses as yet unrecognised. In fact, much of the basic information necessary for the development of a vaccine is already available and it may well be that investigations of antigenic variation and cell mediated responses are irrelevant to immunity to clinical malaria.

Immunity to malaria can be explained in terms of an antibody directed against the merozoites while they are outside the red blood cells. The evidence for this comes from three sources. First, *in vitro* experiments carried out by Cohen and his colleagues at Guy's Hospital Medical School have demonstrated that parasites within their red cells take up ³H-leucine in the presence of immune sera as well as they do in normal sera (*Trans. R. Soc. trop. Med. Hyg.*, **65**, 125; 1971) but that in immune sera the invasion of fresh blood cells is inhibited. They have also shown that merozoites bind to suitable cells (*Nature*, **244**, 40; 1973) and the prevention of this binding by immune

sera could form the basis of immunity. Second, Jerusalem, Weiss and Poels, in a paper that has not received the attention it deserves (*J. Immunol.*, **107**, 260; 1971), showed that fluorescein-labelled antiserum injected intravenously into mice bound only to merozoites and not to intracellular parasites. Third, nobody has observed any damage or signs of changes in parasites within their cells during the immune response.

If it is only the merozoites that are important in immunity, why is it that the antibodies associated with antigenic variation agglutinate infected cells? Part of the answer to this question comes from the work of Brown (*Nature*, **242**, 49; 1973) and Brown and Hills (*Trans. R. Soc. trop. Med. Hyg.*, **68**, 139; 1974). They have found that in *Plasmodium knowlesi* infections in monkeys, infected cells are agglutinated by a non-protective antibody that is easily separated from an opsonising antibody associated with protection. Brown postulates that the agglutinating antibody serves to trigger a switch in antigens and this change allows the parasites to survive in what should have been an immune host. In other words this antibody has been manipulated by the parasite for its own ends. The opsonising antibody is important in protection and is effective against all antigenic variants and the relative rates of synthesis of these two antibodies determine the outcome of the infection.

The protective immune response to malaria is weak and largely ineffective and it is unlikely that any vaccine could produce a better immunity than the natural one. Nevertheless, acquired immunity to malaria does occur and children who survive the first few years of their lives in malarious areas tend to be immune to clinical malaria if not to the infection itself. A vaccine that could be used to see children through their first few years would be invaluable. There is no evidence to suggest that cell mediated responses are important in immunity to malaria so the induction of an antibody against merozoites could well be effective. Possibly all that is required is the antigen involved in binding merozoites to their host cells. As antigenic variation involves an antibody that has nothing to do with protection it is unlikely that antigenic variation would interfere with any protection produced as a result of vaccination against merozoites.

Correction

In the News and Views article "Biogenesis of surface membranes" (*Nature*, **249**, 414; 1974), Dr G. Kreibich's name was misspelled in the penultimate and last paragraphs.

Anthill and tiger counting in the Soviet Union

from Vera Rich

JUDGING from the numerous Soviet press and agency reports, one of the major tasks of Soviet ecologists and conservationists is the taking of censuses of various species of wild life. This applies not only to those species threatened by extinction, but of any that are or might prove of value to the economy. Thus the 1972 census of anthills in Estonia was undertaken, not because the Estonian ant was in imminent danger of disappearing, but because it had been found that an optimum of 3-6 anthills per hectare in stands of timber checks the multiplication of various forest pests. Similarly, the census currently being taken of sables in the Amur taiga will be used for improving the system of distributing hunting licences. Even in the case of threatened species, practical advantages are remembered: the Black Sea dolphin census (1971), which revealed that the dolphin population had doubled since the ban on hunting was introduced in 1965, evoked the remark from its director, A. Chepurnov, at a press conference: "The time is not far distant when dolphins will be used to drive fish into nets, to carry out marine rescue operations and to communicate with underwater laboratories".

The census-taking methods vary considerably in complexity. Counting anthills is a relatively simple task, which could conveniently be delegated to students as part of their practical work, but the estimation of most species is much more difficult. The latest (1973) Black Sea dolphin census, which gave a total of 800,000 (three times the 1965 figure), required aircraft and seagoing ships and took 3 months to complete. The grandiose estimation of the total dry-land flora of the world, carried out by the Botanical Institute of the Academy of Sciences of the USSR, giving a total 2,625,000 M tonne of phytomass, presumably involved satellite data. Sometimes a quantitative result seems impossible to obtain. In the case of the jeiran (a variety of antelope) of southern Tadzhikistan, although "large herds" are now reported where, before protection, only a few isolated specimens were observed, no figures, either absolute or relative, seem to have been established. Presumably, so far, no reliable method of counting them has been devised.

The same difficulty, it might be thought, would arise in the census of Amur tigers made from 1971-73 in the Primor'e region of the Soviet Far East, but an ingenious solution was found—

the investigators counted the tracks of tigers in the snow. Although at first glance this proposal seems to contain a source of confusion in the possible crossing and recrossing of its tracks by the same tiger, the photographs published (*Priroda*, No. 12, 82-88; 1973), show that although snow is not the ideal medium for revealing the fine details of the pad with fingerprint clarify, nevertheless, in practice, little or no confusion arises. Not only did this census give a total of some 110-120 tigers in the area (the figure for the 1940s was 30-40), but it revealed a number of significant details about the habits of tigers in the region.

Now that the Primor'e is being increasingly opened up for forestry, and the like, the tigers seem to be entering into a cautious symbiosis with man. (The Amur tiger is not a man-hunter; on the contrary, it is easily scared off by shouting and arm-waving.) The tigers make use of forest roads used for hauling timber, and seem to have no fear of tractors and automobiles. They appear, and may even hunt, relatively close to settlements and motor roads, and observe human activity from a distance of several metres. If disturbed after hunting, they will drop their prey, but are ready to return to it, even if it has been touched by human hand. And, since the presence of tigers causes a rapid disappearance of wolves from the area, the opening up of the Primor'e is in no way hampered by the protection of the tigers—indeed, their presence may prove an advantage. Providing, therefore, that no illegal tiger shoots take place—a condition which, it is admitted, is hard to guarantee—there seems no reason in principle why the tigers should not continue to flourish in these new conditions of coexistence.

Membrane proteins and serum lipoprotein

from a Correspondent

UNTIL recently, discussions of membrane structure centred around the concept of the unit membrane. The model envisaged was a lipid bilayer with hydrophilic proteins restricted to the outer surfaces where they were attached by electrostatic, non-covalent forces. Although this model was useful for explaining the similarities in physical and chemical properties between artificial lipid bilayers and natural membranes, it failed to account for all the proteins known to be associated with the membrane. This led several workers, notably Singer and Nicolson (*Science*, **175**, 720; 1972), to postulate that globular proteins penetrate into or through the lipid bilayer. This idea has been widely accepted as

a sound working hypothesis, but the mode and distribution of the proteins in the plane of the membrane are still controversial. It has been convenient to divide membrane proteins into two groups; extrinsic proteins remain largely on the aqueous side of the membrane attached by electrostatic forces which can be disrupted by dilute salt solutions whereas intrinsic proteins make substantial contact with the hydrocarbon region of the lipid bilayer and can only be disrupted by use of ionic detergents. In practice, however, this distinction is difficult to apply to any protein because of the lack of structural information.

The human erythrocyte has occupied a central position in membrane research due to the ease of obtaining large quantities of membrane ghosts free of haemoglobin. By SDS gel electrophoresis, more than 20 membrane protein bands have been resolved (see, for example, Fairbanks *et al.*, *Biochemistry*, **10**, 2606; 1971) but, with few exceptions, the types and functional roles of the proteins have remained obscure. In particular, the presence of proteins with an apparent molecular weight in excess of 200,000 and apparently not dissociable into smaller subunits by treatment with thiols or detergents has been rather enigmatic. One would expect that intrinsic proteins would have more non-polar amino acids than do hydrophilic globular proteins, but differences have not been impressive. Furthermore, intrinsic proteins should have relatively short hydrophilic sequences capable of reacting with lipids. Glycophorin, the major glycoprotein of the human erythrocyte, contains a peptide chain of 203 residues which completely spans the membrane and has a portion of 23 residues devoid of charged groups (Segrest *et al.*, *Biochem. biophys. Res. Commun.*, **49**, 964; 1972); but the applicability of this situation to intrinsic protein in general cannot be assessed at present.

The erythrocyte membrane is not the only system where one can find lipids and proteins in close association readily accessible for extensive study. The circulating plasma lipoproteins are important sources of avidly bound lipids and proteins that are not membrane bound. In a recent issue of *Biochimica Biophysica Acta* (**342**, 213; 1974), Langdon now reports that the apoproteins of plasma lipoproteins are major constituents of the human erythrocyte membrane.

Langdon's evidence comes from both immunological and chemical analysis of the membrane proteins. Purified, packed erythrocyte ghosts were treated with sodium dodecyl sulphate to solubilise intrinsic proteins. Then the preparation was subjected to double

Tuberculated beetle



This strange-looking beast is a male dung beetle new to science. It has been named *Amphistomus primonactus* by Matthews of the South Australian Museum, Adelaide, who discovered it in the Dorrig National Park. Its reduced hind body and exaggerated tuberculosity may be a consequence of wing reduction. It is inconspicuous and secretive, like the other wingless species of *Amphistomus*, and is abundant in closed forest leaf litter. It seems to have a very localised distribution (see *Aust. J. Zool.*, Suppl. Ser., No. 24, 1-211; 1974).

immunodiffusion on agar plates against antiserum to human density lipoprotein (apo HDL) and low density lipoprotein apoprotein (apo LDL). Strong precipitin lines indicated that the membrane proteins are related immunologically to both types of apoprotein (appropriate controls eliminated the possibility of non-specific precipitation). To determine whether the antigenic determinants resided in more than one molecular weight class, the membrane proteins were fractionated by Sephadex gel chromatography and examined by immunodiffusion. Although each protein class contained several proteins it was nevertheless noteworthy that antigenic determinants for both apo HDL and apo LDL antisera were present in all of them. Further tests showed that nearly half of the total membrane proteins are accounted for by the sum of the proteins reacting as the apo HDL and apo LDL and that probably antigenic determinants for both tested apoproteins are present in at least one protein molecule.

In order to fortify the immunological evidence with chemical evidence, end group analyses were performed. The membrane proteins were dansylated and hydrolysed and the resultant amino acids were analysed by quantitative two-dimensional polyamide chromatography. As expected, the major NH_2 terminals were aspartic

and glutamic acids, characteristic of apo HDL and apo LDL, respectively. Together they constituted 40% of the NH_2 terminals, a fact which agrees well with the immunological data, if it is assumed that most of the glutamic and aspartic acids are present as the NH_2 terminals of the apoproteins in the membrane. COOH terminals were determined by standard techniques and the results showed that these too were rich in terminal residues characteristic of the serum lipoprotein apoproteins. To discover whether the apoprotein NH_2 terminals were distributed over various molecular weight sizes, a portion of dansylated membrane proteins was fractionated by SDS electrophoresis and the amino acids from each band chromatographed on polyamide. The results showed that the characteristic NH_2 terminals of serum apoproteins were distributed over a wide range of molecular sizes, in agreement with the immunological data.

The pattern of the NH_2 terminal amino acids together with certain of the immunological data and the high molecular weights observed led Langdon to consider that the apoproteins may be linked in the membrane to form larger molecules. Previous work had shown that disulphide links are not present in membrane proteins so Langdon investigated the possibility of lysine-derived crosslinks, more usually found in connective tissue (*Biochem. biophys. Acta*, **342**, 292; 1974). He indeed found positive evidence for such links using chemical techniques similar to those used for examining proteins like collagen and elastin.

Langdon's data pose some intriguing and important questions. Are serum lipoprotein apoproteins the major intrinsic proteins of the erythrocyte by virtue of their affinity for lipids? And do they form a structural basis for the covalent attachment of enzymes and other protein types? Are the apoproteins synthesised in the haemopoietic system during erythropoiesis or are they derived from circulating lipoproteins? If Langdon's results are confirmed, they could have far-reaching implications in both membrane biochemistry and in cardiovascular research.

• Functions of the cell periphery

by Peter Newmark

By shrewd invention of a title the organisers of a discussion meeting held at the Royal Society on June 19–20 managed to attract a diverse range of speakers on the pericellular environment and its regulation in vertebrate tissues. Within this framework many topics were touched on although most

contributions were concerned with connective tissue cells and matrix.

The problems of understanding the metabolism of the matrix are based partly on the complexity of its component proteoglycans, glycosaminoglycans and tensile fibres and partly on separating the synthetic and degradative parts that connective tissue cells play in the turnover of the matrix. H. Muir (Kennedy Institute for Rheumatology, London) reported on the disproportionate importance of the minute amounts of hyaluronic acid recently shown to be present in cartilage matrix. It seems that the distinct proteoglycan aggregates found there are formed by virtue of the affinity of one end of the proteoglycan's core protein for multiple sites on the hyaluronic molecules. An additional important role for hyaluronic acid in cartilage is implied by the demonstration that it inhibits the production of proteoglycans by chondrocyte suspensions, possibly by means of a cell membrane effect.

The role of the membrane in matrix turnover was also raised by the studies of J. T. Dingle (Strangeways Research Laboratory, Cambridge). In addition to reporting on two new cathepsins that may play a part in proteoglycan breakdown, he also elegantly demonstrated that cathepsin D is localised on or around the cell membrane during its stimulated secretion from cells. A functional importance of this phenomenon was suggested by it also being demonstrated in cartilage taken from human rheumatoid arthritic joints. The importance and site of action of the cathepsins, all of which have an acidic pH optimum, however, remain to be elucidated.

P. Davies (Clinical Research Centre, Harrow) drew parallels between the development of glomerular nephritis and asbestosis. The activation of lysosomal enzymes by immune complexes in the first case and by asbestos particles in the second disease can result in the breakdown of the basement membrane. This, perhaps with contributory mechanisms, can trigger off the overproliferation of epithelial cells and thickening of the basement membrane that is associated with both diseases. Cell proliferation, in this case of the arterial smooth muscle cells, was also one of the topics discussed by R. Ross (University of Washington). When grown in culture, these cells, like so many others, only proliferate in the presence of serum. It seems, however, that the important growth factor(s) is initially derived from platelets. The importance of this factor could be that, when released from platelets at a site of arterial injury, it causes the local proliferation of subendothelial cells in atherosclerosis.

In a speculative vein J. E. Scott

(MRC Rheumatism Unit, Taplow) contemplated the evolutionary forces that may have determined the universality of polyanionic carbohydrates in cell membranes and matrix. In prebiotic times they would have produced an excellent shield against radiation-produced hydrated electrons; with the advent of aerobic conditions they would still be very stable; and, finally, they possess excellent properties for withstanding the wear and tear to which extracellular molecules are subjected.

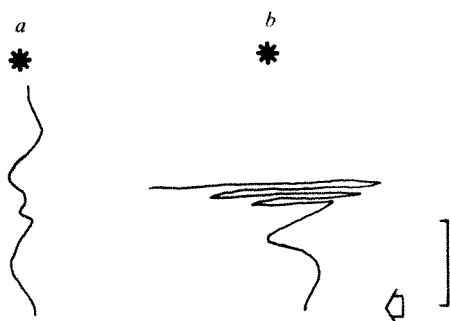
In his continuing studies of cell-cell recognition, M. M. Burger (University of Basel) has recently turned his attention to a model system, the sponge. Suitable treatment of sponge cells results in their releasing an aggregation factor and a base plate. If the latter is covalently attached to cell-sized solid beads, the addition of the aggregation factor and calcium results in bead aggregation. This neat system promises to assist in the elucidation of the molecular basis of cell interactions.

Orientation to odours by insects

from our Insect Physiology Correspondent

It has long been known that insects fly upwind to attractive odours—mosquitos finding their hosts, drones locating the queen bee, fruit flies seeking fermenting fruit—and that the maintenance of the direction of flight is a visual response to the pattern of the ground below. In other words that anemotaxis in the flying insect is an optomotor response. But a year or two ago (*Science*, **173**, 67; 1972; **180**, 1302; 1973) Farkas and Shorey claimed that in response to the sex pheromone of the female, flying males of the pink bollworm moth *Pectinophora* are guided by a chemotactic mechanism which can operate in still air. The existence of such a mechanism of oriented flight in the absence of a moving air stream (as opposed to a kinetic mechanism dependent on gradients in the concentration of odours, or a visually oriented mechanism in an air stream) could be disturbing for accepted theories of orientation by flying insects.

Kennedy and Marsh now point out (*Science*, **184**, 999; 1974) that in the experiments of Farkas and Shorey the males were already in flight and may already have been oriented anemotactically before the air stream was stopped. They have therefore repeated the experiments on males of the dried fruit moth *Plodia interpunctella* and related moths; the movements of the insects were accurately recorded in a wind tunnel and the results were analysed statistically. The flight of male insects in a stream of air in a wind tunnel could be completely controlled by the



Two 3 s flight tracks (based on videotape records) of the same male *Cadra cautella* after its arrival by upwind flight at a point 0.50 m from a source of female scent. To the left (a): the source remained throughout the upwind position marked by the star. To the right (b): the source was removed as soon as the male reached the level of the arrowhead so that the male entered unscented air after travelling approximately 0.15 m further upwind. (Scale: 0.15m.)

movement of black and yellow stripes on the floor. If the stripes were stationary the males flew upwind against the stream of odour from the female; if the stripes moved downwind above a certain velocity the flying male also was carried downwind. If the odour was suddenly removed while the male was in flight, but the odourless air stream continued, the male arrested its upwind flight and flew at right angles to the air stream, casting to and fro over a progressively widening band (see figure). This response was unchanged if one antenna was removed, which would not have been the case if a chemotropotactic mechanism were involved.

It seems, therefore, that an odour-controlled optomotor anemotaxis is still the most plausible mechanism to account for the response of the male moth to the sex pheromone of the female; and that the side to side casting when the odour trail is lost, which had been noted in the past, is an important element in the optomotor reaction.

Anchoring the Ig molecule

from a Correspondent

It is generally accepted that immunoglobulin (Ig) acts as the antigen recognition unit at the surface of bone marrow-derived (B) lymphocytes. The chemical homology between the membrane-bound Ig and the molecules secreted by the activated antibody-forming B cell is unknown although this must be considerable since the surface-bound Ig component can be detected if serum reagents specific for both variable and constant regions are used. This raises the problem of how a soluble Ig glycoprotein molecule is

adapted to perform a function which requires firm attachment to the cell surface membrane. In many integral membrane proteins and glycoproteins a small hydrophobic segment of peptide anchors the rest of the molecule into the lipid bilayer of the membrane. It is possible certainly that the polypeptides of surface Ig contain an extra stretch of amino acids suitable for this purpose. If the two or three well studied examples of membrane proteins are considered, these amino acids would be located at the carboxyl terminal of heavy chains in the constant (Fc) portion of the molecule.

An alternative method for anchoring an essentially water soluble component to a membrane involves attachment to an integral membrane component with a recognition site for some part of the soluble protein. This method is favoured by Ramasamy *et al.* (*Nature*, **249**, 573; 1974) for surface-bound Ig.

B lymphocytes as well as macrophages, mast cells and polymorphonuclear cells possess surface receptors for the constant (Fc) portion of Ig. It is proposed that these receptors tightly bind Ig molecules to the B cell surface in such a way that the antigen binding sites of the variable region of Ig extend into the extracellular space and are available to specific antigen. The murine plasma cell tumour MOPC 21 (P3) secretes IgG1 kappa molecules in culture, a small proportion of which may be assumed to bind to and saturate the putative Fc surface receptors. Indeed, no Fc receptors are detectable on these cells. Mutants blocked in Ig secretion, however, can be obtained and these also carry no surface Ig molecules. Ramasamy *et al.* argue that in these cells the Fc surface receptors should be demonstrable and this proved to be the case. That is to say there is in general an inverse correlation between the presence of surface Ig and Fc receptors.

The surface Ig of mouse B lymphocytes is probably monomeric IgM, and it seems that the secreted pentameric IgM may have decreased affinity for Fc receptors since secreted IgM only weakly inhibits Fc rosettes on B cells. The interaction of Ig with Fc receptors also seems in some cases to require intact Ig carbohydrate units. Williams *et al.* (*J. Immunol.*, **111**, 1690; 1973) have treated rabbit IgG preparations with an endo- β -N-acetylglucosaminidase from *Diplococcus pneumoniae*. About a half of the carbohydrate, present largely in the Fc portion of the Ig molecule, is removed. The bacterial agglutinating activity of anti-pneumococcal IgG was unaffected by this treatment whereas complete loss of opsonic activity occurred. Similarly several treated IgG preparations had markedly decreased ability to inhibit

rosette formation between human monocytes (carrying surface Fc receptors) and IgG-coated erythrocytes. Not all IgG preparations were affected in this way, however, (for example, anti-streptococcal), although similar amounts of carbohydrate were removed by the glycosidase.

The reason for this finding is not clear. Since it cannot yet be excluded that the detailed carbohydrate structure of Ig molecules may differ according to species or possibly with the particular cell clones activated to produce antibody, it seems likely that the type of carbohydrate structure left behind on the treated Ig molecule may also vary. In some cases the biologically relevant sugar sequences might not be removed by glycosidase treatment. Further work using additional enzymes will be required to remove more than half of the carbohydrate, leaving the Ig polypeptide chains intact. Only then will it be feasible to decide if significant differences in the mechanisms for opsonisation and phagocytosis of different bacteria exist rather than the simpler explanation that differences in carbohydrate structure occur in Ig molecules of different specificities.

Terminal sequences of animal virus RNAs

from Alan E. Smith

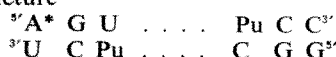
To date, attempts to sequence the RNA of animal viruses have lacked the spectacular success of bacteriophage RNA sequence work. This is largely because of the difficulties encountered with animal viruses in obtaining either highly labelled RNA for analysis by the Sanger technique or large amounts of RNA for sequence determination by more classical methods. Nevertheless, methods are being developed to overcome these problems and among the most successful are the techniques for the enzymatic introduction of radioactive phosphate into specific positions within viral RNA molecules.

Silkworm cytoplasmic polyhedrosis virus (SCPV) contains ten pieces of genomic double-stranded RNA, and in this and other respects it is very similar to the more familiar reoviruses. Some time ago the nucleotides present at the ends of reovirus double-stranded RNA were determined by isolating 32 P-labelled viral RNA and subjecting this to Sanger RNA sequencing techniques. All ten fragments of reovirus were found to have a 5' terminal guanosine 5' diphosphate followed by a pyrimidine residue (*J. molec. Biol.*, **61**, 643-653; 1971) and a 3' terminal cytosine (*Nature new Biol.*, **232**, 114-115; 1971). Each genome fragment therefore

seems to have a perfect duplex form.

In a recent issue of the *Journal of Molecular Biology* (85, 31-48; 1974) Miura, Watanabe and Sugiura report a similar analysis of silkworm cytoplasmic polyhedrosis virus. To avoid the difficulties in labelling silkworms with ^{32}P to produce radioactive virus, the isolated viral RNA was treated with phosphomonoesterase to remove any 5' phosphates and then labelled in the 5' OH position using polynucleotide kinase and [$\gamma^{32}\text{P}$] ATP. The native double-stranded RNA molecules proved very difficult to label by this method, however, and to achieve efficient labelling Miura *et al.* first removed chemically two or three residues from the 3' end of the molecules, thus exposing a short single-stranded 5' terminal region. Presumably such single-stranded tails are much more accessible to enzymatic modification. The labelled molecules were then analysed by ribonuclease digestion followed by column chromatography. Two 5' termini were found and were identified as A*GU and GGC. The identity of the 5' terminal base A* is as yet uncertain but it is probably an adenosine residue with a modified 2' OH group. Such modified bases, although a common feature in transfer RNA molecules, are rare in viral RNAs. The 5' terminal sequences are consistent with perfect duplex formation in the SCPV genome since the same authors have previously identified cytosine and uridine as the 3' terminal bases.

To determine whether all of the double-stranded genome segments contain both 5' terminal sequences or if some fragments contain symmetrical ends, all of the ten genome segments were separated on polyacrylamide gels and then individually subjected to end-group analysis. This showed that every fragment contains both sequences and suggests that each has the structure



As with reovirus, silkworm cytoplasmic polyhedrosis virus particles contain an RNA-dependent RNA polymerase which actively transcribes the double-stranded RNA into messenger RNA *in vitro*. All ten mRNA molecules made *in vitro* by the reovirus enzyme begin with ppG (Banerjee, Ward and Shatkin, *Nature new Biol.*, 230, 169-172; 1971). Further sequence data on highly labelled mRNA made from the smaller double stranded RNA segments has been presented by Nichols, Hay and Joklik (*Nature new Biol.*, 235, 105-107; 1972). Some 5' terminal guanosine triphosphate as well as diphosphate was detected in this study, but no protein synthesis initiation codon was present within the first 25

A larger Gondwanaland?

from Peter J. Smith
Geomagnetism Correspondent

ALTHOUGH the principal features of the ancient supercontinent of Gondwanaland are now widely accepted, there are still some details (if whole countries may be so regarded) and disagreements to be settled. One outstanding problem, for example, concerns the present position of the continental crust which once lay between Australia and India. Somewhat less of a problem in many people's eyes is the nature of the present join between India and the rest of Asia. Dewey and Bird (*J. geophys. Res.*, 75, 2625; 1970) suggested that the Himalayas resulted from continental collision; and such has been the influence of the article in which this proposal appeared that some of the difficulties with this interpretation have perhaps not been given the attention they deserve.

At least, that seems to be the view of Crawford (*Science*, 184, 1179; 1974) who cites Meyerhoff's claim (*J. Geol.*, 78, 1; 1970) that there is evidence against India's colliding with the rest of Asia. Certainly, there are problems here, which is why Powell and Conaghan

(*Earth planet. Sci. Lett.*, 20, 1; 1973) recently proposed that although the Himalayan mountain chain is basically of the collision type, it was not formed as a direct result of the collision. But Crawford, who at the time of writing was apparently unfamiliar with the Powell-Conaghan model, goes further in proposing that the Himalayas formed intracontinently and arguing against collision on the grounds that the Indus Suture Line, "if it is the relic of oceanic subduction preceding collision, lies on the wrong side of the Himalayas".

In Crawford's reconstruction, Gondwanaland was originally larger than is usually supposed and included Tibet, the Tarim Basin block and parts of northern China. It was Tibet, in fact, which lay between India and western Australia in the form of submerged continental crust. The Indus Suture Line is then seen as "a relic of a Permojurassic oceanic opening to the mantle" which closed at the end of the Jurassic. The Himalayas later developed along fractures which had formed parallel to the opening of the Indus Suture Line.

Precursors of strike-slip faulting

from Peter J. Smith
Geomagnetism Correspondent

THE travel-time residuals of seismic waves—the differences between observed arrival times and the corresponding arrival times computed using a standard Earth model—are due (in addition to random experimental errors) partly to imperfect source determination and partly to differences in velocity between the real Earth and the assumed model. But if at a particular seismograph station the residuals from many sources at many different locations are averaged, the random errors associated with particular wave paths and particular sources should cancel out, thereby making it possible to isolate the common part of the residuals arising from velocity deviations in the crust beneath the station. If the seismic events concerned are also well distributed in time, it should then be possible to determine any secular variations in the near-station contribution to the residuals, and even to determine changes in the near-station crustal velocity (given the source volume).

5' terminal residues sequenced.

Shimotohno and Miura (*J. molec. Biol.*, 85, 21-30; 1974) now report that the 5' sequence of the RNA made by the silkworm virus enzyme is ppAGPy, and this unique sequence is present in the mRNA transcribed from each of the genome fragments. This indicates that transcription to make mRNA begins at the first base of each molecule, reading from the strand with the 3' OH sequence PuCU. Perhaps the unusual base at the 5' end of the complementary chain plays some part as a recognition site for the binding of the polymerase molecule.

It would be interesting to determine whether the 5'-terminal A in mature mRNA is modified, since the presence or absence of such a modification might be used to distinguish between molecules destined to be messengers from those making up progeny genomic RNA. So far studies on reovirus multiplication have shed no light on the distinction between transcription to give mRNA and replication to give double-stranded RNA, nor on how they are controlled relative to one another. Apparently, the presence of poly (A) in the mRNA species cannot be used as a distinguishing feature since reovirus mRNA does not contain this appendage (*J. biol. Chem.*, 248, 7993-7998; 1973).

Applying this technique to 3,000 P wave residuals for events in the Matsushiro area of Japan during the period 1960-1968, Wyss and Holcomb (*Nature*, **245**, 139; 1973) were able to show that the Matsushiro series of earthquakes of 1965-1966 had been preceded by an anomalous decrease in P wave velocity in the source region. Specifically, the P wave residuals began to increase significantly from the long term average of -1.25 s around October 1962 (about 3 years before the earthquake series began), representing a decrease in P wave velocity near the Matsushiro station of about 20% (assuming a source volume with a radius of about 16 km). The residuals then returned to within one standard deviation of the long term average about 330 d before the Matsushiro swarm began and about 770 d before the swarm's principal energy release.

At the time that Wyss and Holcomb were carrying out their analysis they were apparently aware that premonitory changes in seismic velocity in earthquake source regions had already been observed in the Soviet Union, New York State and the Transverse Ranges of southern California; and so in that sense they were neither reporting a new phenomenon nor pointing out for the first time its possible application to earthquake prediction. On the other hand, the Matsushiro result was more than just another example of a known effect, for it was the first time that premonitory velocity changes had been observed before strike-slip faulting.

Not the least interesting aspect of this discovery was that, at about the same time, several groups of workers were attempting, and apparently failing, to find comparable premonitory changes along the active central section of the San Andreas fault—and Nur *et al.* (*Stanford Univ. Publs. geol. Sci.*, **13**, 391; 1973) had even suggested that the explanation for velocity changes in terms of dilatancy may not be applicable generally to strike-slip faults.

But all that has now changed, for Robinson *et al.* (*Science*, **184**, 1281; 1974) report that, using P wave residuals as indicators, they have been able to observe changes preceding the Bear Valley earthquake of February 24, 1972 (surface magnitude, 5.0). The travel-time residuals analysed were from small local earthquakes which occurred along the Calaveras fault north-west of the Bear Valley region between June 1, 1971 and May 30, 1972 and which had been located precisely using the US Geological Survey's network of seismograph stations, excluding the three stations (BVL, which lies almost above the focus of the Bear Valley earthquake, and the nearby EKH and JHC) at which the residuals them-

selves were observed. In all, 49 shocks were observed sufficiently well at BVL and a slightly smaller number at each of the other two stations.

The expected range of 'normal' residuals about their mean value was ± 0.15 s; and for most of the one year observation period, residuals were indeed within these limits. But between late December 1971 and late January 1972 the residuals at BVL were anomalously high at about 0.3 s above normal values and, in the statistical sense, significantly higher on average than the mean for the preceding 7 months. As the eight events within the anomalous period covered a distance range of 50 km (20-70 km), a magnitude range of 2.7 (0.7-3.4) and a depth range of 7 km (2-9 km), and were apparently no different in such respects from the other events, there seems little reason to doubt that their anomalous nature represents a genuinely precursory indication of the February 24, 1972 earthquake.

Unfortunately, data from the other two stations were ambiguous, with only two of the seven residuals within the December-January period at EKH and none of the five corresponding residuals observed at JHC lying outside the 0.15 s limit, and so to that extent confirmation was lacking. But accepting the BVL results, Robinson and his colleagues conclude that the anomalous changes in residuals are equivalent to a 10-15% decrease in P wave velocity within a volume having the same radius (7 km) as the observed aftershock zone. This decrease began 60-53 d before the Bear Valley shock itself, which is consistent with the time scale deduced for a magnitude 5.0 event using other methods. And as seems to be usual in such circumstances, the velocity returned to its normal value before (in this case several weeks before) the onset of the main event, although the data are not sufficient to indicate whether this return was sudden or gradual.

Naturally, this result raises the interesting question of why other workers (using different methods) have failed to observe premonitory changes, even when investigating the same earthquake: McEvilly and Johnson (*Science*, **182**, 588; 1973) for example, examined the stability of P wave travel times along a path passing about 10 km north of Bear Valley, and concluded that the 1972 Bear Valley shock was preceded by no significant changes. In this case, however, failure seems to have been simply a matter of bad luck. McEvilly and Johnson made use of quarry blasts as sources; but unfortunately, as Robinson *et al.* point out, no blast actually took place during the anomalous period now recognised from the BVL residuals.

Octupole states in cadmium

from Peter E. Hodgson

Nuclear Theory Correspondent

THE energies, spins and wavefunctions of low lying nuclear states can often be calculated from a simple model, and it then becomes particularly interesting to see if they can be found experimentally. If some of them are predicted by one model but not by another, they become important for deciding which of the models is to be preferred.

An example of such a state is the 3^- octupole state expected on the collective vibrational model at about 2 or 3 MeV in heavy nuclei. Such states had been found in the even isotopes of cadmium, and this was accepted as evidence for the collective nature of these nuclei.

Collective states are strongly excited by inelastic scattering. The energies of the inelastically scattered particles give the energies of the nuclear states, and their angular distributions and intensities give the quantum numbers and strengths of the states. In the case of vibrational nuclei the strength of a state is related to the dynamic deformation. The work on ^{110}Cd showed a 3^- state at 1.9 MeV with a strength similar to that expected from the collective model.

This has now been challenged by some accurate new measurements by Gill and colleagues at the University of Alberta (*Phys. Rev. Lett.*, **32**, 889; 1974) of the energies of the γ rays emitted after the inelastic scattering of neutrons by ^{110}Cd . As shown in Fig. 1, they found γ rays of 1,404, 1,409 and 1,416 keV, corresponding to the decay of states at 1,917, 1,923 and 1,930 keV to the state at 513 keV. None of these states decays to the ground state to a detectable degree. Thus, what was previously interpreted as a single 3^- state is now resolved into three separate states with quite different properties. As shown in Fig. 2, the sum of the inelastic scattering cross sections to these three states is similar to that expected from a single

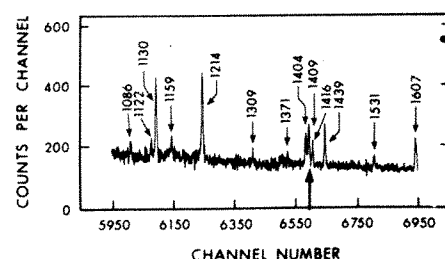


Fig. 1 Spectrum of γ rays after the inelastic scattering of neutrons by ^{110}Cd , showing the line at about 1,410 keV resolved into three peaks.

3^- state, but individually they correspond to 0^+ states. Thus the evidence previously interpreted as indicating a single 3^- state is now re-interpreted in quite a different way. There is no other state in the required energy region that could plausibly be interpreted as the required 3^- collective state, so the evidence for the collective nature of this nucleus is weakened.

This result is important for the understanding of the structure of cadmium, but it has several wider implications. First, it is a reminder of the importance of precision in physical measurement. Time and again increased precision does not merely sharpen the picture, but reveals wholly unexpected details that oblige one to adopt a completely new interpretation. Second, it is a reminder of the provisional nature of much of the day-to-day details of scientific work, particularly on the frontiers of knowledge. In so many cases scientists build ideas on quite flimsy and scattered evidence; it is right for them to do this provided they realise how subject it is to revision in the light of new information.

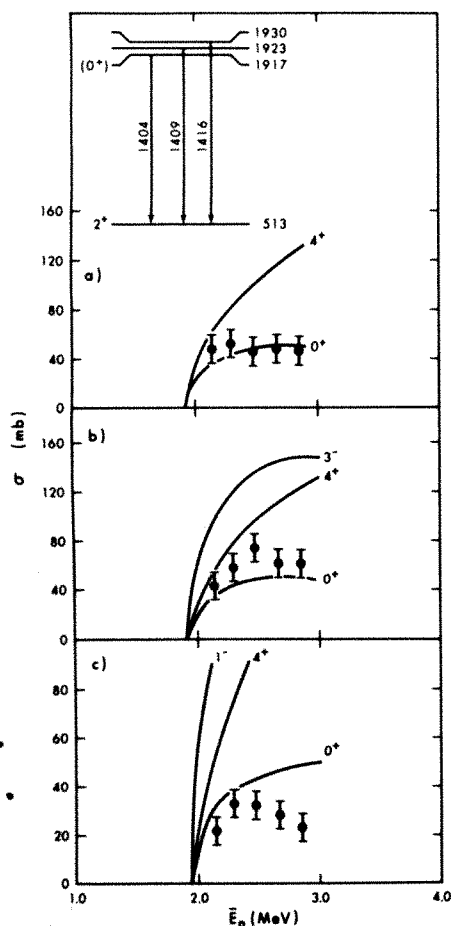


Fig. 2 Excitation functions for the total cross sections for the excitation of the states of 1,917, 1,923 and 1,930 keV by inelastic neutron scattering. The sum of the individual intensities is compatible with 3^- , but individually they cannot be 3^- and are likely to be 0^+ .

It is never sufficient to be satisfied with an interpretation that explains the incomplete set of experimental data available. Every consequence of an interpretation needs to be tested as rigorously as possible, and even then one must be prepared for the new unexpected result that calls it all in question again.

Acceptable radiation exposure

from J. R. A. Lakey

THE chairmen of both the International Commission on Radiological Protection (ICRP) and its counterpart on Radiation Units and Measurements (ICRU) were opening speakers at the Society for Radiological Protection International Symposium held at Aviemore, Scotland from June 2 to 6. These two commissions provide the ground rules for all aspects of radiological protection and both place considerable stress on the measurement of radiation and its interpretation.

C. G. Stewart (ICRP) opened the symposium with a statement on the ICRP recommended Investigation Levels and Derived Working Limits and described these as signposts to direct action which could also be used as criteria for discarding unnecessary information. Radiation exposures exceeding these levels demand more careful consideration and higher accuracy of measurement. J. Dunster (National Radiological Protection Board, Harwell) endorsed this thesis and said that errors of a factor 2 are acceptable in some radiation measurements but this does not excuse lack of clarity in thought on the part of the health physicist.

P. J. Campion (National Physical Laboratory, Teddington) described metrology as the cornerstone on which all advances in radiological protection could be built. In addition to the necessity for instrument calibration he stressed the need for a strict hierarchical structure so that the calibration of a particular instrument could be traced back to the national standard. R. Maushart (B. F. Vertriebes, Karlsruhe) appealed for standardisation of the methods of using instruments so that calibration is not an end in itself.

H. L. Wyckoff, the chairman of the ICRU (Washington DC) presented an elegant resumé of the quantity of dose equivalent which has been jointly developed by his commission and the ICRP. This quantity is expressed in the rem unit, derived from the absorbed dose with corrections depending on the nature of the radiation.

R. H. Mole (MRC Radiobiology Unit, Harwell) agreed that a relation-

ship must exist between the absorbed dose of radiation and the degree of probability of an effect on the exposed person. Philosophical problems emerge when risks to population are computed by the summation of risks to individual members. It is particularly difficult to place these computed population risks into perspective and a great deal has yet to be learned about public acceptance of these estimates. On the other hand the concept of risk or detriment to an individual organ of the body is useful for the summation of radiation exposure to the body due to intake of a single radionuclide. J. Vennart (MRC Radiobiology Unit, Harwell) said that this estimate of risk could be derived from the forthcoming ICRP recommended Maximum Permissible Annual Intake which is to replace the less satisfactory Maximum Permissible Body Burden. He said that the commission would also publish Derived Air Concentrations but there was to be no attempt to give limits for drinking water for occupationally exposed persons, who, after all, consume the same water as the public.

The legal attitude to radiation protection varies between countries to the extent that the radiation worker could be restricted in mobility and so the European Economic Community aims to improve this situation through its directives to member countries. These proposals, summarised by P. Recht (EEC), are expected to involve some changes in United Kingdom legislation in spite of the common ICRP basis. A. W. Kenny (Department of the Environment, London) also showed that the EEC would become a new factor in the control of radioactive waste. The UK approach presented by A. Preston (Fisheries Laboratory, Lowestoft) is to limit radioactive waste disposal by restricting radiation exposure to members of a critical population group. The main difficulty in the application of this procedure is the need to find a completely objective method of selecting the critical group. W. D. Rowe (US Environmental Protection Agency, Washington DC) described an alternative approach which makes use of comprehensive mathematical models which include source terms, environmental transport pathways and human metabolism to predict the dose to members of the public. These models require some form of measurement for their periodic validation but might avoid some of the problems of the critical group method.

Preprints of most of the papers which were presented can be obtained on payment of a small fee from Mr K. B. Shaw, National Radiological Protection Board, Harwell, Didcot, Berkshire OX11 0RQ.

Chronological and ecological implications of the fossil Bovidae at the Sterkfontein Australopithecine site

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Bovid remains indicate the presence at Sterkfontein of three faunal phases, accumulated at different times and probably under different climatic conditions. These phases are variously associated with such well known Sterkfontein finds as the type locality australopithecine assemblage, the West Pit (extension locality) hominid remains which may include Homo and numerous stone artefacts.

SEVERAL hundred bovid cranial fragments, mostly dentitions, hailing from the various localities at the Sterkfontein excavation site (see Fig. 1), were available for this study. Associated with the famous australopithecine material at the Sterkfontein main quarry (type locality—henceforth referred to as STS)—were 111 specimens. Forty-two specimens came from the West Pit (extension locality, SE), which was excavated by J. T. Robinson in 1957 and 1958 (ref. 2). Some 200 specimens came from rubble dumps D1, D2, D3, D5, D6, D8, D12-16 and H2, excavated by P. V. Tobias and A. R. Hughes, who placed these materials at my disposal for study. These dumps, STS and SE will be referred to throughout this work as site units. The bovid species identified at Sterkfontein are shown in Table 1.

Of primary interest in this study is what information the Bovidae can provide about a possible time difference between the two main hominid-bearing site units, STS and SE. It has been suggested that SE may belong to a later time period than STS. Robinson² wrote of the presence at Sterkfontein of an earliest Lower breccia including STS, which he believed to be unconformably overlain by a later Middle breccia including SE. He also recognised a third, youngest Upper breccia. Tobias⁴ felt that unlike STS, the SE assemblage includes at least a trace

of a hominid more advanced than *Australopithecus*. An important contribution to any discussion of the affinities of STS and SE is the fact that many stone tools have been recovered from SE. They were first discovered there by Brain⁵, and have since been found in numbers totalling hundreds at SE and in several dumps. None has been found in association with *Australopithecus* at STS. The first published suggestion that some major South African fossil assemblages can be arranged in a succession of Faunal Stages (this was later amended to Faunal Spans⁶) came from Wells⁷, who included STS in the earlier Sterkfontein stage and SE in a later Swartkrans stage. This is the first time that Sterkfontein faunal remains have been included in the

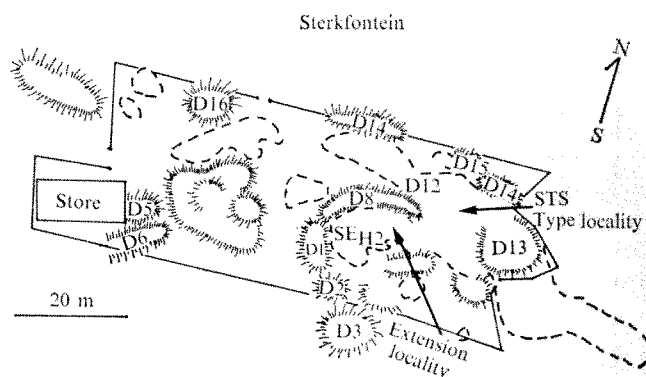


Fig. 1 Plan of the Sterkfontein Excavation Site (after Tobias and Hughes¹). SE, West Pit; D1-16, rubble dumps; H2, material excavated under SE; - - - caves, holes and excavations.

Table 1 Minimum numbers of individuals in bovid species at Sterkfontein. Taxonomy of extant Bovidae as in ref. 3; E, extinct; IR, indistinguishable from recent; 'larger small' as in my unpublished work

	Species	STS	SE	H2	D1	D2	D3	D5	D6	D8	D12	D13	D14	D15	D16
1	<i>Syncerus</i> cf. <i>acoelotus</i>	E	1												
2	<i>Taurotragus</i> cf. <i>oryx</i>	IR		1								2			1
3	<i>Tragelaphus</i> cf. <i>strepsiceros</i>	IR								2					
4	<i>Tragelaphus</i> sp. aff. <i>angasi</i>	E	1												
5	<i>Tragelaphus</i> cf. <i>scriptus</i>	IR													2
6	<i>Pelea</i> cf. <i>capreolus</i>	IR													3
7	<i>Redunca</i> cf. <i>arundinum</i>	IR	1												
8	<i>Kobus</i> cf. <i>ellipsiprymnus</i>	IR			1										
9	<i>Hippotragus</i> cf. <i>equinus</i>	IR	2												
10	<i>Hippotragus</i> cf. <i>niger</i>	IR							1						4
11	cf. <i>Hippotragus</i> sp. aff. <i>gigas</i>	E	8	1								5	1	1	
12	cf. <i>Megalotragus</i> sp.	E	1												
13	<i>Connochaetes taurinus</i> lineage	E + IR	1	1	3					1		1			4
14	Medium-sized alcelaphines	E + IR	7	6	2	2		1							1
15	<i>Damaliscus</i> sp. ('larger small')	E	7	3	2	2		2		1	1	2			5
16	<i>Damaliscus</i> cf. <i>dorcas</i>	IR		3			1	2		1					11
17	<i>Antidorcas</i> cf. <i>recki</i>	E	2	3											
18	<i>Antidorcas</i> cf. <i>marsupialis</i>	IR			1					1					2
19	<i>Antidorcas bondi</i>	E	1	2	1	1				3					5
20	cf. <i>Gazella vanhoepeni</i>	E	1									2			
21	<i>Oreotragus major</i>	E		1								1			
22	<i>Ourebia</i> cf. <i>ourebi</i>	IR								1					3
23	<i>Raphicerus</i> cf. <i>campestris</i>	IR						1							2
24	<i>Makapania</i> cf. <i>broomi</i>	E	9	1								4		1	
Minimum numbers of individuals of all species		42	22	8	7	1	1	6	1	10	1	17	1	2	40

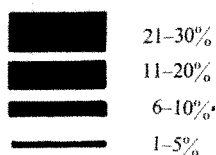
discussion of the contemporaneity or otherwise of STS and SE.

To date there has been no published attempt to relate the fossil content of the rubble dumps to the *in situ* breccia of Sterkfontein. If the Sterkfontein fauna was really assembled during successive time periods, whether in a series of breccias as visualised by Robinson or as part of a continuous breccia,

it has to be concluded that the contents of the rubble dumps may be mixed with respect to such time periods. Nonetheless any one dump should contain a high proportion of fossils from a single part of the deposit, and so from a single time period. An attempt is made here to group these dumps, on the basis of their bovid fossil content, with each other and with STS and SE.

	Faunal phase A	Faunal phase B	Species also present (X) or probably present (?X) at Makapans- gat Lime- works
	STS	SE	
<i>Hippotragus</i> cf. <i>equinus</i> (IR roan antelope)			
cf. <i>Megalotragus</i> sp. (E large hartebeest type)	-----		
<i>Redunca</i> cf. <i>arundinum</i> (IR reedbuck)			?X
<i>Syncerus</i> cf. <i>acoelotus</i> (E buffalo)			?X
<i>Tragelaphus</i> sp. aff. <i>angasi</i> (E nyala)			?X
cf. <i>Gazella vanhoepeni</i> (E gazelle)			X
<i>Makapania</i> cf. <i>broomi</i> (E muskox type)	=====	-----	X
cf. <i>Hippotragus</i> sp. aff. <i>gigas</i> (E large hippotragine)	=====	-----	X
<i>Antidorcas</i> cf. <i>recki</i> (E springbok type)		=====	
<i>Antidorcas bondi</i> (E springbok type)	-----	=====	
<i>Oreotragus major</i> (E klipspringer)			X
<i>Taurotragus</i> cf. <i>oryx</i> (IR eland)			
<i>Damaliscus</i> cf. <i>dorcas</i> (IR blesbok)			
<i>Damaliscus</i> sp. 1 or <i>Parmularius</i> sp. (E blesbok type)	=====	-----	
<i>Damaliscus</i> sp. 2 (E blesbok type)	-----	=====	
<i>Connochaetes taurinus</i> lineage (E and IR? blue wildebeest)			?X
Medium-sized alcelaphines (E and IR)	=====	=====	?X
Total number of cranial and dental specimens	111	42	
Total minimum number of individuals	42	22	
Number of extinct : indistinguishable from recent species	12 : 2	8 : 3	
% constituted by minimum number of definitely extinct individuals of total minimum number	$\frac{39 \times 100}{42 \times 1} = 93\%$	$\frac{17 \times 100}{22 \times 1} = 77\%$	
% constituted by minimum number of alcelaphines and antilopines of total minimum number	$\frac{20 \times 100}{42 \times 1} = 48\%$	$\frac{18 \times 100}{22 \times 1} = 82\%$	

Line thicknesses represent minimum number percentages per species per site as follows:



----- Only one or two specimens present which are suspected of belonging to a different faunal phase

Fig. 2 Distribution of bovid species between STS and SE. The specimens here represented are only those deriving from *in situ* STS and SE breccia. *Damaliscus* sp. of Table 1 and Fig. 4 is divided into *Damaliscus* sp. 1 or *Parmularius* sp., and *Damaliscus* sp. 2; nearest extant relative given in each case; E, extinct; IR, indistinguishable from recent.

Statistical methods and results

In site units STS and SE species, as represented in Table 1, were separated into five tribes or tribal groupings: Hippotragini; Ovisovini; Alcelaphini; Antilopini and Neotragini; Bovini, Tragelaphini and Reduncini. A chi-square test showed that, in terms of the minimum number contents within these tribal groups, STS differs significantly from SE ($\chi^2_4 = 11.84$; $\chi^2_4(0.05) = 9.488$).

The tribes Alcelaphini and Antilopini are widely recognised as generally forming the bulk of any open plains-grassland fauna. For STS and SE the percentages which the added minimum numbers of these two tribes constitute of the total minimum numbers were found to be 48% and 82% respectively (Fig. 2). A chi-square test performed on these data showed that the SE assemblage has a highly significantly larger proportion of alcelaphines and antilopines ($\chi^2_1 = 7.17$; $\chi^2_1(0.01) = 6.635$) than has the STS assemblage.

Two types of distance functions were used to express affinity, in terms of minimum numbers per species, between all pairs of site units in Table 1. The first, here named FCD for 'faunal composition difference' coefficient, was arrived at as follows: if the taxa (species) are coded $i = 1, \dots, I$ and the site units $j = 1, \dots, J$ and if x_{ij} denotes the minimum number of individuals of species i at site unit j , then the FCD between site units m and n , denoted by D_{mn} , is calculated as

$$D_{mn} = \sum_{i=1}^I |P_{im} - P_{in}|$$

where $P_{ij} = \frac{x_{ij}}{x_{.j}}$, $j = 1, \dots, J$, where $x_{.j}$ denotes the sum of x_{ij}

over i . FCDs range from 0 to 2. The second distance function used was χ^2 . Each of the two functions was calculated (a) for all pairs of site units over all separate species categories 1–24; (b) for all pairs of site units over separate species categories 1–11 and 16–24, but lumping 12–15 into a single category; (c) for all pairs of site units with total minimum numbers ≥ 3 over all separate species categories 1–24; (d) for all pairs of site units with total minimum numbers ≥ 3 over separate species categories 1–11 and 16–24, but lumping 12–15 into a single category. The resulting matrices of FCDs and χ^2 values were clustered by the Weighted Pair Group Method Clustering Procedure⁸ into several dendrograms. An example is given in Fig. 3. Without exception the dendrograms showed the most fundamental split to be between STS, together with D13, D14 and D15, and the rest. A further split among the remaining site units was consistently indicated between SE together with H2, D1 and D5 on the one hand, and D16 together with D8 and D6 on the other. The placement of D2, D3 and D12, each with a total minimum number of 1, varied, but they were always separate from the STS cluster. Figure 4 shows the three faunal phases represented by site units, as suggested by these methods. It must be borne in mind that the low minimum number content of some of the site units limits the confidence that can be felt in the accuracy of their placement.

Bovoid faunal phases

A comparison between Figs 1, 3 and 4 shows an interesting correspondence between the geographic location of site units and their grouping on bovid content, with the possible exception of D5 and D8. Nonetheless there remains a greater measure of uncertainty with respect to the homogeneity of the dump assemblages than is the case with STS and SE. It is therefore useful to look first at the bovid species distribution between faunal phases A and B as represented only by STS and SE (Fig. 3). The dominant species at STS are: *Makapania broomi*, which is also among the most common species at the Makapansgat Limeworks⁹; a large new hippotragine species, which could be ancestral to *Hippotragus gigas* known throughout the Olduvai Beds and at Elandsfontein in South Africa; and a small alcelaphine which could be close to, or specifically identical to, a species from the East Rudolf *Metridiochoerus*

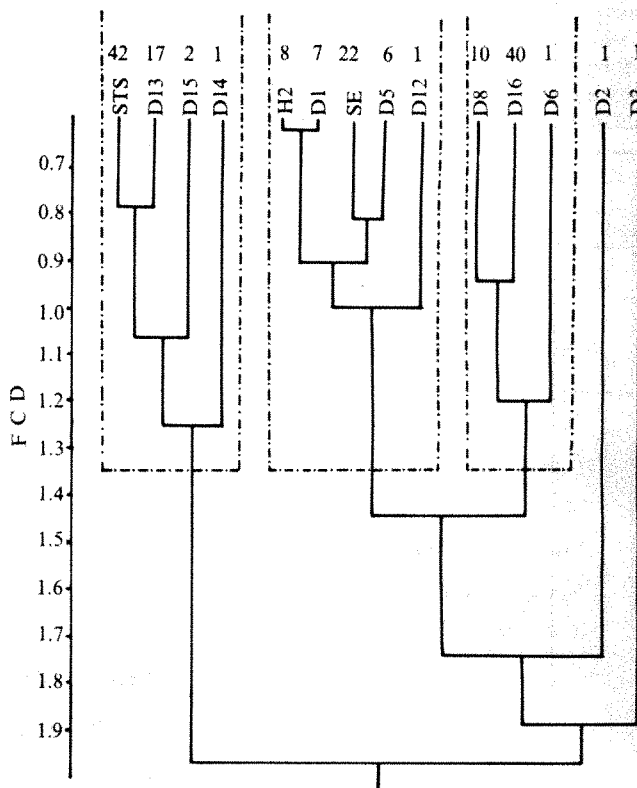


Fig. 3 Dendrogram of the relations among 14 site units based on WPGM clustering procedure⁸ using FCD values. Site units as in Fig. 1, each accompanied by its minimum number content.

andrewsi zone¹⁰. Each of the three species is represented doubtfully in the SE assemblage by a single broken specimen. At SE can be seen the first occurrence in the Sterkfontein context of the distinctive dentitions indistinguishable from the extant *Damaliscus dorcas*. *Antidorcas bondi* and *Damaliscus* sp. 2, which is likely to be *Damaliscus niro* (my unpublished work), probably also make their first appearance at SE, if the single specimen of each from STS is indeed misplaced in that assemblage as I strongly suspect for several reasons. On the whole, in view of the markedly greater affinity to Makapansgat of STS than of SE, and the implications of the χ^2 test results, the bovid assemblages of STS and SE seem to be essentially different and successive in time.

Figure 4 shows that inclusion of the dumps amplifies differences between faunal phases A and B, and adds a third phase. Faunal phase C, as represented mainly by D16 is undoubtedly distinct in its general character from phase B, and probably considerably later in time. There is here the advent at Sterkfontein of several species that are indistinguishable from recent forms. Only two phase C species are definitely extinct: *A. bondi* and the 'larger small' *Damaliscus* sp., which again in phase C very likely belongs to *D. niro* (my unpublished work). *A. bondi* and *D. niro* are generally among the few extinct bovid species at southern African Middle Stone Age sites.

It seems that at least a large part of the differences between these three bovid faunal phases, accumulated in close geographical proximity and signalling the advent of new 'indistinguishable-from-recent' species with each successive phase, is due to time. Phase A, associated with the STS australopithecine assemblage, belongs with Makapansgat in the Sterkfontein Faunal Span. Phase B, associated with the SE hominids which might include at least some *Homo*, resembles both Kromdraai A (the faunal site) and Swartkrans with respect to its high alcelaphine-antilopine content, although it is perhaps closer to the latter in terms of species present. It could belong to the Swartkrans or to the Cornelia Span. Relevant here is the simi-

larity of tool forms from both SE and Swartkrans to the developed Oldowan assemblages¹¹. Phase C could belong to the Florisbad⁷ or Florisbad-Vlakkraal Faunal Span⁶ of Middle Stone Age time. In my thesis for the University of Cape Town, I am making a more detailed attempt at bovid faunal correlation

between the Sterkfontein phases and the other Krugersdorp sites. Tobias¹² has reviewed the dating of the South African australopithecine sites, linking the results of new geomorphological studies to what has so far been concluded on the basis of faunal comparisons.

	Faunal phase A	Faunal phase B	Faunal phase C	Species also present (x) or probably present (?) at Makapans- gat Lime- works
Primary site units; min. nos. > 15 :	STS D13	SE	D16	
Secondary site units; min. nos. = 5-15:		H2 D1 D5	D8	
Tertiary site units; min. nos. = < 5 :	D14 D15	D3 D12 D2	D6	
<i>Hippotragus</i> cf. <i>equinus</i> (IR roan antelope)				
cf. <i>Megalotragus</i> sp. (E large hartebeest type)				
<i>Redunca</i> cf. <i>arundinum</i> (IR reedbuck)				?X
<i>Syncerus</i> cf. <i>acoelotus</i> (E buffalo)				?X
<i>Tragelaphus</i> sp. aff. <i>angasi</i> (E nyala)				?X
cf. <i>Gazella vanhoepeni</i> (E gazelle)				X
<i>Makapania</i> cf. <i>broomi</i> (E muskox type)				X
cf. <i>Hippotragus</i> sp. aff. <i>gigas</i> (large hippotragine)				X
<i>Oreotragus major</i> (E klipspringer)				X
<i>Antidorcas</i> cf. <i>recki</i> (E springbok type)				
<i>Antidorcas bondi</i> (E springbok type)				
<i>Damaliscus</i> cf. <i>dorcas</i> (IR blesbok)				
<i>Kobus</i> cf. <i>ellipsiprymnus</i> (IR waterbuck)				
<i>Raphicerus</i> cf. <i>campestris</i> (IR steinbok)				
<i>Antidorcas</i> cf. <i>marsupialis</i> (IR springbok)				
<i>Ourebia</i> cf. <i>ourebi</i> (IR oribi)				
<i>Pelea</i> cf. <i>capreolus</i> (IR Vaal ribbok)				
<i>Tragelaphus</i> cf. <i>scriptus</i> (IR bushbuck)				
<i>Hippotragus</i> cf. <i>niger</i> (IR sable antelope)				
<i>Tragelaphus</i> cf. <i>strepsiceros</i> (IR greater kudu)				
<i>Taurotragus</i> cf. <i>oryx</i> (IR eland)				
<i>Connochaetes taurinus</i> lineage (E and IR blue wildebeest)				?X
<i>Damaliscus</i> sp. (larger small) (E blesbok type)				
Medium-sized alcelaphines (E and IR)				?X
% constituted by min. no. of alcelaphines and antilopines of total min. no. in a faunal phase:	$\frac{25 \times 100}{62 \times 1} = 40\%$	$\frac{37 \times 100}{43 \times 1} = 86\%$	$\frac{32 \times 100}{51 \times 1} = 63\%$	
% constituted by min. no. of definitely extinct individuals of total min. no. in a faunal phase:	92%	58%	23%	

Line thicknesses represent minimum number percentages per species per faunal phase as follows:

20-29%

10-19%

5-9%

1-4%

Only 1 or 2 specimens present which are suspected of belonging to a different faunal phase

Fig. 4 Suggested distribution of bovid species throughout all site units at Sterkfontein. E, extinct; IR, indistinguishable from recent.

Are there differences between phases A, B and C as here constituted, other than those resulting from time? In phases A and C, rather than B, there seem to predominate forms that are identical with or close to extant species that are to a greater or lesser extent bush-loving and water-dependent, like the roan antelope, reedbuck, buffalo, nyala, sable antelope and bushbuck. This is correlated with the significantly higher percentage of alcelaphines and antilopines in phase B. Although Fig. 4 makes it clear that some degree of open grassland existed near Sterkfontein during all three time periods, the data strongly suggest that during phase B there was proportionately less bush cover than during A and C. One possible cause of such vegetational change could be a decrease in rainfall between A and B with a subsequent increase in phase C. Brain⁵ concluded, on the basis of determinations of the sand grain angularity and chert-quartz ratios of the australopithecine breccias, that a general increase in rainfall, with minor fluctuations, occurred in the Sterkfontein valley from STS times onwards. An explanation for the bovid data in Fig. 4 invoking bush cover changes as a result of rainfall changes would thus seem to stand in contradiction to Brain's findings between phases A and B, while agreeing with them between B and C. Another strong possibility is that the apparent vegetation changes are largely or entirely due to a drop in temperature between phases A and B with a subsequent increase towards C.

From this perspective, therefore, it can be said that the Sterkfontein bovid fossils show evidence of having been accumulated not only at three different times but also under varying ecological, more specifically bushcover, conditions, the latter probably resulting from temperature and/or rainfall changes.

A further difference between phases A and B must be mentioned here. Individuals in phase A have a larger average body-size than do those in phase B. Although it is not impossible that

this effect is at least to some extent correlated with the ecological differences that were deduced on the basis of species content, it leads to speculation about a possible accumulation difference: for instance, the phase A bovid remains could largely be the result of the activities of a predator, such as a sabre-tooth cat, specialised for eating large prey. Perhaps the predominant influence contributing to the phase B bovid assemblage was the advanced hominid, who in spite of his tools may have been confined to small to medium-sized prey. Detailed studies by C. K. Brain of the mode of accumulation of the australopithecine bone assemblages, and my further studies of their bovid faunas, may throw light on such questions.

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- ¹ Tobias, P. V., and Hughes, A. R., *S. Afr. archaeol. Bull.*, **24**, 158 (1969).
- ² Robinson, J. T., *S. Afr. archaeol. Bull.*, **17**, 87 (1962).
- ³ Ansell, W. F. H., part 15 in *The Mammals of Africa: An Identification Manual* (edit. by Meester, J., and Setzer, H. W.), 11-84 (Smithsonian Institution Press, Washington, 1971).
- ⁴ Tobias, P. V., *S. Afr. archaeol. Bull.*, **20**, 167 (1965).
- ⁵ Brain, C. K., *Transv. Mus. Mem.*, **13**, 1 (1958).
- ⁶ Cooke, H. B. S., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.) 175 (University of Chicago Press, Chicago and London, 1967).
- ⁷ Wells, L. H., *Ann. Cape prov. Mus.*, **11**, 37 (1962).
- ⁸ Sokal, R. R., and Sneath, P. H. A., *Principles of Numerical Taxonomy* (W. H. Freeman, San Francisco and London, 1963).
- ⁹ Wells, L. H., and Cooke, H. B. S., *Palaeont. afr.*, **4**, 1 (1956).
- ¹⁰ Maglio, V. J., *Nature*, **239**, 379 (1972).
- ¹¹ Leakey, M. D., *Nature*, **225**, 13 (1970).
- ¹² Tobias, P. V., *Nature*, **246**, 79 (1973).

Radiometric ages of late Cainozoic basalts from northern Israel: chronostratigraphic implications

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K-Ar dating of volcanics in the Jordan rift allows an estimation of the ages of widespread stratigraphic events in the eastern Mediterranean region. The data provide a link between Pleistocene deposits of Europe and Africa suggesting, in particular, age limits to the early Acheulian and Mousterian cultures.

BASALT volcanism of late Cainozoic age is widespread in the western part of the Arabian peninsula, along a zone trending NNW from the Red Sea into Turkey. One of the most prominent occurrences of this volcanic phase is the Jebel Druze basalt province, in which lavas, covering about 48,000 km², extend south-eastwards from Lebanon to Israel, through Syria

and Transjordan, into Saudi Arabia. K-Ar ages have been determined on basalts occurring in the north-western extremity of the province and five eruptive phases, interbedded with late Cainozoic sediments of the northern Jordan Valley and the Valley of Jezre'el, are distinguished. Our data include representative samples of the chief units throughout the late Cainozoic volcanic sequence exposed in this region, with the possible exception of some of the most recent flows from the Golan Plateau (Figs 1 and 2). The present dating project thus enables radiometric ages to be allocated to many late Cainozoic sedimentary formations and stratigraphical events on the eastern seaboard of the Mediterranean.

K-Ar age determinations

The rocks investigated here have the chemical and mineralogical characteristics of alkali olivine basalts, closely similar to those described from the Jebel Druze and other Cainozoic localities

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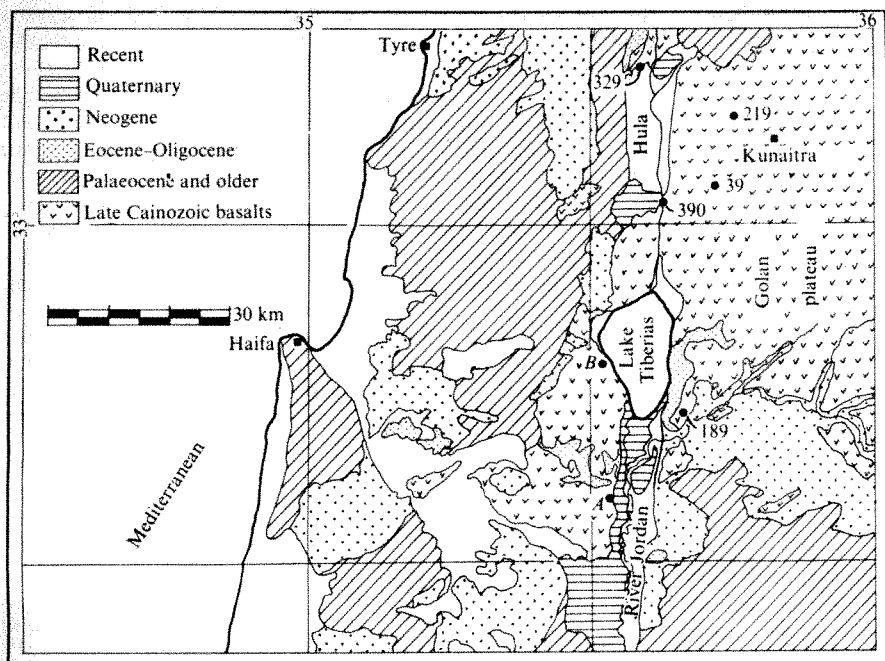


Fig. 1 Geological sketch map of northern Israel. Numbers indicate the locations of dated specimens. A, Belvoir section; B, Tiberias section.

in Transjordan^{1,2}. The analysed specimens from the northern Jordan Rift, represented in Table 1, are nonamygdaloidal, fine grained and commonly porphyritic. The phenocrysts are mostly olivine which occurs either alone or accompanied by augite and labradoritic plagioclase in various proportions. The matrix includes plagioclase in the range andesine-labradorite, augite, olivine and, in some varieties, nepheline with analcite (ref. 3, and I. Brenner, unpublished work).

Argon isotope ratios were measured statically on a Varian GD-150 mass spectrometer, equipped with a vibrating reed electrometer, using an Ar-38 enriched spike. A correction of 295.5 for the atmospheric ⁴⁰Ar : ³⁶Ar ratio was used in calculating the ages. Peak magnitudes were calculated by extrapolation

from successive scans back to the opening of the Ar-extraction line to the mass spectrometer. The decay constants adopted were

$$\lambda \beta = 4.72 \times 10^{-10} \text{ yr}^{-1}$$

and

$$\lambda e = 0.584 \times 10^{-10} \text{ yr}^{-1}$$

and the ⁴⁰K : K ratio = 1.19×10^{-2} atom %. Potassium was determined by atomic absorption on a Perkin-Elmer 303 instrument, using a bracketing procedure for calculating the results. The errors in the individual ages (Table 1) reflect analytical uncertainty only and were calculated for each analysis at the one-sigma level, following the method outlined by McDougall *et al.*⁴.

Table 1 Occurrence, locations K-Ar analytical data, and calculated ages of late-Cainozoic basalts from northern Israel

Sample no.	Rock type and field occurrence	Formation	Location (height above sea level in brackets)	Lat. N	Long. E	Geo-magnetic polarity	K (weight %)	Radiogenic Ar (10 ⁻¹¹ mol/g ⁻¹)	Radiogenic ⁴⁰ Ar / Total ⁴⁰ Ar (%)	Calculated age (Myr)	Stratigraphic age
1B-219	Basalt flow	El Furn	Golan Plateau, El Furn (+900 m)	33°09'	35°45.5'	N	1.410	0.019	0.8	0.079 ± 0.013	Riss-Würm
1B-39	Basalt flow	Aleka	Golan Plateau, Aleka (+520 m)	33°03'	35°42.3'	N	1.192	0.012	0.5	0.064 ± 0.013	
1B-329	Basalt flow	Hasbani	Northern Jordan Valley, Qiriat Shemoa (+100 m)	33°13.5'	35°35.5'		1.056	0.014	0.6	0.073 ± 0.014	
1B-189	Basalt flow	Yarmuk	Eastern Jordan Valley, above Yarmuk River (+220 m)	32°42.5'	35°39'		0.709	0.085	4.9	0.68 ± 0.05	Late Mindel or early Mindel-Riss
1B-390	Basalt flow	Yarda	Eastern Jordan Valley, near B'not Yaakov (+100 m)	33°01'	35°58'	N	0.938	0.107	6.9	0.64 ± 0.12	
1B-48	Basalt flow, near top	Cover Basalt	Jordan Valley, Tiberias section (+180 m)	32°47.1'	35°31.6'	R	1.222	0.33	15.7	1.7 ± 0.1	Preglacial Pleistocene
1B-274	Basalt flow, near base	Cover Basalt	Jordan Valley, Belvoir section (+250 m)	32°35.5'	35°32.3'	R	0.687	0.26	8.8	2.0 ± 0.1	
1B-82	Basalt flow	Intermediate Basalt	Jordan Valley, Belvoir section Single flow-samples about 100 m apart (+200 m)	32°35.5'	35°32.3'	N	1.245	1.06	14.7	4.7 ± 0.2	Middle Tabernian
1B-185	Basalt flow						0.668	0.60	7.1	5.0 ± 0.3	
1B-187	Basalt flow						0.886	0.74	2.2	4.7 ± 0.5	
1B-180	Basalt flow, uppermost	Lower Basalt	Jordan Valley Belvoir section (+90 m)	32°35.5'	35°32.3'	N	1.109	2.45	17.8	12.5 ± 0.5	Middle Miocene
1B-172	Basalt flow	Lower Basalt	Jordan Valley, Belvoir section (-150 m)	32°35.5'	35°32.3'	N	1.172	3.06	38.5	14.6 ± 0.4	
1B-86	Basalt flow, lowermost	Lower Basalt	Jordan Valley, Belvoir section (-200 m)	32°35.4'	35°32.3'	N	1.259	2.90	67.6	13.0 ± 0.3	
1B-84	Basalt flow, lowermost	Lower Basalt	Jordan Valley, Tiberias section (-90 m)	32°47.1'	35°31.6'	N	0.826	1.78	24.1	12.2 ± 0.4	

In spite of the large errors shown for the Quaternary basalts, which mainly reflect the high level of atmospheric argon contamination, the calculated ages are clustered closely into groups differing by an order of magnitude, and are in excellent agreement with available stratigraphic evidence. Thus, on the present data, the pattern of the post-Middle Pliocene volcanism in the northern Jordan Valley region seems to have comprised several intense but discrete episodes.

Stratigraphy

The earliest manifestations of Cainozoic volcanism in the Jebel Druze igneous province are provided by several basalt horizons of Eocene and Miocene age penetrated by boreholes in Transjordan¹. Near Tripoli and Damascus, Neogene volcanism began in the Middle Miocene⁵ and on the northern side of Wadi Sirhan, in north-western Saudi Arabia, several extensive basalt flows have yielded an average K-Ar age of 12.3 Myr (ref. 6). In northern Israel some 500 m of Lower Basalt flows of Middle Miocene age (Table 1) interdigitate with fluvial sediments of the Herod Formation⁷. The latter correlates with the Hazeva Formation of the Negev, which interfingers in the Be'er Sheva area with Middle Miocene marine sediments of the Ziqiaq Formation^{8,9,25}. The Herod and Hazeva Formations are regarded as representing the maximum extent of the Middle Miocene marine transgression²⁵. Sediments from the Barada Valley near Damascus, which resemble the Herod Formation in age and lithology, and include interbedded basalt flows, have been described by Dubertret⁵. The dated flows of Lower Basalt occur on the western rift scarp of the northern Jordan Valley, at the Belvoir and Tiberias sections (Fig. 1) and are 12.2 to 14.6 Myr old. The flows may be regarded as essentially coeval, with a mean age of 13.1 Myr.

The Herod Formation is unconformably overlain by the Bira and Gesher Formations, within which several flows of the Intermediate Basalt are intercalated. The latter has been described by Schulman⁷ for the central Jordan Valley, and by Horowitz¹⁰ and Fleischer (Subsurface geology of the Hula, Geol. Surv. Ist. Rep., 1968. Unpublished) for the Hula Valley and Korazim Block, respectively. The Bira Formation, mainly lagoonal chalks and marls, interfingers westward in the Yizre'el Valley with marine sediments bearing a typical Pliocene fauna^{8,11,12}. Bira sediments of the central Jordan Valley, where the dated flow is located, are interpreted as representing the Tabianian stage of the Mediterranean Pliocene²⁵, as recently defined¹³. At Belvoir a single flow was sampled at three locations about 100 m apart in order to test the reproducibility of our measurements. Individually calculated ages of the Intermediate Basalt agree to within about 3% of their mean of 4.8 Myr.

Volcanism recommenced throughout the Jebel Druze province with the formation of plateau basalts which inundated wide areas in north-western Transjordan, south-western Syria, Lebanon, and north-eastern Israel. In the last of these areas the Cover Basalt commonly attains thicknesses of 100 to 150 m and most sections show at least 10 flows, each 10 to 20 m thick⁷. The Cover Basalt is separated by an erosional unconformity from underlying Pliocene formations and is itself overlain by Guenzian sediments of the Jordan Valley^{10,25}. No sediments interfinger with the Cover Basalt, but it has been stratigraphically correlated with the preglacial Pleistocene formations of the Coastal Plain, Judea and the Negev in Israel²⁵. Ages determined on flows from near the top of the Belvoir and Tiberias sections agree closely and have an average age of 1.8 Myr.

The results given here enable us to distinguish two age groups in the post-Cover Basalt volcanics: one with an average age of 0.66 Myr, the other much younger at near 72×10^3 yr. Carbon-14 dates from bones buried within the youngest lavas on the Syrian Jebel Druze indicate an age of 4.5×10^3 yr (ref. 14). In the Hula Valley, Quaternary basalts occur in tilted blocks, interbedded with Pleistocene deposits and, on the northern border, as flat-lying sheets which can be traced to the Golan

Plateau. The Yorda Basalt^{11,15} flowed over the Korazim-Gadot block, south of the Hula Valley, filling erosional features in the Guenzian Gadot Formation and in the Mindelian Mishmar Hayarden Formation. The faulted and tilted Yorda basalt is unconformably overlain by the Rissian Benot Ya'akov Formation. Its stratigraphical age should, therefore, correspond to the late Mindel or the early stage of the Mindel-Riss interpluvial. The Yorda Basalt has been correlated by Horowitz¹⁰ with the Yarmuk Basalt¹¹ of the central Jordan Valley. Radiometric ages of these basalts are 0.64 and 0.68 Myr respectively.

The youngest extrusive episode studied by us is represented by two flows from the Golan Plateau and one from the northern Hula Valley, all of which are coeval within the analytical error, with a mean age of 72×10^3 Myr and normal magnetic polarity (D. Mor, personal communication). The Hasbani Basalt covers the upper Hula Valley¹⁵, extending northwards into Lebanon and eastwards to its probable source in the Golan. This basalt, sampled as a surface flow near Qiriat Shemona (1B-329), has been traced to the Banias waterfall¹⁰ where it separates two travertine layers. The underlying Kefar

Specimen Number	K Ar ages ($\times 10^6$ yr)	Chrono-stratigraphy	Hula Basin	Central Jordan Valley
		Holocene	Malaha Formation	Tabgha Formation
		Würm	Dan Travertine	Lisan Formation
39 329 219	0.064 0.073 0.079	R-W	Hasbani Basalt and Hulata Formation	Roqqad (Naharayim) Basalt
		Riss	Benot Ya'akov Formation and Kefar Yuval Travertine	Naharayim Formation
		M-R	Ayelet Hashahar Formation	
390 189	0.64 0.68		Yorda Basalt	Yarmuk Basalt
		Mindel	Mishmar Hayarden Formation	Ubeidiya Formation
		G-M	Palaeosol	
48 274	1.7 2.0	Günz	Gadot-Hazor Fm.	Erk el-Ahmar Fm.
		Preglacial Pleistocene	Cover	Basalt
		Pliocene	Piacencian	Tanur Conglomerate
82 185 187	4.8	Tabianian	Tel Hai Limestone	Fejjas Tuff
				I. Basalt
				Um Sabune Cgl.
				Gesher Fm.
				Bira Fm.
84 180 86 172	12.2 12.5 13.0 14.6	Miocene		Lower Basalt
				Herod Fm.

Fig. 2 Upper Cainozoic sections for northern Israel, showing stratigraphical positions of dated basalts. (I. Basalt = Intermediate Basalt).

Yuval Travertine contains late Acheulian implements and corresponds stratigraphically to the Rissian Benot Ya'akov Formation of the Hula Valley¹⁰. The Dan Travertine overlies the Hasbani Basalt and can be correlated with the Würmian Ashmura Formation of the Hula Valley which contains implements of Mousterian to Epipalaeolithic age, and with the Roqqad (or Naharayim) Basalt¹⁶ of the central Jordan Valley with which it is stratigraphically analogous¹⁰. The Hasbani Basalt thus constitutes a precise marker for the calibration of the Riss-Würm interpluvial. The age of 73×10^3 yr indicated for this phase agrees with estimates of sedimentation rates and radiocarbon age determinations of the Würmian sequence in Israel^{8,10,17}. The sampled flows from the Golan Plateau do not interdigitate with any sediments and are not subject to direct stratigraphic control, but their radiometric ages clearly show that they correlate with the Hasbani Basalt.

Discussion

The maximum development of the Miocene marine transgression in the Mediterranean region, allocated a Middle Miocene age by Gignoux¹⁸, has been shown by our dating of the Lower Basalt to have occurred between 12.2 and 14.6 Myr ago, which is in good agreement with evidence from other regions¹⁹. The Tabianian marine transgression, represented in Israel by sediments of the Pliocene Bira Formation, which are about 4.8 Myr old, is thought to have been very rapid, and to have reached its maximum extent within about 0.5 to 0.75 Myr (ref. 20).

The age of the Cover Basalt is relevant to the dating of the preglacial and the beginning of the Pleistocene glacial stages. The dated specimens were collected from the upper flows of this volcanic sequence and represent the end of the preglacial Pleistocene, about 1.7–2.0 Myr ago. The reversed magnetic polarity of the upper section of the Cover Basalt, and the consistently normal polarity of its lowermost flows^{21,22}, strongly suggests that its eruption straddled the transition from the Gauss Normal to the Matuyama Reversed epochs, estimated at 2.4 Myr (ref. 23). The beginning of the preglacial Pleistocene therefore predates the polarity transition and we propose, provisionally, to allocate it an age in the range 2.6 to 3.0 Myr—a date which also corresponds to the close of the Pliocene.

The radiometric dating of the Yarden and Yarmuk Basalts, stratigraphically of late or post-Mindel age, indicates a minimum age of 0.66 Myr for the Mindel. This age limit provides an important link between the early Acheulian cultures of Europe and Africa and has been discussed in detail²⁴. Radiometric dates from Pleistocene deposits in Africa are fairly numerous, but in Europe there is almost no dateable Pleistocene material. Furthermore, climatic correlation between the European and African Pleistocene is poor, whereas the correlation between the European and Israeli Pleistocene seems well established¹⁷. Dating of the Israeli Pleistocene thus provides a sound basis for Pleistocene geochronology in Europe.

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- ¹ Bender, F., *Geologie von Jordanien* (Gebrüder Borntraeger, Berlin, 1968).
- ² Van den Boom, G., *Geol. Jb.*, **85**, 489 (1968).
- ³ Oppenheim, M. J., *Bull. Res. Coun. Israel*, **11G**, 58 (1962).
- ⁴ McDougall, I., Polach, H. A., and Stipp, J. J., *Geochim. cosmochim. Acta*, **33**, 1485 (1969).
- ⁵ Dubertret, L., *Notes Mém. Moyen-Orient*, **VIII**, 250 (1966).
- ⁶ Brown, G. F., *Phil. Trans. R. Soc.*, **A267**, 75 (1970).
- ⁷ Schulman, N., *Bull. Res. Coun. Israel*, **8**, 63 (1959).
- ⁸ Gvirtzman, G., *Geol. Surv. Israel Rep.*, **OD/5/67** (1970).
- ⁹ Derin, B., and Reiss, Z., *Israel Inst. Petrol., Rep.* 4/71 (1971).
- ¹⁰ Horowitz, A., *Israel J. Earth Sci.*, **22**, 107 (1973).
- ¹¹ Picard, L., *Hebrew Univ. geol. Dep. Bull.*, **1B**, (1943).
- ¹² Moshkowitz, S., *Israel J. Earth Sci.*, **12**, 97 (1964).
- ¹³ Colalongo, E. A., *Boll. Soc. geol. Ital.*, **91**, 489 (1972).
- ¹⁴ deVries, H. L., and Barendsen, G. W., *Nature*, **174**, 1138 (1954).
- ¹⁵ Picard, L., *Geol. Soc. Am. Spec. Paper*, **84**, 337 (1965).
- ¹⁶ Michelson, H., *Water Planning for Israel, Rep. HR/72/037* (1972).
- ¹⁷ Horowitz, A., *Pollen Spores*, **XII**, 2,255 (1971).
- ¹⁸ Gignoux, M., *Stratigraphic Geology* (Freeman, New York, 1955).
- ¹⁹ Eicher, D. L., *Geologic Time* (Prentice-Hall, New Jersey, 1968).
- ²⁰ Hsü, K. J., *Scient. Am.*, **227**, 6,27 (1972).
- ²¹ Freund, R., Oppenheim, M. J., and Schulman, N., *Israel J. Earth Sci.*, **14**, 37 (1965).
- ²² Nur, A., and Helsley, C. E., *Earth planet. Sci. Lett.*, **10**, 375 (1971).
- ²³ Cox, A., Doell, R. R., and Dalrymple, C. B., *Q. Jl. geol. Soc. Lond.*, **124**, 53 (1968).
- ²⁴ Horowitz, A., Siedner, G., and Bar Josef, O., *Nature*, **262**, 186 (1973).
- ²⁵ Horowitz, A., *Israel J. Earth Sci.* (in the press).

Vegetation classification by reference to strategies

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It is suggested that there are three major determinants of vegetation—competition, stress and disturbance—and that each has invoked a distinct strategy on the part of the flowering plant. A method is described whereby it is possible to distinguish types of herbaceous vegetation by reference to the relative importance of the three strategies in the genotypes of the component species.

THE electronic computer has been a mixed blessing to vegetation classification. On the one hand, it has facilitated the development of methods of numerical and multivariate analysis. On the other, it has contributed to the decline in confidence in

established methods but has yet to replace them with a new *lingua franca*. The resultant confusion in the field of vegetation classification is unfortunate in that it coincides with an unprecedented demand for standardised botanical information which can be readily interpreted and assimilated into plans to reclaim or manage the landscape.

This is not to argue for a return to the older methods of phytosociology which apart from their subjectivity are often difficult to apply in a world landscape experiencing increasingly diverse and disruptive interference by man. The current requirement is for methods of classification which can include recent or unstable vegetation, avoid unnecessary abstraction and provide data intelligible to nonspecialists. An approach to the classification of herbaceous vegetation has been made with these considerations in mind.

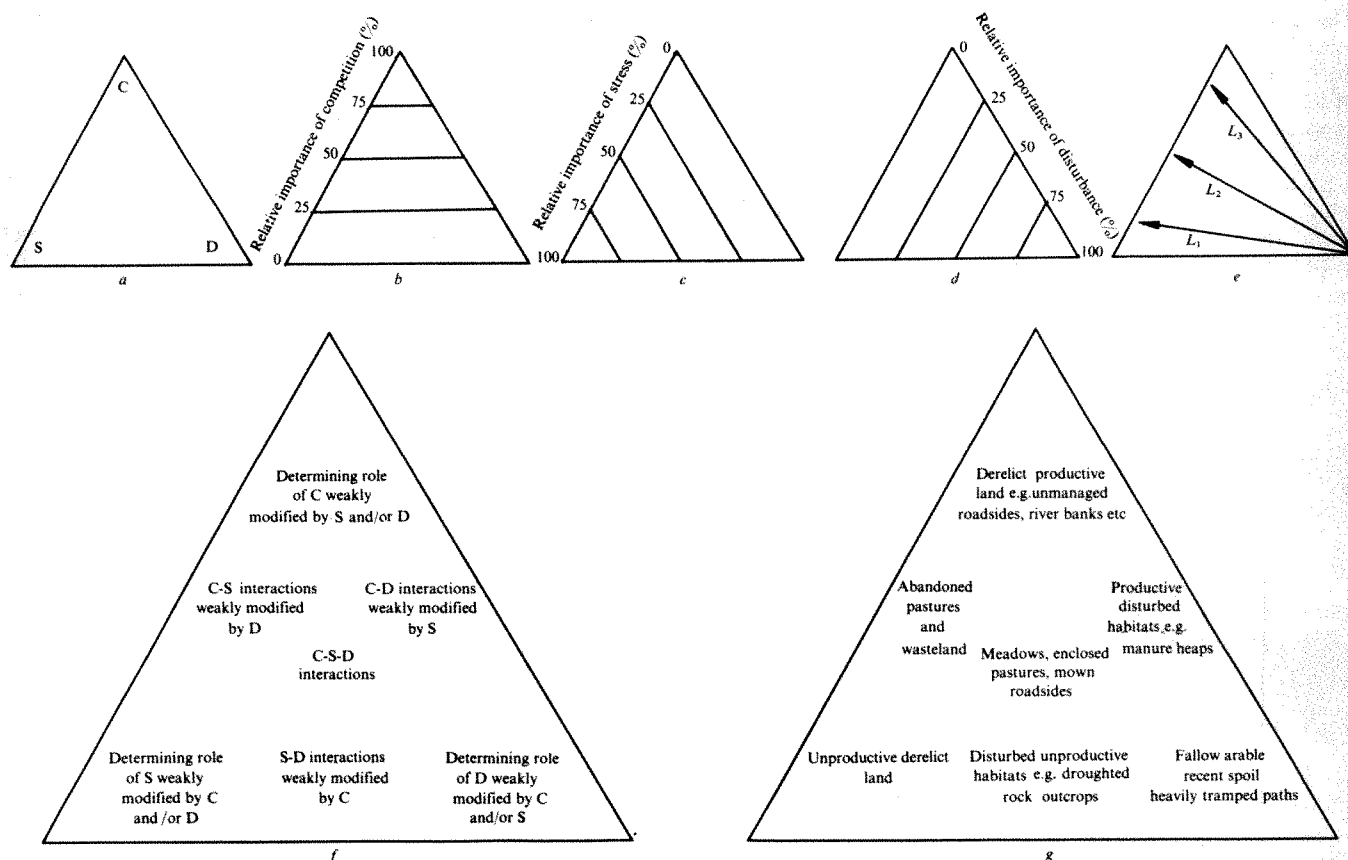


Fig. 1 A triangular model of herbaceous vegetation. *a*, Identification of the corners at which competition (C), stress (S) and disturbance (D) are exclusive determinants. *b-d*, Contours in percentage contribution of competition, stress and disturbance, respectively. *e*, Course of vegetation succession. The arrows correspond to lines of succession of low (L_1), moderate (L_2) and high (L_3) productivity. *f*, Interaction of competition, stress and disturbance. *g*, Location of selected habitat types.

Triangular ordination

The method depends upon the assertion that, basically, there are three determinants of herbaceous vegetation—competition, stress and disturbance—and that each has invoked a distinct strategy on the part of the flowering plant. The possibility arises therefore that an ordination of vegetation types can be based on measurements of the relative importance of the three strategies among the component species. More specifically, it is suggested that the spectrum of herbaceous vegetation types may be accommodated in a model (Fig. 1*a*) consisting of an equilateral triangle in the corners of which the relative importance of competition (C), stress (S) and disturbance (D) reach their respective maxima. In Fig. 1*b-d*, the gradients in relative importance of each of the three determinants are indicated by means of contours and in Fig. 1*f* the interactions between competition, stress and disturbance are summarised. Figure 1*g* illustrates the predicted location of selected habitat types within the triangle. The triangular model also appears to incorporate some of the dynamic features of herbaceous vegetation. The course of succession (Fig. 1*e*) is from the right-hand corner of the triangle towards the opposite side which constitutes the interface with woody vegetation. Increase in the angle of elevation of the line of succession (L_1 – L_3) above the horizontal is associated with a progressive increase in productivity. Species density (number of species per unit area) would be expected to show a progressive decline towards the apex as a result of competitive exclusion.

To derive from this model a practical method of ordination, the minimum requirement is to find measurable attributes of the flowering plant which vary in accordance with any two of the three sets of contours in Fig. 1*b-d*. To explore the possible means by which this requirement may be fulfilled it is necessary to consider the essential nature of competition, stress and disturbance and the strategies which they seem to have invoked.

Competition, stress and disturbance

Competition may be defined as the attempt by neighbouring plants to utilise the same units of light, water, mineral nutrients or space¹. By definition, therefore, competition exerts its maximum impact as a determinant of vegetation in circumstances where the competition is resolved perhaps even to the extent that the habitat is occupied by one species, possibly one individual plant. Stress and disturbance together comprise those phenomena which prevent the resolution of competition. At moderate intensities this intervention has the effect of creating spatial or temporal niches; at their most severe both stress and disturbance may so suppress plant development that individual plants scarcely impinge on each other and competition is occluded. The difference between stress and disturbance lies in the fact that whilst both inhibit the development of a large standing crop the former does so by restricting primary production, the latter by damage to the vegetation. Whereas stress is usually imposed by the physical environment (shortages of light, water, mineral nutrients, suboptimal temperatures, soil and toxins), disturbance arises from the activities of grazing animals, pathogens, man (trampling, mowing and ploughing) and from physical phenomena such as soil erosion. The same environmental factor, drought for example, may cause both stress and disturbance. In certain situations it is difficult to distinguish between competition and stress. In particular, problems of definition arise where herbaceous vegetation derives shade or phytotoxins from a remote tree canopy.

Three strategies

Many plant attributes have been implicated in adaptations to particular forms of competition, stress or disturbance. Here attention is focused on general characteristics of the competitive, stress-tolerant and ruderal strategies.

The competitive strategy. From both field and laboratory investigations of competition²⁻¹⁵, it seems that a number of plant attributes are conducive to the efficient capture and utilisation of light, water, mineral nutrients and space. These include an elevated leaf canopy, the capacity for extensive lateral spread both above and below ground and the tendency to accumulate a thick layer of litter on the ground surface, all characteristics which are especially prominent in herbaceous species such as *Pteridium aquilinum* and *Epilobium hirsutum*, which frequently occupy extensive areas of vegetation to the virtual exclusion of

other species. The ability of these three attributes to distinguish the competitive strategy is suggested by their very low incidence in environments subjected either to severe stress or to continuous disturbance¹⁶.

The stress tolerant strategy. In addition to small stature, a general characteristic of herbaceous plants in environments experiencing continuous and severe stress is a low potential relative growth rate¹⁷. This generalisation appears to hold for a wide variety of stresses including those associated with nutrient deficiencies on basic and acidic soils¹⁸⁻²³, shading^{24,25} and

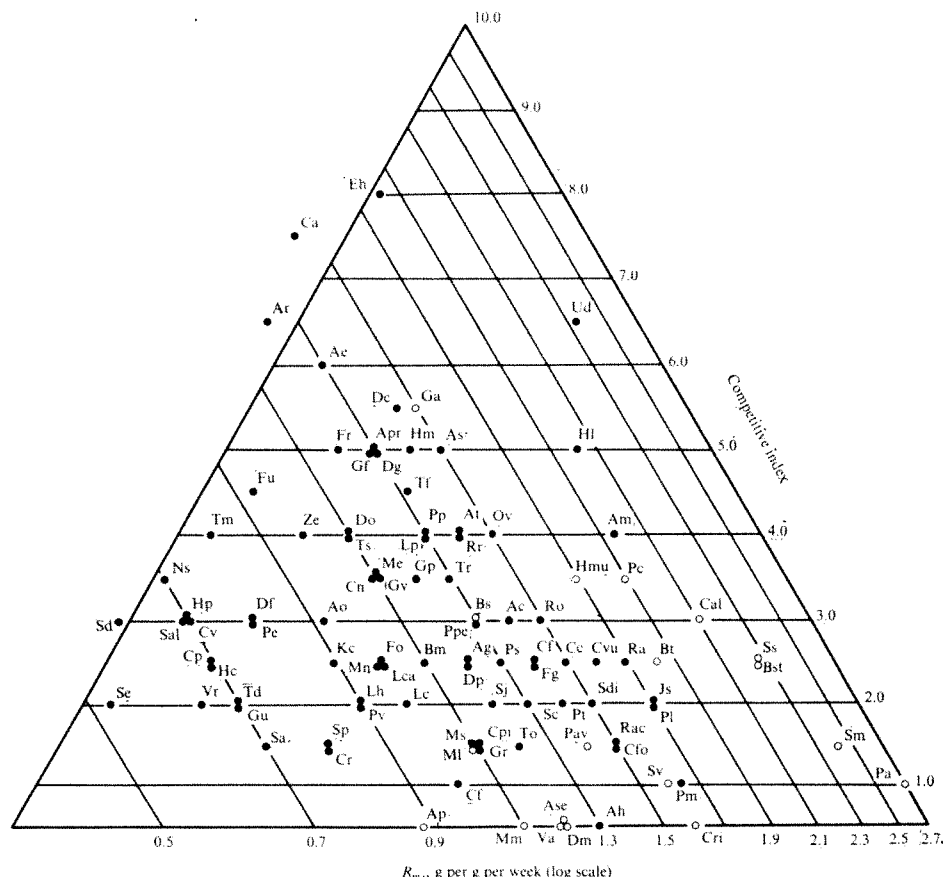


Fig. 2 A triangular ordination of herbaceous species. ○, Annuals; ●, perennials (including biennials). The competitive index (CI) was calculated from the formula $CI = (a + b + c)/2$ where a , estimated maximum height of leaf canopy (1, < 12 cm; 2, 12–25 cm; 3, 25–37 cm; 4, 37–50 cm; 5, 50–62 cm; 6, 62–75 cm; 7, 75–87 cm; 8, 87–100 cm; 9, 100–112 cm; 10, > 112 cm); b , lateral spread (0, small therophytes; 1, robust therophytes; 2, perennials with compact unbranched rhizome or forming small (< 10 cm diameter) tussock; 3, perennials with rhizomatous system or tussock attaining diameter 10–25 cm; 4, perennials attaining diameter 26–100 cm; 5, perennials attaining diameter > 100 cm); c , estimated maximum accumulation of persistent litter (0, none; 1, thin, discontinuous cover; 2, thin, continuous cover; 3, up to 1 cm depth; 4, up to 5 cm depth; 5, > 5 cm depth).

Key to species: The value in brackets refers to the 95% confidence limit for R_{max} .

Ac, *Agrostis canina*, ssp. *canina* (± 0.18); Ae, *Arrhenatherum elatius* (± 0.16); Ag, *Alopecurus geniculatus* (± 0.14); Ah, *Arabis hirsuta* (± 0.28); Am, *Achillea millefolium* (± 0.57); Ao, *Anthoxanthum odoratum* (± 0.17); Ap, *Aira praecox* (± 0.14); Apr, *Alopecurus pratensis* (± 0.22); Ar, *Agropyron repens* (± 0.11); As, *Agrostis stolonifera* (± 0.22); Ase, *Arenaria serpyllifolia* (± 0.30); At, *Agrostis tenuis* (± 0.25); Bm, *Briza media* (± 0.14); Bs, *Brachypodium sylvaticum* (± 0.28); Bst, *Bromus sterilis* (± 0.25); Bt, *Bidens tripartita* (± 0.28); Ca, *Chamaenerion angustifolium* (± 0.17); Cal, *Chenopodium album* (± 0.73); Cc, *Cynosurus cristatus* (± 0.13); Cf, *Carex flacca* (± 0.24); Cfl, *Cardamine flexuosa* (± 0.18); Cfo, *Cerastium fontanum* (± 0.19); Cn, *Centaurea nigra* (± 0.15); Cp, *Carex panicea* (± 0.25); Cpr, *Cardamine pratensis* (± 0.18); Cr, *Campanula rotundifolia* (± 0.36); Cri, *Catapodium rigidum* (± 0.38); Cv, *Clinopodium vulgare* (± 0.09); Cvu, *Cirsium vulgare* (± 0.56); Dc, *Deschampsia cespitosa* (± 0.12); Df, *Deschampsia flexuosa* (± 0.18); Dg, *Dactylis*

glomerata (± 0.14); Dm, *Draba muralis* (± 0.15); Do, *Dryas octopetala* (± 0.47); Dp, *Digitalis purpurea* (± 0.34); Eh, *Epilobium hirsutum* (± 0.13); Fg, *Festuca gigantea* (± 0.41); Fo, *Festuca ovina* (± 0.14); Fr, *Festuca rubra* (± 0.16); Fu, *Filipendula ulmaria* (± 0.26); Ga, *Galium aparine* (± 0.21); Gf, *Glyceria fluitans* (± 0.19); Gp, *Galium palustre* (± 0.15); Gr, *Geranium robertianum* (± 0.13); Gu, *Geum urbanum* (± 0.38); Gv, *Galium verum* (± 0.30); Hc, *Helianthemum chamaecistus* (± 0.24); Hl, *Holcus lanatus* (± 0.28); Hm, *Holcus mollis* (± 0.16); Hmu, *Hordeum murinum* (± 0.35); Hp, *Helictotrichon pratense* (± 0.09); Js, *Juncus squarrosus* (± 0.17); Kc, *Koeleria cristata* (± 0.11); Lc, *Lotus corniculatus* (± 0.14); Lca, *Luzula campestris* (± 0.13); Lh, *Leontodon hispidus* (± 0.14); Lp, *Lolium perenne* (± 0.13); Me, *Milium effusum* (± 0.16); Ml, *Medicago lupulina* (± 0.13); Mm, *Matricaria matricarioides* (± 0.22); Mn, *Melica nutans* (± 0.11); Ms, *Myosotis sylvatica* (± 0.12); Ns, *Nardus stricta* (± 0.16); Ov, *Origanum vulgare* (± 0.22); Pa, *Poa annua* (± 0.42); Pav, *Polygonum aviculare* (± 0.12); Pc, *Polygonum convolvulus* (± 0.56); Pe, *Potentilla erecta* (± 0.23); Pl, *Plantago lanceolata* (± 0.13); Pm, *Plantago major* (± 0.19); Pp, *Poa pratensis* (± 0.14); Ppe, *Polygonum persicaria* (± 0.13); Ps, *Poterium sanguisorba* (± 0.38); Pt, *Poa trivialis* (± 0.25); Sal, *Sesleria alba* (± 0.11); Sc, *Scabiosa columbaria* (± 0.16); Sd, *Siegingia decumbens* (± 0.14); Sdi, *Silene dioica* (± 0.17); Sj, *Senecio jacobaea* (± 0.13); Sm, *Stellaria media* (± 0.23); Sp, *Succisa pratensis* (± 0.15); Ss, *Senecio squalidus* (± 0.17); Sv, *Senecio vulgaris* (± 0.36); Td, *Thymus drucei* (± 0.20); Tf, *Tussilago farfara* (± 0.17); Tm, *Trifolium medium* (± 0.08); To, *Taraxacum officinale* (± 0.12); Tr, *Trifolium repens* (± 0.16); Ts, *Teucrium scorodonia* (± 0.21); Ud, *Urtica dioica* (± 0.13); Va, *Veronica arvensis* (± 0.28); Vr, *Viola riviniana* (± 0.16); Ze, *Zerna erecta* (± 0.08).

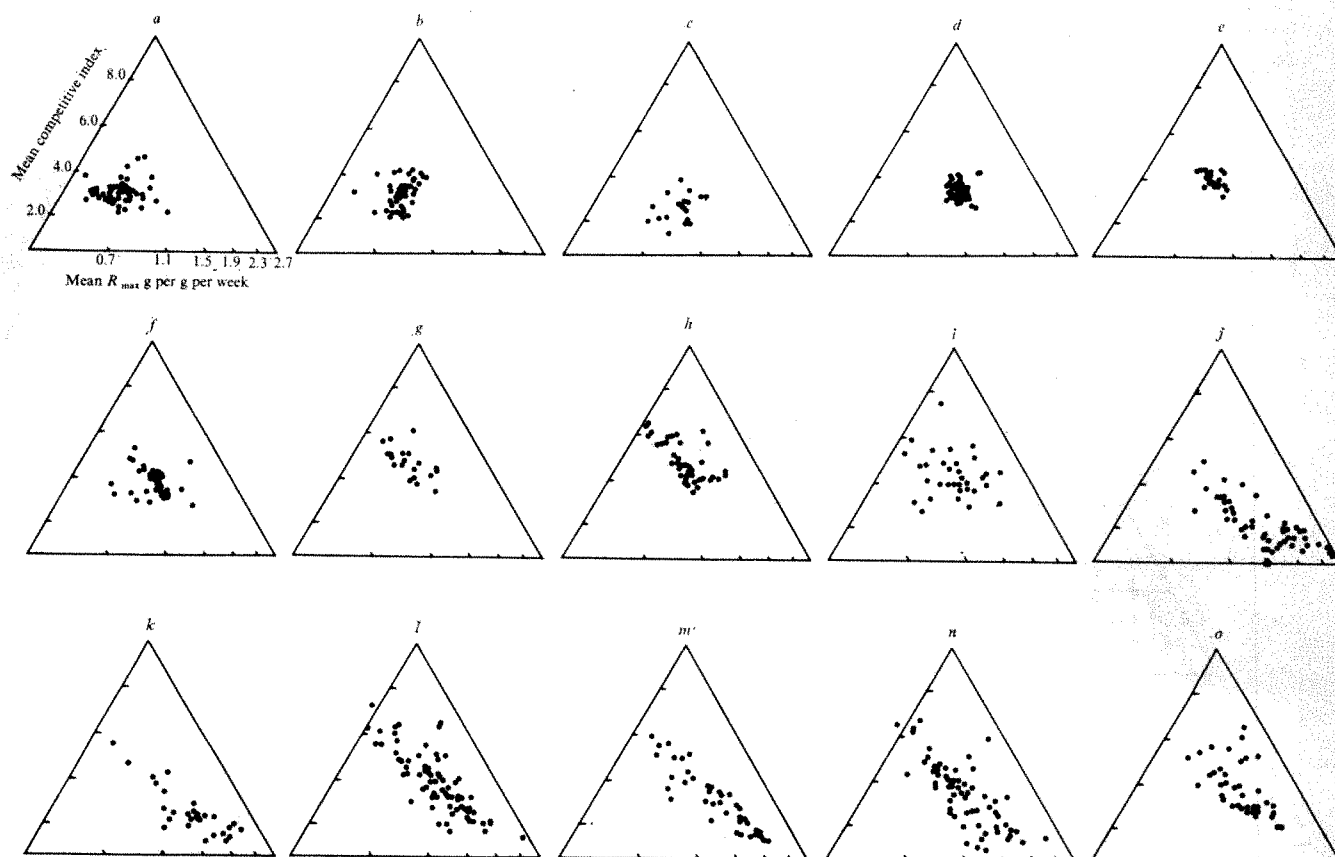


Fig. 3 Triangular ordinations of m^2 samples of herbaceous vegetation from fifteen habitats. Axes are mean competitive index and mean R_{max} each derived as in Fig. 2 and weighted according to the relative frequency of the species in the sample. a, Unenclosed sheep pastures on acidic strata; b, Unenclosed sheep pastures on limestone; c, limestone outcrops; d, meadows; e, road verges, mown frequently; f, enclosed pastures; g, road verges, mown infrequently; h, hedge bottoms; i, derelict banks of rivers, ponds and ditches; j, paths; k, fallow arable; l, heaps of mineral soil (such as building sites); m, demolition sites (brick and mortar rubble); n, cinders (tips and railway ballast); o, manure heaps and sewage sludge.

desiccation²³. In marked contrast, both the competitive and the ruderal strategies are associated with high potential relative growth rates^{23, 26, 27}.

The ruderal strategy. The majority of herbaceous species in highly disturbed habitats are annuals or short-lived perennials. A result of disturbance is the release of space and relief from stress and competition. It is not surprising, therefore, to find that a characteristic of many ruderals is the capacity for rapid seedling establishment and growth²³. A related feature is the tendency for a high proportion of the photosynthate to be directed into seeds and, under conditions of stress, for seed production to be maintained at the expense of vegetative growth^{28, 29}.

Ordination of species

To test the practical value of ordination by strategy, tests were carried out using data from an unpublished survey of the vegetation of the Sheffield region. As a first step, a triangular ordination was carried out on 100 herbaceous plants prominent in the data. One axis in the ordination was designed to correspond to the relative importance of the competitive strategy and was a numerical index based upon estimates of the maxima in height of canopy, lateral spread and litter accumulation derived from the field observations of Dr J. G. Hodgson and myself. The results of numerous investigations (for example refs 5–8, 11 and 13) suggest that in a majority of competitive species height of canopy is of greatest importance. For the purposes of this investigation, therefore, the maximum possible score for height of canopy was arranged to be twice that allowed for either lateral spread or litter accumulation (legend of Fig. 2). The competitive index used here differs from a predecessor¹⁶ both in the introduction of a weighting system and in the fact that relative growth rate is not incorporated. The second axis,

on a log scale, referred to stress tolerance and was R_{max} , the maximum relative growth rate of the species recorded during the period 2–5 weeks after germination in a standardised, productive growth-room environment²³. The values of R_{max} should be regarded as first estimates and may be subject to revision as data become available for other seed sources and conditions of growth.

In view of the provisional nature of the data, it is reassuring to find that the majority of the species fall within the triangle (Fig. 2) and, with certain exceptions, for example, *Origanum vulgare*, *Scabiosa columbaria* and *Tussilago farfara*, lie in close proximity to species with which they have strong ecological affinities. The left-hand corner is occupied by species tolerant of desiccation, for example *Sedum acre*, or shade, for example *Sanicula europaea*, or frequent in nutrient-deficient habitats whether acidic (*Deschampsia flexuosa*, *Nardus stricta*), calcareous (*Helictotrichon pratense*, *Sesleria albicans*) or associated with a wider range in soil pH (*Sieglingia decumbens*, *Viola riviniana*). Annual plants are concentrated in the 'ruderal corner' and show a significant ($P < 0.001$) and anticipated³⁰ rise in seed weight with increasing competitive index. Species of productive, derelict habitats occur towards the apex of the triangle. The unique position in the ordination of *Urtica dioica* is due to the unusually rapid growth rate of the species and is consistent with the sensitivity of the species to mineral-nutritional stress^{21, 23, 31}. The location of the winter annuals *Aira praecox*, *Veronica arvensis*, *Arenaria serpyllifolia* and *Draba muralis* coincides with that predicted for disturbed, unproductive habitats (Fig. 1g) and is quite distinct from that of the perennial plants such as *Festuca ovina* and *Koeleria cristata*, with which the annuals occur on limestone outcrops. This suggests that in such habitats the patches of bare soil occupied by the annuals constitute a distinct spatial and temporal niche.

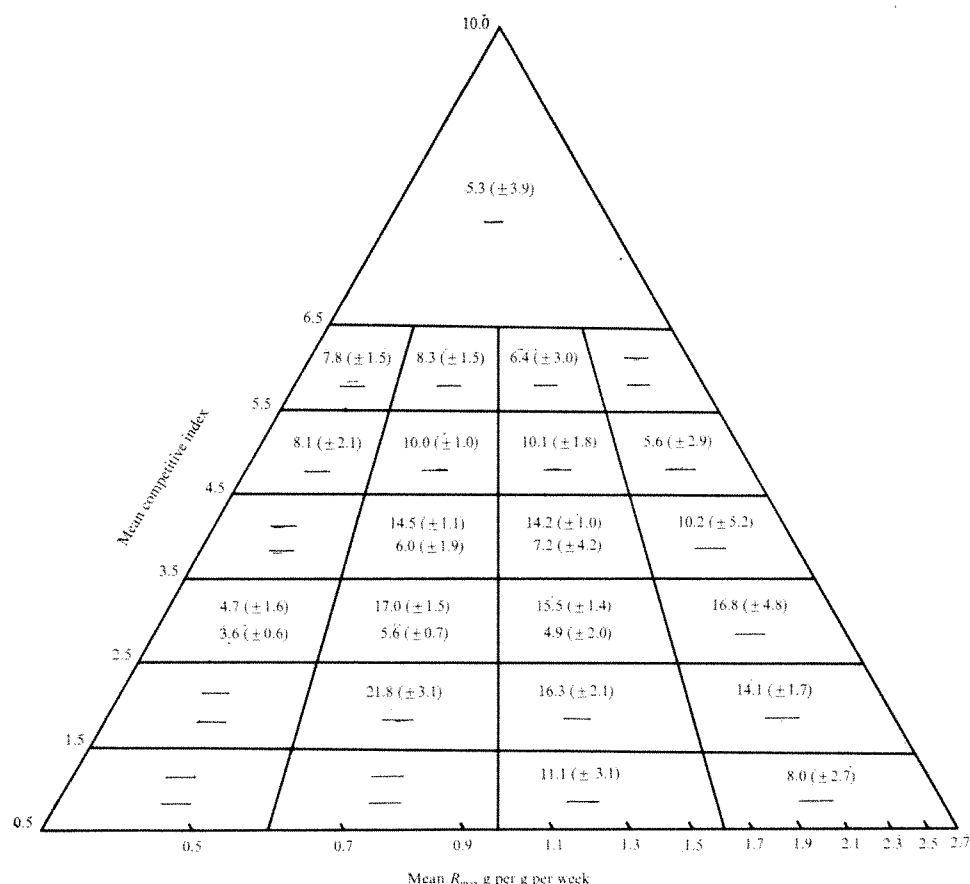


Fig. 4 Pattern of species density (number of species m^{-2}) obtained by ordination of 925 samples of herbaceous vegetation from a wide range of habitats. Axes are the same as in Fig. 3. In each cell the uppermost values are the mean and 95% confidence limit for samples from sites with a surface soil pH > 4.0. The lower values refer to samples of pH < 4.1.

* Cells containing less than five samples.

Ordination of vegetation samples

The second step in evaluating the approach was to ordinate vegetation samples from a wide variety of habitats. The axes were based on mean values for individual quadrats of both the competitive index and R_{max} , with R_{max} again plotted on a logarithmic scale. In the case of the competitive index, the values for each species which contributed towards the mean for the quadrat were weighted according to the relative importance of the species in the m^2 sample. An identical system of weighting was applied in the calculation of mean R_{max} for the quadrat although here data were necessarily restricted to those species for which growth analyses had been carried out (> 50%) and including the more frequent species in the majority of samples). A selection of the ordinations is presented in Fig. 3. The various types of vegetation occupy positions in quite close agreement with those predicted in Fig. 1g. The figures illustrate the tendency of samples from stressed habitats (a, b, c), disturbed environments (j, k) and semiderelict but productive sites (g, h, i) to extend into respective corners of the triangle. Vegetation types experiencing a moderate intensity of orderly disturbance (d, e, f) tend to occupy compact areas in the centre of the diagram. By contrast, samples from spoiled land (l-o) show an attenuated distribution which seems to represent the course of vegetation succession in these new habitats (compare Fig. 1e).

Figure 4 illustrates the pattern of species density which was obtained by pooling data from 25 ordinations (925 samples). When 103 samples from extremely acidic soils (surface soil pH < 4.1) are discounted the predicted decline in species density, towards the apex of the triangle, is apparent. The fall in species density in the lowest rank of cells is probably related to the scarcity of species adapted to extremes of stress and disturbance¹⁶.

Future developments

The consistent patterns evident in Fig. 3 are encouraging, particularly in view of the fact that the samples in each ordination have been drawn from localities scattered over a wide geo-

graphical area. However, there is obvious scope for refinement of the method. There is a need for more accurate estimations of R_{max} and for an assessment of the extent to which this attribute and others used in the ordination is subject to intraspecific variation. It is also necessary to evaluate alternative criteria with respect to both the competitive and the stress-resistant strategies. In addition, the possibility of a 'ruderal axis', perhaps related to 'reproductive effort'³² remains to be examined.

None of the species in Fig. 2 occurs within the two areas of the triangle corresponding to extreme competition or severe stress. This may be due to deficiencies in the axes or in the data. An alternative explanation is that it is necessary to explore beyond a temperate herbaceous flora in order to find the necessary conjunctions of R_{max} and competitive index.

The value of this triangular ordination to vegetation management lies at present in the ability to detect the intensity of competition and stress and to predict the intensity of disturbance at particular sites. If the approach is to be put to maximum use, however, it must be complemented by techniques which identify particular forms of competition, stress and disturbance.

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¹ Grime, J. P., *Nature*, **244**, 311 (1973).

² Olsen, C., *J. Ecol.*, **9**, 1 (1921).

³ Greig-Smith, P., *J. Ecol.*, **36**, 339 (1948).

⁴ Monsi, M., and Saeki, T., *Jap. J. Bot.*, **14**, 22 (1953).

⁵ Watt, A. S., *J. Ecol.*, **43**, 490 (1955).

⁶ Black, J. M., *Aust. J. agric. Res.*, **9**, 299 (1958).

⁷ Donald, C. M., *Aust. J. agric. Res.*, **9**, 421 (1958).

⁸ Black, J. N., *Aust. J. agric. Res.*, **11**, 277 (1960).

⁹ Palmer, J. H., and Sagar, G. R., *J. Ecol.*, **51**, 783 (1963).

¹⁰ Ellenberg, H., *Vegetation Mitteleuropas mit den Alpen* (Eugen Ulmer, Stuttgart, 1963).

- ¹¹ Willis, A. J., *J. Ecol.*, **51**, 353 (1963).
- ¹² Grime, J. P., *J. Ecol.*, **36**, 339 (1963).
- ¹³ Thurston, J. M., In *Ecological Aspects of the Mineral Nutrition of Plants* (edit. by Rorison, I. H.) (Blackwell, Oxford, 1969).
- ¹⁴ Smith, C. J., Elston, J., and Bunting, A. H., *J. Br. Grassld Soc.*, **26**, 213 (1971).
- ¹⁵ Maarel, E. Van der., in *The Scientific Management of Animal and Plant Communities for Conservation* (edit. by Duffey, E. and Watt, A. S.) (Blackwell, Oxford, 1971).
- ¹⁶ Grime, J. P., *Nature*, **242**, 344 (1973).
- ¹⁷ Parsons, R. F., *Am. Nat.*, **102**, 595 (1968).
- ¹⁸ Bradshaw, A. D., Chadwick, M. J., Jowett, D., and Snaydon, R. W., *J. Ecol.*, **52**, 665 (1964).
- ¹⁹ Clarkson, D. T., *J. Ecol.*, **55**, 111 (1967).
- ²⁰ Parsons, R. F., *Oecologia Plantarum*, **3**, 121 (1968).
- ²¹ Rorison, I. H., *New Phytol.*, **67**, 913 (1968).
- ²² Higgs, D. E. B., and James, D. B., *J. Ecol.*, **57**, 553 (1969).
- ²³ Hunt, R., thesis, Univ. Sheffield (1970).
- ²⁴ Grime, J. P., *J. Ecol.*, **51**, 391 (1963).
- ²⁵ Loach, K., *New Phytol.*, **69**, 273 (1970).
- ²⁶ Clatworthy, J. N., and Harper, J. L., *J. exp. Bot.*, **13**, 307 (1961).
- ²⁷ Myerscough, P. J., and Whitehead, F. H., *New Phytol.*, **66**, 785 (1967).
- ²⁸ Salisbury, E. J., *The Reproductive Capacity of Plants* (Bell, London, 1942).
- ²⁹ Harper, J. L., in *Mechanisms in Biological Competition* (edit. by Milthorpe, F. L.), **1** (Cambridge University Press, London, 1961).
- ³⁰ Harper, J. L., Lovell, P. H., and Moore, K. G., *Adv. Ecol. Systemat.*, **1**, 237, (1970).
- ³¹ Pigott C. D., and Taylor, K., *J. Ecol.*, **52**, (suppl.), 175 (1964).
- ³² Ogden, J., thesis, Univ. Wales (1968).

Computer-aided three-dimensional reconstruction and quantitative analysis of cells from serial electron microscopic montages of foetal monkey brain

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Computer-aided reconstruction of immature brain cells with highly irregular shapes from serial electron microscopic montages gives three-dimensional images and quantitative data on surface areas and volumes, providing new classes of data on cell behaviour during development.

KNOWLEDGE of structural relationships between cells in the central nervous system has reached a level of precision that requires efficient high resolution methods of quantitative three dimensional analysis. The classic Golgi method^{1,2} provides a blackened image of the complete silhouette of individual cells against an almost colourless background, and steps have been taken to obtain quantitative cytological data with computer aid³. But this method lacks adequate resolution and usually does not facilitate visualisation of relationships between contiguous cells. The transmission electron microscope provides appropriate resolution and facilitates visualisation of the profiles of cell bodies and processes, and their relationships with other cells in a given field of view. Preparation of several hundred consecutive serial sections is now feasible and computer-aided reconstruction methods have been applied to electron microscopic analysis of the organisation of invertebrate neural tissue (refs 4 and 5 and personal communication from S. Brenner). We describe here the application of a quantitative computer-aided method to the analysis of cell shapes,

volumes and surface areas as visualised in electron micrographs of a sector of foetal monkey cerebrum.

A 58-d embryo was removed from an anaesthetised *Macacus rhesus* monkey by hysterotomy and perfused through the circulatory system with a mixture of 1% formaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer⁶. Blocks of tissue 1×1×0.5 mm were postfixed in OsO₄, stained with uranyl acetate, dehydrated and embedded in Maraglas. Precisely oriented transverse sections 1 μm thick were cut from a sector of cerebral wall approximately at the border of the occipital and temporal lobes (Fig. 1a). The block was remounted and trimmed to a rhomboid shape, yielding a final block face that extended from the ventricular zone to the base of the cortical plate (Fig. 1b) and included a sector of sub-ventricular zone about 100 μm thick and intermediate zone 300 μm thick (neuroembryological terminology recommended by Boulder Committee⁷). A set of 170 consecutive serial sections, each about 80 nm thick, was mounted on Formvar film on one-hole (1×2 mm) grids and stained with uranyl acetate and lead citrate. From every third section, 16–20 overlapping fields were photographed at ×2,800 with a Hitachi electron microscope and montages were prepared of prints at a final magnification of ×8,400. Selected cells and their nuclei were outlined on transparent acetate sheets, and minor adjustments in alignments were made by reference to nearby transversely cut cell processes and capillaries. Registration marks were placed on each transparency for alignment

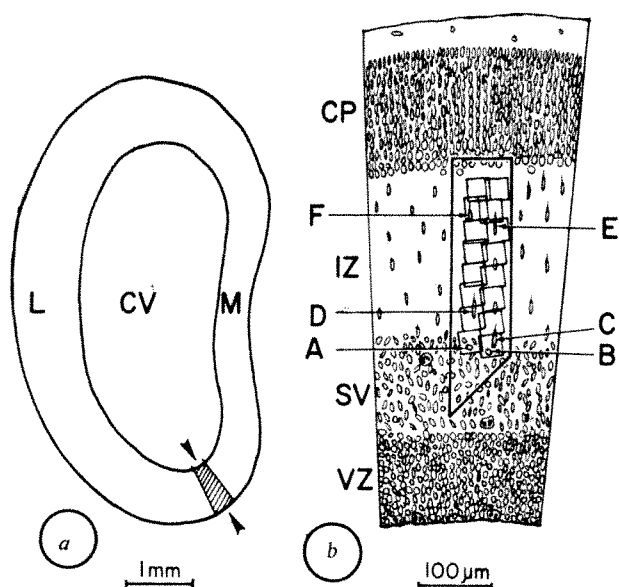


Fig. 1a Outline of a coronal section across the occipital lobe of a 58-d-old monkey embryo. The cerebral ventricle (CV) is relatively large at this stage and the lateral wall (L) is much thicker than the medial wall (M) on which the incipient calcarine fissure is slightly indented. The shaded area between the arrowheads indicates the position of the block of tissue that was processed for electron microscopy. **b**, Drawing of a 1 μ m thick, toluidine blue-stained section across the entire area shaded in (a). The cerebral wall from the ventricular surface at the bottom to the external surface at the top of the drawing consists of ventricular zone (VZ), subventricular zone (SV), intermediate zone (IZ) and cortical plate (CP). The area outlined by the rhomboid was cut serially for electron microscopy. Overlapping squares represent individual fields photographed for the reconstruction on photomontages. Arrows A to F point to the positions of six cells reconstructed in this study.

purposes. A representative electron microscopic field from which cell outlines were traced is illustrated in Fig. 2a. Considerable human judgement is involved in the choice of valid cell contours, and we thought it not worthwhile to attempt to automate this step.

Computer analysis

The cell and nuclear contours on the serial section transparencies were digitised as follows. The registration marks on successive transparencies were carefully aligned with pre-marked registration indicators on the digitiser to establish the x and y axes, and the height of the serial section above a reference level was dialled into the digitiser to provide elevation information (z axis). In the case of cells with complex shapes, more than one contour sometimes appeared on a transparency. Each contour on the transparency was traced with the cursor of the digitiser and the coordinates of selected points were recorded by the operator. The x-y coordinates of each point, the z coordinate of the transparency, and an identifying integer were transferred automatically to punched cards. Each closed membrane contour constituted a data set. Scaling information was recorded separately and used to convert the x-y-z data into absolute physical units.

The basic software for processing was prepared for an IBM 360/75 computer. The software reads the individual sets of data into memory, recognises multiple contours on a level, scales the data and computes the volume and surface area of each cell and nucleus as represented by the digital images. Registration is not necessary to obtain volume and surface area, but is necessary for graphic presentation.

Graphic software has also been written which gives the viewer the choice of observing the cell from any desired angle of rotation about the axis of the image (Fig. 2b-d). Standard Eulerian and projective transformations accomplish this efficiently. Presentation of the cell or nucleus as a three dimensional solid in space required the suppression of points hidden from the observer's eye. The requirement for a fixed number of points per contour is too strict a condition for the type of data considered⁸. A new method was developed which has no constraints on the number of points per contour or the number of contours per level. An arbitrary maximum can be set on the total number of points needed to specify the cell. The visibility algorithm depends only on the property of whether a point falls inside or outside the contour of visible points which preceded it. Visibility is determined data set by data set beginning with the first contour on the level closest to the observer and proceeding to the last on the contour furthest away.

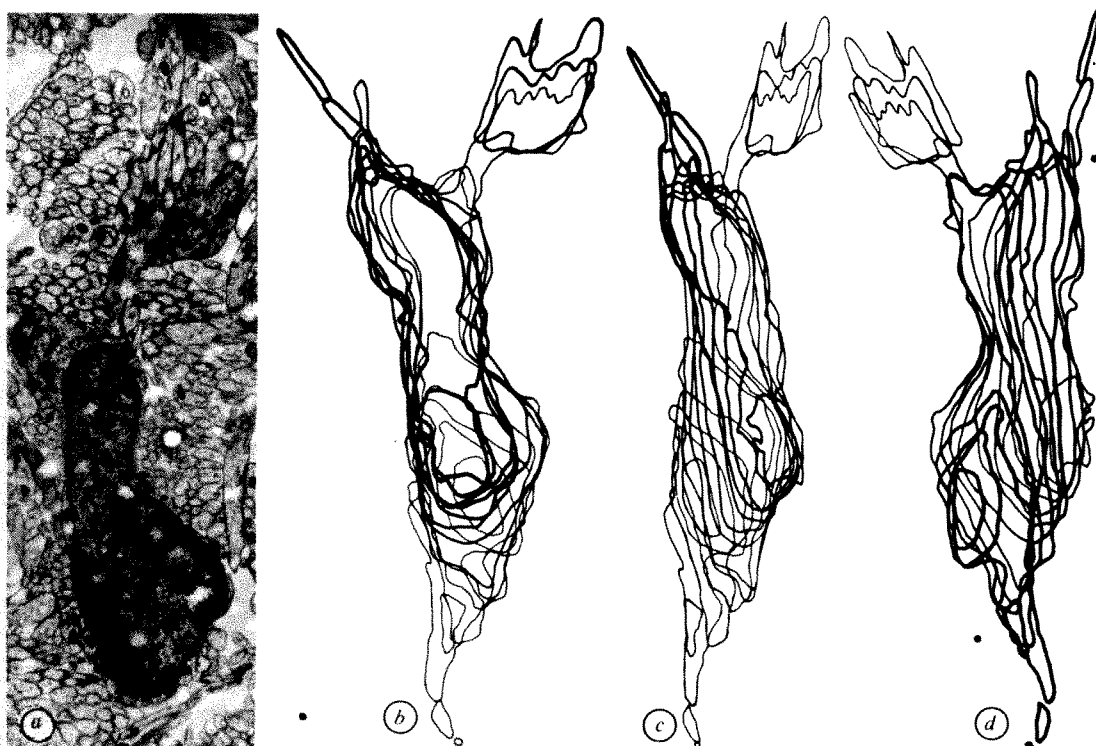


Fig. 2a Electron micrograph of cell C in serial section no. 83. Although the processes of all cells are in close apposition, it is relatively easy for the human observer to trace the irregular outline of a given cell. **b-d**, Cell C, as reconstructed from several fields, including that shown in (a). Only every ninth outline is represented, to make diagrams legible. In the three drawings, the cell is rotated on the axis running from pole to pole by 0°, 45° and 135°, respectively.

Considerable savings in computer time can be effected with a variation of the method. The intensity of each segment making up a contour is weighted according to its distance from the observer; nearer contours are reproduced in progressively thicker and darker lines, further ones are thinner and lighter (Fig. 2*b-d*). The effect achieved is similar to full hidden line suppression. To illustrate the savings achieved with this method, two trial cases each having 800 points of coordinate data representing 25 levels of contour data were processed using (1) full hidden line suppression, and (2) intensity weighting. Full hidden line suppression required 2.5 min of Central Processing Unit time on an IBM 360/75 computer compared with 0.5 min for intensity weighting. Therefore we used the latter mode of presentation in the preparation of the computer graphics presented here.

Three dimensional reconstruction

Of 24 cells reconstructed, six were examined in detail: two in the subventricular zone (Fig. 1*b*, cells A and B), two in the inner part of the intermediate zone (cells C and D) and two in the outer part of the intermediate zone (cells E and F).

volume though not in shape (Fig. 3*b, d* and *i-l*). The nucleocytoplasmic volume ratios and the cell surface area-volume ratios express numerically the relatively round compared with the elongated configurations of the cells (Table 1). The most interesting quantitative feature is the striking difference in cytoplasmic volume between cells E and F, which migrated the greatest distance, and cells C and D, which migrated less. If these four cells belong to the same postmitotic neuronal class, the data suggest considerable cell growth during migration. Alternative possibilities are that the cells are not statistically different in size or that cells C and D are smaller because they belong to a different population, destined for a different cortical layer¹⁴. Another important quantitative feature is the increase in surface area during elongation of the cells to bipolar shapes (compare cells A and B with C and D in Table 1) and a possible further increase in surface area as cells approach the cortical plate (cells E and F), an indication of net synthesis of cell membrane during migration. When more significant statistical samples are obtained new concepts may well emerge concerning the mechanisms and significance of cell movement in the developing central nervous system.

Figures 3*e-h* show that at the stage of cortical development under study, every migrating neurone examined is in contact with radially oriented fibres (compare refs 6 and 10). The leading processes of the migrating neurones have complex

Table 1 Quantitative cellular data

Cell	Surface area (μm^2)	Total volume (μm^3)	Ratio: area volume	Cytoplasmic volume (μm^3)	Nuclear volume (μm^3)	Ratio: nuclear volume cytoplasmic volume
A	140.1	155.0	0.904	44.7	110.3	2.47
B	177.7	239.6	0.741	50.5	189.1	1.83
C	266.7	200.9	1.33	75.1	125.8	1.68
D	212.0	179.8	1.18	63.5	116.3	1.83
E	305.4	264.7	1.15	121.3	143.4	1.18
F	235.9	239.8	0.984	103.2	136.6	1.32

From their positions and morphology as represented in random sections, we had suspected that cells A and B were subventricular (neuronal or glial precursors, possibly still proliferating), while cells C-F were postmitotic young neurones whose positions along the migration trajectory to the cortical plate should, in a general way, reflect their ages^{6,9}. Three-dimensional computer displays of the contours of cell C reconstructed from many sections, and viewed from three different angles are illustrated in Fig. 2*b-d*.

Drawings of all six cells, their nuclei and some contiguous bundles of fibres of relatively small calibre are shown in Fig. 3. These were made manually to scale directly from the tracings, with frequent reference to the original electron micrographs. Comparison of Figs 2*d* and 3*e* shows that the manual and computer reconstructions give similar results.

Volumes and surface areas

The computerised method of cell reconstruction reported here represents a departure from the slower and more laborious wax plate procedure adopted in earlier electron microscopic studies^{9,12,13}. It requires less time and labour, and provides a set of quantitative data of biological interest (Table 1). Although the sample of data is small, it suggests certain trends. Of the two subventricular cells, B has a much larger nucleus, which seems to be in a different class from those of the other cells, and we suspect both from this measurement and its electron microscopic appearance that cell B has just entered mitosis. The other nuclei resemble one another in

forms, reminiscent of the appearance of cells observed directly during migration in tissue culture¹¹. Leading processes commonly extend along more than one radial fibre (Fig. 3*e* and *h*). They terminate within the intermediate zone and in the sample of 24 cells, they have not been seen to extend into the cortical plate or through it to the external surface of the cerebrum. The cell shape (Fig. 3*g*) suggests that the leading processes are plastic, some withdrawn and others extended further as the cell migrates toward the cortex; if the number and form of the leading processes were fixed, this cell would be unable to advance beyond the fibre bundle that lies across the migration path and passes in front of one apical process and behind the other. Trailing processes were followed in some instances to their tapered terminations (for example, cell E) or to a point where they pass from the serially cut tissue (for example, cell C). It is also noteworthy that the rounded subventricular cells A and B, which do not seem to be migrating, likewise contact radial fibres (Fig. 3*a* and *c*). Such cells were not examined in the earlier studies^{6,10}, and it is not clear from this small sample whether or not subventricular cells usually relate to radial fibres.

Further prospects

Investigators may have some difficulty in estimating their computer requirements in relation to the complexity of their biological data. On the one hand, a mini-computer such as the PDP-11/05 with graphic accessories would permit the recording and display of a set of serial section contours but would allow only limited image rotation or data storage for large complex cells. On the other hand, a computer of the

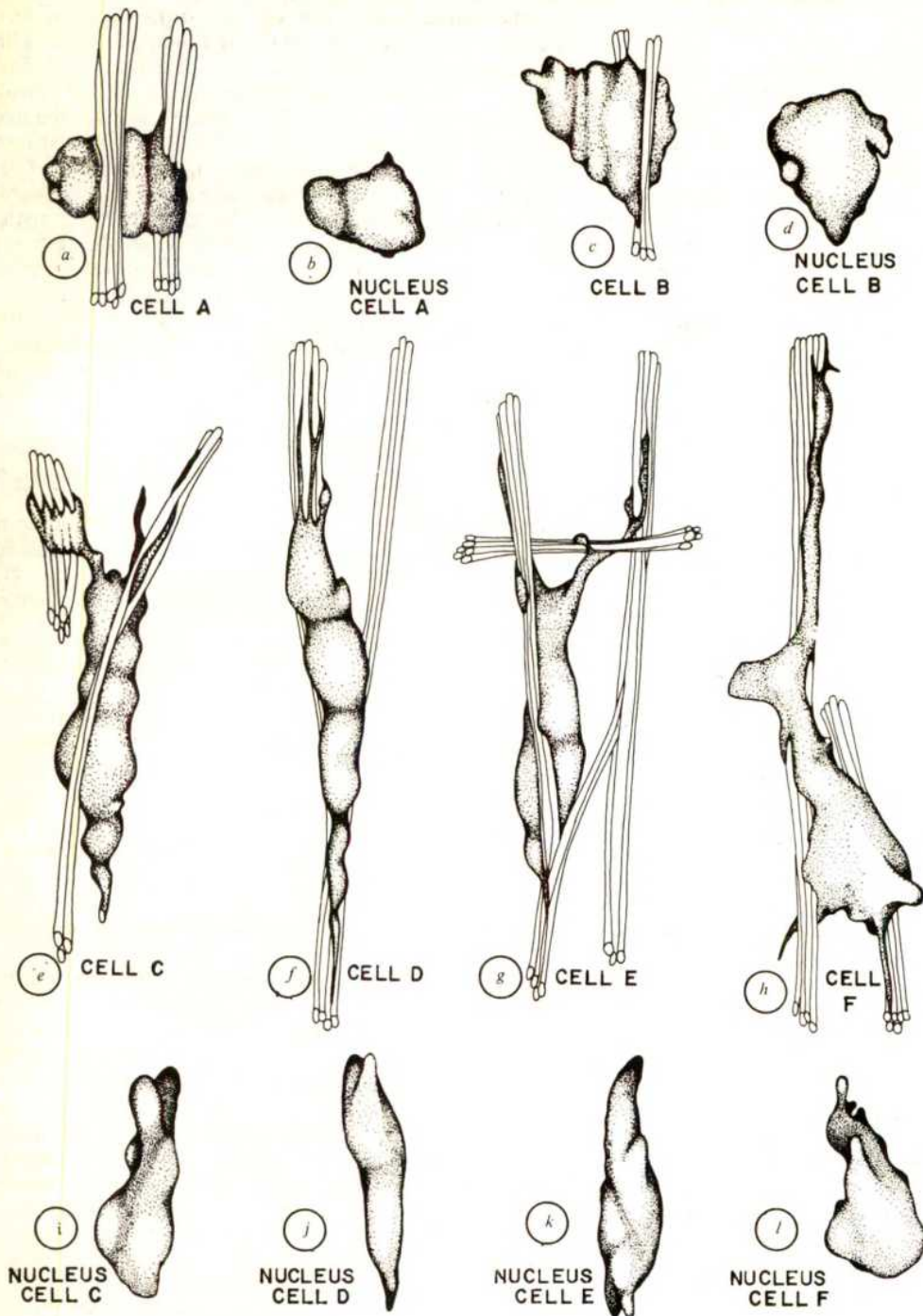


Fig. 3 a, c, e-h represent reconstructions of cells A to F respectively. Cells were drawn with the aid of superimposed outlines of cell profiles in serial sections at different levels traced onto transparent Mylar sheets. Some of the fibres which lie in contiguity with the migrating cells were also traced and reconstructed but most of the neighbouring cells and processes were omitted. b, d and i-l represent similarly reconstructed nuclei of cells A to F, respectively.

- IBM 360/75 type which we used has a larger capacity than we needed. Its greater storage capability will be necessary, however, to handle more complex issues such as spatial relationships among several cells that are changing their positions relative to one another in the developing brain. It will be needed also for analysis of the shapes and relationships of mature neurones, particularly for quantitative plotting and three dimensional display of synaptic inputs.

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¹ Golgi, C., *Sulla fina anatomia degli organi centrali del sistema nervoso*, 1883, republished in *Opera Omnia* (Hoepli, Milan, 1903).

- ² Scheibel, M. E., and Scheibel, A. B., *Int. Rev. Neurobiol.*, **13**, 1 (1970).
- ³ Wann, D. F., Woolsey, T. A., Dierker, M. L., and Cowan, W. M., *Inst. Elect. Electron. Engng, Trans. Biomed. Engng*, **20**, 233 (1973).
- ⁴ Levinthal, C., and Ware, R., *Nature*, **236**, 207 (1972).
- ⁵ Macagno, E. R., Lopresti, V., and Levinthal, C., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 57 (1973).
- ⁶ Rakic, P., *J. comp. Neurol.*, **145**, 61 (1972).
- ⁷ Boulder Committee, *Anat. Rec.*, **166**, 257 (1970).
- ⁸ Willey, T. J., Schultz, R. L., and Gott, A. H., *Inst. Elect. Electron. Engng, Trans. Biomed. Engng*, **20**, 288 (1973).
- ⁹ Stensaas, L. J., and Stensaas, S. S., *Z. Zellforsch.*, **91**, 341 (1968).
- ¹⁰ Rakic, P., *Brain Res.*, **33**, 471 (1971).
- ¹¹ Bray, D., and Bunge, M. B., *Locomotion of Tissue Cells, Ciba Foundation Symp.*, **14**, 195 (1973).
- ¹² Sjöstrand, F. S., *J. ultrastruct. Res.*, **2**, 122 (1958).
- ¹³ Karlsson, U., *J. ultrastruct. Res.*, **16**, 482 (1966).
- ¹⁴ Rakic, P., *Science*, **183**, 425 (1974).

LETTERS TO NATURE

PHYSICAL SCIENCES

Was Jupiter the protosun's core?

THE differences between Jupiter and conventional stars are apparently due solely to its small mass. Therefore in looking for possible means of formation of the Solar System it is natural to attempt to establish the origin of the Jupiter-Sun binary system. The other planets could be just a by-product of the evolution of this system.

I shall start from the hypothesis that the close binaries form as a result of the rotational-exchange instability of the protostar (my unpublished work). This hypothesis can be formulated as follows.

Immediately after the collapse of the prestellar cloud, convection begins in the protostar formed¹. The initial differential rotation is replaced in a zone of convective mixing by uniform rotation (mixing can be caused by an instability of the differential rotation proper during the onset of quasihydrostatic equilibrium in the protostar). Because of the transfer of angular momentum, the centrifugal force can exceed the gravitational force in the outer layers. These layers will then become separated, forming a massive ring rotating around the protostar. A fast (adiabatic) loss of mass from the convective zone (the politrope index being 1.5) produces an increase in its size². As a result, the transfer of mass on to the ring, just as in the later stages of close binary evolution, proceeds on a 'dynamic' time-scale until the convective envelope is totally lost³. The ring is unstable, breaking up and collapsing into one or several bodies at some stage.

In this way a multiple system is formed, and the mass ratio of its components should thus depend on that of the convective zone and of the stable core in the protostar. The fraction of the total mass involved in convection depends critically on the density of the prestellar cloud, but it should generally be larger the smaller is the mass of the protostar⁴. The accounts for the observed decrease (ref. 5 and my unpublished work) of the number of binaries with a short period ($P \lesssim 5-7$ d) and a small component mass ratio ($q < 0.8$) as one goes from B to F stars. Apparently the masses of the convective envelope and of the stable core in protostars forming close binaries with F components are, on the average, the same. The systems here have a $q \approx 1$.

With less massive protostars, systems with $q < 1$ should appear again (my unpublished work) but here the more massive component is formed by the lost matter. The loss of a larger mass releases a larger angular momentum J contained in the protostar. The system period increases at a fixed total mass, $P \propto J^3(1+q)^6 q^{-3}$ becoming longer than $5-7^6$. Such systems are difficult to find.

When convection extends throughout the protostar, practically all the initial mass should become transferred on to the object formed by ring condensation.

Indeed, convection penetrates into the star from outside, so that fast mass transfer begins, as a rule, before convection has reached the centre. Matter involved in convection is lost continuously so that the protostar retains only a small fraction of its initial mass at the time when the centre regains radiative stability after becoming convective.

The major factor determining the completion of transfer

is apparently the cooling of the protostar remnant caused by its fast expansion and radiation of energy. Nonvolatile components condense, forming in the protostar remnant a rocky core (and probably bodies orbiting in the outer rarified zone of the remnant).

Thus the above process of breakup of the protostar and of the mass exchange between its fragments results in the formation of a fairly wide system consisting of a newly formed massive star and the remnant of the protostar, its mass being much less than the initial mass. We now have a star with a satellite, that is with a planet.

Turning now to the Solar System, it is natural to assume Jupiter to be such a remnant of a protostar. This provides an easy explanation for the origin of the terrestrial planets and for their great difference from the massive Jupiter. These planets condensed and acquired angular momentum from the matter streaming from Jupiter—the protostar (the primary component)—to its satellite—the Sun (the secondary component).

This matter carries some angular momentum and therefore forms a rotating disk around the secondary. Keplerian motion in the disk is upset by friction transferring angular momentum to the disk's periphery. From here, the matter is thrown out by centrifugal force which carries away the momentum excess. The matter from the disk's interior is deposited on to the secondary⁶.

The gas dynamics of such a rotating disk have not been studied in detail but it is evident that the physical parameters should vary over a wide range, from thousands of K and tens and more atmospheres in the stream flowing from the primary⁷ and in shock waves generated by its motion, to hundreds of K and fractions of an atmosphere far away from both components. Within such a wide range one can easily envisage the realisation of the conditions necessary for the production of some chemical compounds and of their condensates which are needed for the formation of the terrestrial planets^{8,9}.

As for the planets beyond Jupiter, they could have formed from fragments of already sufficiently dense matter which remained from (or was thrown out in) the collapse of the ring separated from the protosun. The majority of the fragments coalesced and/or dispersed in collisions with one another and the parent bodies. The remaining fragments could have acquired arbitrary orientation of axial rotation (Uranus).

Afterwards, when the disk rotated around the Sun giving birth to the terrestrial planets, the loss of excess angular momentum from it resulted in the matter concentrating beyond the Jupiter-Sun system. Part of the matter was captured by the massive fragments-planets, while another part (just as in the inner disk but at a lower temperature) could condense to form planets similar in many respects to those of the Earth group, as well as comets (Oort's cloud).

The reasons for the slow rotation of the Sun, just as in the case of close binary stellar systems (my unpublished work), should be looked for in the effect of the rotation of the primary on the magnitude (and even sign) of the angular momentum of matter streaming away from it¹⁰. If the equatorial velocity of the primary greatly exceeds the

orbital velocity then the streaming matter will not form a disk, and the secondary (the Sun) will increase in mass with almost no increase of the momentum of its axial rotation.

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- ¹ Hayashi, Ch., *A. Rev. Astr. Astrophys.*, **4**, 171-192 (1966).
- ² Chandrasekhar, S., *An Introduction to the Study of Stellar Structure* (University of Chicago Press, 1939).
- ³ Paczinski, B., and Sienkiewicz, R., *Acta astr.*, **22**, 73-91 (1972).
- ⁴ Bodenheimer, P., *Rep. Prog. Phys.*, **35**, 1-54 (1972).
- ⁵ Drobyshevski, E. M., *Astrofizika* (in the press).
- ⁶ Prendergast, K. H., and Burbidge, G. R., *Astrophys. J.*, **151**, L83-87 (1968).
- ⁷ Drobyshevski, E. M., and Reznikov, B. I., *Acta astr.*, **24**, No. 2 (1974).
- ⁸ Safronov, V. S., *Evolution of the Protoplanetary Cloud and the Formation of the Earth and Planets* (in Russian), (Nauka, Moscow, 1969).
- ⁹ Anders, E., *A. Rev. Astr. Astrophys.*, **9**, 1-34 (1971).
- ¹⁰ Kruszewski, A., *Acta astr.*, **17**, 297-310 (1967).

Gamma rays from black holes

As a result of electron ion decoupling, the compression of interstellar matter falling on to an isolated black hole results in ion temperatures greater than 100 MeV as the matter approaches the Schwarzschild radius. Here, experimental pion production cross sections are used to calculate the rate of production of γ rays from this hot gas. A characteristic γ -ray spectrum is produced, which peaks at 18 MeV regardless of the mass of the black hole or the interstellar density.

Matter accreting on to a black hole may be heated to very high temperatures and radiate γ rays^{1,2}. The accretion of matter onto a black hole can be treated as a hydrodynamic flow if a weak magnetic field is carried with the accreting gas^{1,3}. In the hydrodynamic model the accreting gas is rapidly compressed as it falls inwards, causing a significant fraction of the gravitational potential energy to be converted into thermal energy. Moreover, if the mass of the black hole is not too large ($\leq 10^4 M_\odot$) the accreting gas radiates away only a small fraction of the available thermal energy^{1,2}, so the compression of the accreting gas will be very nearly adiabatic. The temperature of the accreting gas at a distance r from the centre of the black hole is given by:

$$\theta(r) \approx 0.5 \theta_0 (r_1/r)^{3(\gamma-1)/2} \quad (1)$$

where γ is the average adiabatic index, r_1 is the distance at which the gas begins to fall inwards, and θ_0 is the temperature of the gas as it begins to fall. The distance r_1 will be given by⁴:

$$r_1 = (2GM/c^2) (m_p c^2 / \gamma \theta_0) \quad (2)$$

where M is the mass of the black hole and m_p is the proton mass (assuming that the interstellar medium is predominantly hydrogen).

The temperature of the accreting gas near the gravitational radius, $r_g = 2GM/c^2$, depends markedly on whether the gas is ionised when it begins to fall inwards. If the gas is not initially ionised, then the temperature of the accreting gas does not get hotter than about 10^9 K (ref. 2). The gas near a black hole may, however, be ionised with $\theta_0 \sim 1$ eV (ref. 3), in which case (using a one-temperature approximation and assuming an average adiabatic index of 13/9 when the electrons become relativistic) there is a gas temperature of 10^{11} K near the Schwarzschild radius². The electron-ion coupling time, however, turns out to be much longer than the radial infall time so that it is more appropriate to use different temperatures and different adiabatic indices for the ions and electrons. As the ions are non-

relativistic at temperatures less than 100 MeV $\gamma = 5/3$ can be used, and the ion temperature is¹:

$$\theta_i(r) \approx 0.3 m_p c^2 (r_g/r) \quad (3)$$

Thus, the ion temperature exceeds 100 MeV as the accreting gas approaches the gravitational radius. The ion density is¹:

$$n(r) = 6.10^{12} n_0 (r_1/r)^{3/2} \quad (4)$$

Considering the change in adiabatic index for the relativistic electrons, they approach a temperature of about 10 MeV near the gravitational radius if $M \leq 10^4 M_\odot$. The ion-electron Coulomb coupling time for $r < 100 r_g$ is $> 10^6$ s, which is far longer than the radial infall time $\lesssim 10^{-3}$ s, so the ions and electrons are indeed uncoupled. This is not altered by the presence of a turbulent magnetic field¹ if there is approximate equipartition of the magnetic field energy and the gas kinetic energy. The turbulent magnetic field tends to heat the ions at the expense of infall kinetic energy. The validity of the hydrodynamic approximation depends only on the smallness of the proton cyclotron radius relative to scales of interest.

At temperatures in excess of 100 MeV proton-proton collisions result in copious pion production, and γ rays will be produced because of the decay $\pi^0 \rightarrow 2\gamma$. This process is the main contribution to the γ -ray luminosity of black holes.

The rate of π^0 production in a hot gas with proper density n is $(n^2/2) \langle \pi^0 \rangle \bar{\sigma} \bar{v}(\theta)$; where $\langle \pi^0 \rangle$ is the average number of π^0 's produced per collision, and σ is the pion production cross section. The quantity $\bar{\sigma} \bar{v}(\theta)$ is related to the laboratory pion production cross section $\sigma(E)$ by:

$$\bar{\sigma} \bar{v}(\theta) = \iint \sigma(E_L) v_L f(\mathbf{p}_1) f(\mathbf{p}_2) d\mathbf{p}_1 d\mathbf{p}_2 \quad (5)$$

where v_L is the laboratory velocity and f is the thermal distribution. Numerical evaluation of equation (5), using the experimental pion production cross sections⁵, and a relativistic Maxwell distribution, provides a curve of $\bar{\sigma} \bar{v}$ against θ (Fig. 1). Knowing the ion temperature and density as a function of r the production of pion γ rays can be calculated as a function

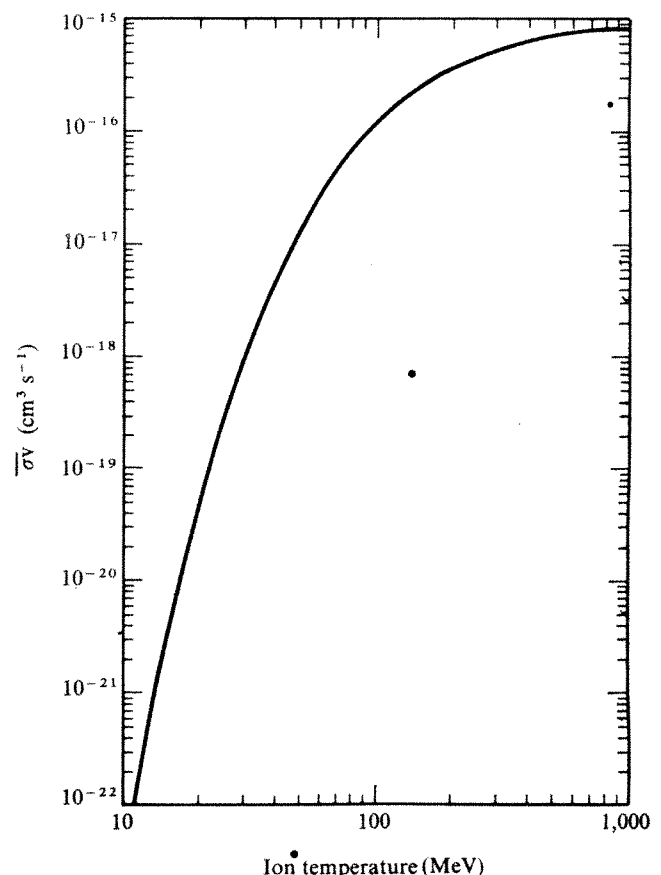


Fig. 1 Relativistic thermal average of $\sigma \bar{v}$, where σ is the cross section for the total pion production.

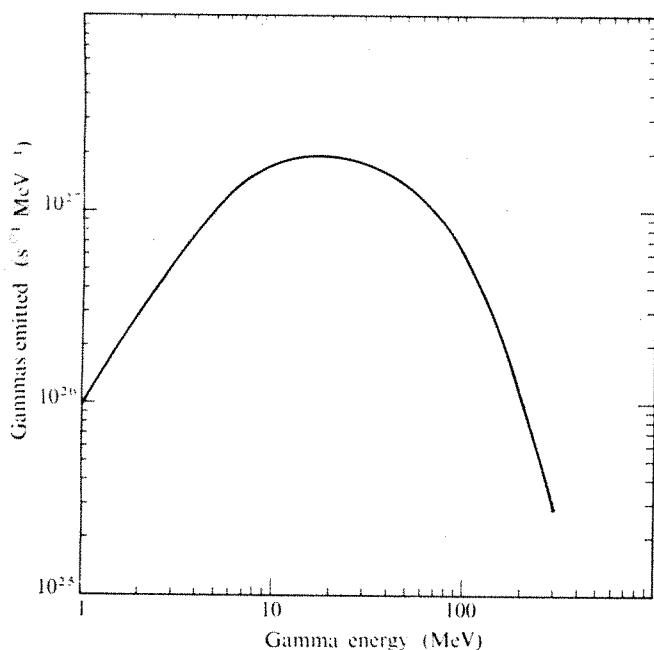


Fig. 2 Differential γ -ray spectrum from a $10 M_{\odot}$ black hole in a region of density $= 1 \text{ cm}^{-3}$, and $\theta_0 = 1 \text{ eV}$.

of r . The total rate of emission of γ rays resulting from spherical accretion is:

$$\dot{N}_{\gamma} = \int_{r_g}^{\infty} \langle \pi^0 \rangle n^2 \bar{\sigma} v(\theta) [1 + (\cos \psi - V/c)/(1 - V/c \cos \psi)] 2\pi r^2 dr \quad (6)$$

where V is the radial infall velocity and ψ is the half angle of the radiation 'escape cone'. Numerical evaluation of the right side of equation (6), using the values of $\bar{\sigma} v(\theta)$ shown in Fig. 1 and equation (3) and (4) gives:

$$\dot{N}_{\gamma} \approx 3 \times 10^{26} (M/M_{\odot})^3 (1 \text{ eV}/\theta_0)^3 n_0^2 \text{ s}^{-1} \quad (7)$$

These γ rays have energies of the order of 20 MeV, so that the γ ray luminosity resulting from spherical accretion is:

$$L_{\gamma} \approx 10^{22} (M/M_{\odot})^3 (1 \text{ eV}/\theta_0)^3 n_0^2 \text{ erg s}^{-1} \quad (8)$$

This is larger than the γ -ray luminosity resulting from bremsstrahlung emission², but it is small compared to the total luminosity of the black hole, which results from the synchrotron radiation of visible light¹. It may be larger than indicated in equation (8) if matter is falling onto an isolated rotating black hole because of the accumulation of matter in orbits near r_g . Accreting disk models in binary systems⁶, have a much lower luminosity γ -ray because the accreted material has time to radiate a significant fraction of its energy, thus invalidating the adiabatic approximation.

The spectrum of γ rays emerging from the accreting gas will be determined by: the energy spectrum of the π^0 's as observed by a comoving observer; the Doppler shift resulting from the radial infall of the gas; and the gravitational redshift. More than half of the γ -ray emission comes from the region $r_g < r < 2r_g$, so the gravitational red shift is an important effect.

The γ -ray spectrum in a comoving frame which results from the energy spectrum of the π^0 's, can be calculated to sufficient accuracy by noting that for laboratory energies of less than 4 GeV almost all pion production occurs through an $N^*(1236)$ intermediate state. Thus, in order to calculate the γ -ray spectrum in a comoving frame only the γ -ray spectrum in the rest frame of an $N^*(1236)$ need be known. It can be obtained⁷ by convoluting the spectrum in the N^* rest frame with the relativistic thermal Doppler spectrum $\phi(\omega) = (2\omega)^{-1} \gamma(\omega) \exp[(m_{N^*} c^2/\theta_1) \gamma(\omega)]/K_1(m_{N^*} c^2/\theta_1)$; where $\gamma(\omega) = (\omega/\omega_0 + \omega_0/\omega)/2$ and ω_0 is the γ -ray energy in the N^* rest frame. If ω_1 is the γ -ray energy in the comoving frame then the frequency measured by a distant observer is:

$$\omega_{obs} = \omega_1 [(1 - v/c)/(1 + v/c)]^{1/2} (1 - r_g/r)^{1/2} \quad (9)$$

(We have neglected the angular dependence of the Doppler

shift because the escape cone light of sight is nearly radial when the infall velocity is largest.) The spectrum obtained by integrating the contributions from all radii has a width of ~ 80 MeV, and falls off approximately as E^{-3} for $100 \text{ MeV} < E < 300 \text{ MeV}$ (Fig. 2).

The spectrum (Fig. 2) is universal in the sense that its shape is independent of the mass of the black hole or the interstellar density. Thus, if many black holes are present in the Universe some kind of anomalous feature could be expected in the γ -ray background in the neighbourhood of 10 MeV. The spectrum from black holes in our Galaxy would have a peak at 18 MeV; the spectrum from black holes in other galaxies would peak below 18 MeV because of cosmological redshift which lowers the peak in the 'background' spectrum to ~ 10 MeV. An anomalous feature in the γ -ray background at about 10 MeV has, in fact, been reported at a level of about $10^{-4} \text{ } \gamma \text{ s cm}^{-2} \text{ sr}^{-1} \text{ MeV}^{-1}$ (refs 8 and 9).

If this anomaly results from isotropic emission from external galaxies with $30 M_{\odot}$ black holes, then about 10^{10} black holes per galaxy are required, assuming equation (7) for the γ -ray luminosity and $n_0 = 100 \text{ cm}^{-3}$. As mentioned, higher γ -ray luminosities may occur, thereby decreasing the number of black holes required. In our Galaxy, upper limits on the emission above 15 MeV from the galactic centre¹⁰ indicate that as many as 10^7 $30 M_{\odot}$ black holes may be in that vicinity if $n_0 = 100 \text{ cm}^{-3}$.

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- Schwartzman, V. F., *Soviet Astr. AJ*, **15**, 377 (1971).
- Shapiro, S. L., *Astrophys. J.*, **180**, 531 (1973).
- Zeldovich, Y. B., and Novikov, I. D., *Relativistic Astrophysics* (University of Chicago Press, Chicago, 1971).
- Bondi, H., *Mon. Not. R. astr. Soc.*, **112**, 195 (1952).
- Lock, W. O., and Measday, D. F., *Intermediate Energy Nuclear Physics* (Methuen, London, 1970).
- Novikov, I., and Thorne, K. S., *Black Holes* (edit. by DeWitt, C., and DeWitt, B. S.), (Gordon and Breach, New York, 1973).
- Stecker, F. W., *Cosmic Gamma Rays*, NASA SP-249 (Scientific and Technical Information Office, Washington DC, 1971).
- Trombka, J. E., Metzger, A. E., Arnold, J. R., Matteson, J. L., Reedy, R. C., and Peterson, L. E., *Astrophys. J.*, **818**, 737 (1973).
- Vedrenne, W., Alberne, F., Martin, I., and Talon, R., *Astr. Astrophys.*, **15**, 50 (1971).
- Share, G. H., Kinzer, R. L., and Seeman, N., *Astrophys. J.*, **187**, 54 (1974).

Do black holes really explode?

THE creation of particles out of the vacuum will occur in regions of space-time where the metric is changing rapidly. Theoretical discussions of this process encounter some interpretational difficulties, however, because the concept of 'particle' is only well understood in Minkowski space. Nevertheless, in some simple cases, for example with the metric of homogeneous cosmologies, or of black holes of the Kerr and Schwarzschild type, the existence of a global timelike Killing vector allows a very plausible extension of the Minkowski space definition of particle. A number of exact results may then be proved. One of these results (ref. 1, and C. J. Isham and J. G. Taylor, personal communication) states that there is no creation of massless particles in the exterior region of a Schwarzschild black hole, which is the static end state reached as a result of spherically symmetric gravitational collapse. This result is not valid for a Kerr (rotating) black hole for essentially classical reasons².

Nevertheless, during the collapse of a spherically symmetric object, the metric near the surface will be changing rapidly on a time scale of $\tau \approx 10^{-5} (M/M_\odot)$ s, so that on general grounds the production of massless particles with energy of order \hbar/τ is expected. Many of these particles will escape from the surface of the object and reach distant observers. The energy removed in this way will slow up the collapse and may even prevent it completely, thus causing the collapsing object to 'explode' in a burst of radiation. Exact calculations of this explosion are not possible with present theory, which does not enable the back reaction of the particle creation on the metric (a quantum gravitational correction) to be evaluated. A rough idea may be obtained, however, by treating quantised fields in a given classical background metric, and estimating the amount of particle emission as the collapse proceeds.

If we assume that the production rate is more or less constant during the collapse, the surface will have a luminosity L_0 , but a distant observer will see a rapidly fading luminosity as the retreating surface falls towards the event horizon:

$$L(t) = L_0 \exp(-t/\tau) \quad (1)$$

Particle creation will only be important for objects with $M \ll M_\odot$, and it follows from equation (1) that in this case the radiation will be emitted in a flash with a duration of much less than a microsecond. The spectrum of radiation will be given (roughly) by the Fourier transform of equation (1), that is, proportional to $v/(v^2 + v_0^2)$ where $v_0 = (2\pi\tau)^{-1}$. This is peaked around $v = v_0$ so that a distant observer might approximate this spectrum with that of a blackbody with a Planck spectrum $v/[\exp(\hbar v/kT) - 1]$, corresponding to a temperature

$$T \approx \hbar v_0/k \approx 10^{-6} (M/M_\odot) \text{ K} \quad (2)$$

where k is Boltzmann's constant. This result has already been obtained in another way³.

Treating the collapsing object as a blackbody radiator with temperature T for the duration of the 'flash', the energy emitted per unit time will be about $A\sigma T^4$, where σ is Stefan's constant, and A the surface area, which will not be greatly different from that of a final black hole of the same mass, that is, $16\pi G^2 M^2/c^4$. Consequently, the total mass loss by the object as a result of massless particle emission will be in the region of

$$(16\pi M^2 \sigma) \times (\hbar v_0/k)^4 \approx \hbar c/MG \quad (3)$$

with a numerical coefficient on the right hand side involving only small powers of 10. This result may be written as a fractional mass loss

$$\Delta M/M \sim (\text{Compton wavelength of object/Planck length})^2.$$

Evidently, black hole explosions with $\Delta M \approx M$ will only occur for an object the Compton wavelength of which is comparable with the Planck length, that is for masses of about 10^{-4} g and densities of 10^{94} g cm⁻³. This is just the region where quantum gravitational effects also become important. Indeed, this result is not surprising because in order to reverse the collapse, the back reaction of the created particles on the dynamics of the collapse needs to be appreciable, and this can only occur when quantum corrections to gravity become important.

Equation (3) is confirmed by the detailed calculations of Zel'dovich and Starobinskii⁴, who have treated the problem of massless particle creation in the context of anisotropic cosmological models. They have verified the formula $\hbar/c^3 t^4$, originally deduced on dimensional grounds, for the energy density of created particles at a time t before collapse to a singularity. If a collapsing anisotropic single object is treated as a region of an anisotropic universe, this formula can be applied to obtain the total created particle energy-density over a time scale of order τ to obtain

$$(\hbar/c^3)\tau^{-4} \times (\text{volume object}) \sim \hbar c/MG$$

as before. Because of the additional tidal forces present in anisotropic collapse, spherically symmetric collapse would not be expected to lead to a greater particle production than this.

The conclusions of this simple treatment of particle production around collapsing objects seems to be in conflict with a recent result by Hawking⁵, who claims that black holes as large

as 10^6 gm would explode in massless radiation in about 0.1 s. Collapsing objects of this mass have an energy density only of the order 10^{-27} of that of quantum gravity fluctuations, so that this 'explosion' is more like a slow 'leak'. The e folding time for collapse is 10^{-29} s at this mass, so that Hawking's result implies that emission of massless particles continues to occur when the static limit of a Schwarzschild field is an exceedingly good approximation. It is not therefore clear what mechanism could be responsible for this emission (on the assumption of a reasonable definition of 'particle'). Any particles produced near the surface of a collapsing object will find it increasingly difficult to escape as the event horizon is approached. The last quantum ever able to reach a distant observer does so after an (asymptotic) time of only about 100 e folding times (10^{-27} s in this case)⁶. Hawking's result is, on the other hand, deduced on the assumption that the black-hole temperature given by equation (2) persists, unaffected by the collapse of the object, in the external asymptotic region, rather than fading out rapidly in accordance with equation (1). In our view it is a rather too literal interpretation of the concept of the 'temperature' of a black hole to apply it to emission processes in this way, and its use should be restricted to the discussion of absorption, as originally suggested by Bekenstein⁷.

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¹ Blum, B. S., thesis, Brandeis Univ. (1973).

² Unruh, W., *Proc. R. Soc.* (in the press).

³ Bardeen, J. M., Carter, B., and Hawking, S. W., *Commun. math. Phys.*, **31**, 161-170 (1973).

⁴ Zel'dovich, Ya. B., and Starobinskii, A. A., *Soviet Phys. JETP*, **34**, 1159-1412 (1972).

⁵ Hawking, S. W., *Nature*, **248**, 30 (1974).

⁶ Misner, C. W., Thorne, K. S., and Wheeler, J. A., *Gravitation*, 873 (Freeman, San Francisco, 1973).

⁷ Bekenstein, J., *Phys. Rev.*, **D7**, 2333-2346 (1973).

Further simultaneous hard X-ray and optical observations of Sco X-1

THE optical and X-ray emissions from Sco X-1 are thought to come from a hot plasma as small as or smaller than a white dwarf¹, but the energy source and the time variations of the radiation are not understood.

In our 1971 observations we found a positive correlation between the optical luminosity and the intensity of hard X rays at the optically enhanced phase of Sco X-1 (refs 2 and 3). Although the optical enhancement seemed to be a flare, a rather poor time resolution of the photographic observation prevented us from identifying it for certain. During a balloon flight on April 16, 1972, an X-ray enhancement was observed simultaneously with an optical flare, the latter being verified by photoelectric observation. With the result of another balloon flight on April 19, 1972, the hard X-ray spectra at several different B magnitudes are available for the study of the correlation between X-ray and optical emissions.

The instruments essentially the same as the one in the previous flight³, were launched on April 16 and 19, 1972, from Hyderabad, India. The balloon floated at the atmospheric depth of 2.9 to 3.0 g cm⁻² for about 4 h in each case. One of the counters in the April 16 flight malfunctioned, and its data were discarded.

Table 1 Results for Sco X-1 in selected periods

Date	Time (UT)	m_B	X-ray intensity at 30 keV ($\text{keV cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$)	Apparent* temperature	τ_{es}	Mass (10^{19}g)
May 1, 1971	2009–2018	12.6	0.060 ± 0.023	4.7 ± 0.7	8.6	1.7
May 1, 1971	2057–2106	12.4	0.12 ± 0.01	5.1 ± 0.3	9.9	2.0
April 16, 1972	2217–2225	12.5	0.12 ± 0.01	5.1 ± 0.4	10.0	1.8
April 16, 1972	2241–2246	12.3	0.20 ± 0.02	5.3 ± 0.5	10.7	2.3
April 19, 1972	2046–2117	12.7	0.035 ± 0.009	4.0 ± 0.7	11.0	1.3

* The plasma temperatures for the first four cases are obtained to be 3.6 keV, whereas 3.0 keV for the last case.

Simultaneously with the balloon flight, Sco X-1 was observed photographically with a 12-inch reflecting astrograph at the balloon launching station at Hyderabad and with an 8-inch astrograph at Nizamiah Observatory, and photoelectrically with a 48-inch reflector at Rangapur Observatory. All these optical data were consistent with each other in overlapping periods. Absolute X-ray intensities in three energy ranges, 17.3 to 22.3 keV, 22.3 to 27.1 keV and 27.1 to 31.8 keV from Sco X-1 on April 16 are shown in Fig. 1, with B magnitudes. They vary in parallel to each other. The optical luminosity increased by about 0.2 mag (around 2245 UT), while the X-ray intensity also increased by a factor of 2. In Fig. 1 we also show the apparent temperature that is derived by fitting the

X-ray spectrum in the energy range 20 to 40 keV to the exponential spectrum. In the previous papers^{2,3} the apparent temperature was defined by fitting the observed X-ray spectrum to the thermal bremsstrahlung spectrum with the energy-dependent Gaunt factor. The apparent temperature found here is lower than that obtained with the method used before.

The hard X-ray spectra obtained in selected periods of the two flights are compared with those of the previous flight on May 1, 1971, in Fig. 2. The B magnitudes, X-ray intensities and apparent temperatures on the assumption of the exponential spectrum in these selected periods are listed in Table 1. A positive correlation between X-ray and optical emissions is clearly observed in the optically bright phase, whereas the apparent temperature shows only a weak positive correlation, in agreement with our previous result^{2,3}. On the other hand, rather strong positive correlations have been reported for lower X-ray energies⁴⁻⁶.

The observed features can be interpreted in terms of thermal bremsstrahlung from a hot, semi-opaque plasma. Thermal bremsstrahlung X rays are subject to Compton scattering by hot electrons. So the spectrum of X rays is considerably modified in the energy range 1 to 10 keV (refs 7–9), whereas only minor modification is predicted in the energy range above 20 keV, as is revealed by a theory of photon transport (unpublished work by J. N.). In the optical region free-free absorption suppresses the intensity of radiation emitted. Essential features of the spectrum are dictated chiefly by the optical depth for electron scattering τ_{es} , and also to some extent by the electron density and the shape of the plasma. For comparison with our experimental data, we have drawn theoretical X-ray spectra for several optical depths (S. H., M. M. and I. K., unpublished). The calculated spectra are in essential agreement with the observed ones in non-flaring and flaring periods, respectively, if the total mass of plasma particles is increased by about 40%. In all cases the plasma temperatures are found to lie in the range 3.5 to 3.8 keV.

Properties of Sco X-1 obtained by the simultaneous X-ray and optical observations are summarised as follows:

(1) The X-ray intensity in the energy range 20 to 40 keV shows a positive correlation with optical luminosity in the bright phase of Sco X-1. In general the amplitude of X-ray variability is much larger than that of optical luminosity.

(2) The apparent temperature of Sco X-1 derived from the spectrum in the energy range 20 to 40 keV does not appreciably change in comparison with considerable spectral variations in the energy range 1 to 10 keV. The plasma temperature does neither appreciably change during our observations.

(3) Flare activity is associated with an increase of the total mass of a hot plasma.

It should, however, be noted that on a few other occasions less steep spectra and higher intensities have been observed in optically less bright periods. Among these cases properties of Sco X-1 in the period of $m_B > 12.9$ may be different from those we are concerned with. An exceptional result was found by Miyamoto *et al.*¹⁰ who observed an intensity twice as high as ours in the flaring period and a less steep spectrum for $m_B = 12.6$. According to Uhuru observations, the correlation between X-ray and optical emissions in some rare periods

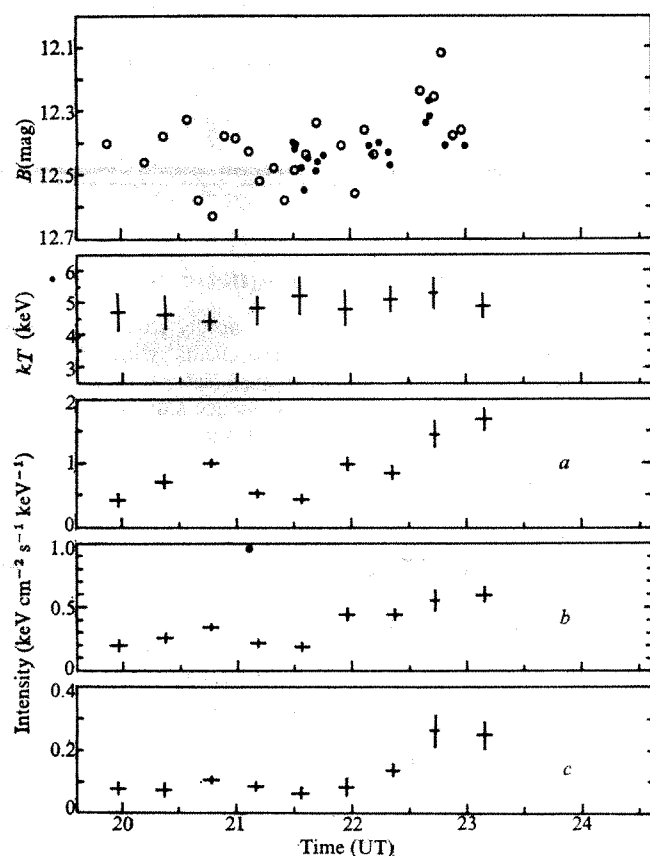


Fig. 1 Time variations of X-ray and optical emissions observed on April 16, 1972. B magnitudes indicated by open circles and black dots were obtained by the photographic observation with a 12-inch reflector and by the photoelectric observation with a 48-inch reflector. The apparent temperature kT is derived by fitting the observed spectrum to the simple exponential form. The X-ray intensities in three energy ranges are obtained by subtracting the background counting rates and correcting for the effective area of the counter and the atmospheric absorption. a, 17.3 to 22.3 keV; b, 22.3 to 27.1 keV; c, 27.1 to 31.8 keV.

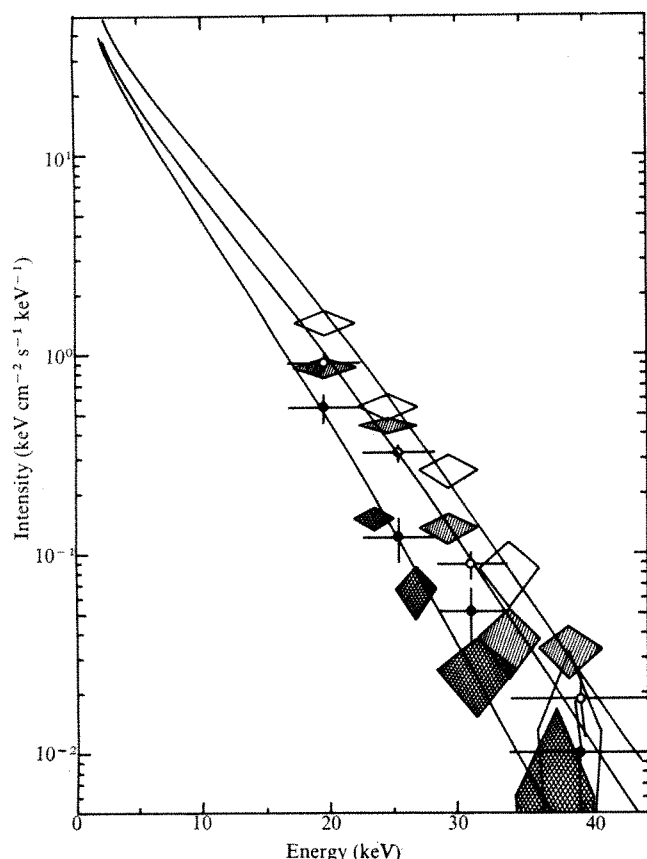


Fig. 2 Hard X-ray spectra observed in five selected periods. For comparison theoretical spectra are given for three periods in the 1972 flights with the values of plasma parameters given in Table 1. May 1, 1971: ●, 2009 to 2018 UT, $B=12.6$; ○, 2057 to 2106 UT, $B=12.4$. April 16, 1972: open diamond, 2217 to 2225 UT, $B=12.5$; hatched diamond, 2241 to 2246 UT, $B=12.3$. April 19, 1972: cross-hatched diamond, 2046 to 2117 UT, $B=12.7$.

is different from that observed in most periods (H. Gursky, unpublished). Thus further comprehensive research will be demanded in order to construct a convincing model for any case of Sco X-1.

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- ¹ Oda, M., and Matsuoka, M., *Progr. Elementary Particle Cosmic Ray Phys.*, **10**, 303 (1971).
- ² Matsuoka M., Fujii, M., Miyamoto, S., Nishimura, J., Oda, M., Ogawara, Y., Ohta, S., Hayakawa, S., Kasahara, I., Makino, F., Tanaka, Y., Agrawal, P. C., Sreekantan, B. V., Hatanaka, Y., and Sreedhar Rao, S., *Nature phys. Sci.*, **236**, 53 (1972).
- ³ Matsuoka, M., Fujii, M., Miyamoto, S., Nishimura, J., Oda, M., Ogawara, Y., Hayakawa, S., Kasahara, I., Makino, F., Tanaka, Y., Agrawal, P. C., and Sreekantan, B. V., *Astrophys. Space Sci.*, **18**, 472 (1972).
- ⁴ Hudson, H. S., Peterson, L. E., and Schwartz, D. A., *Astrophys. J. Lett.*, **159**, L51 (1970).
- ⁵ Evans, W. D., Belian, R. D., Strong, I. B., and Kunkel, W. E., *Astrophys. J. Lett.*, **162**, L115 (1970).
- ⁶ Canizares, C. R., Clark, G. W., Lewin, W. H. G., Schnopper, H. W., Sprott, G. F., Hjellming, R. M., and Wade, C. M., *Astrophys. J. Lett.*, **179**, L1 (1973).
- ⁷ Loh, E. D., and Garmire, G. P., *Astrophys. J.*, **166**, 301 (1971).
- ⁸ Felten, J. E., and Rees, M. J., *Astro. Astrophys.*, **17**, 226 (1972).
- ⁹ Illarionov, A. F., and Syunyaev, R. A., *Astr. J. (Moscow)*, **46**, 58 (1972).
- ¹⁰ Miyamoto, S., Ogawara, Y., Matsuoka, M., and Oda, M., *Space Research*, **13**, 851 (1973).

Origin of neutron star magnetic fields

PULSARS are generally thought to be rapidly rotating neutron stars which have poloidal magnetic fields with intensities consistently in the range 10^{12} – 10^{13} gauss (ref. 1). Most of them are believed to evolve from main sequence stars with original masses of >1.4 – $8 M_{\odot}$. Evolutionary computations^{2,3} indicate that stars in this mass range develop a dense core of carbon and oxygen in which carbon begins to burn when the temperature reaches about 3×10^8 K and the density about 3×10^9 g cm⁻³. The rapid production of energy during this carbon-burning stage drives almost the entire core into convection for an extended time of the order of 10^{11} s (ref. 4), with convective velocities of the order of 10^4 cm s⁻¹ (ref. 5), provided that the rate of cooling through the convectively driven 'Urca process' is sufficiently high to stabilise carbon burning and to prevent the core from detonating and dispersing. If this sequence is a reasonable approximation to reality then the existence of neutron star pulsars suggests that carbon burning is, in fact, stabilised until electron capture, or some other process, causes part of the core to collapse to a neutron star²⁻⁷.

The origin of pulsar magnetic fields with nearly uniform intensities¹ is a matter of some interest. If a main sequence star with dimensions of $\sim 10^{11}$ cm contains a poloidal field, of about 100 gauss in its interior, then conserved magnetic flux during collapse to neutron star dimensions of $\sim 10^6$ cm will produce a magnetic field of the order of 10^{12} gauss. This line of argument is usually relied on in discussions of the origin of the magnetic fields of pulsars. We have argued (not yet published) that turbulent transport and mixing of the magnetic field, during the convective carbon-burning stage, will dominate its behaviour in the core. In this case the simplest notions of conserved magnetic flux do not apply, the accepted line of

reasoning fails and the final magnetic field of the collapsed neutron star is not a unique descendant of the field which the star possessed while on the main sequence.

Also in this connection, Ruderman and Sutherland⁸ have suggested that turbulent convection during the carbon-burning period will produce an equipartition magnetic field with an energy density equal to the kinetic energy density of the convecting fluid. The final state reached by a turbulent, conducting fluid and magnetic field has not, however, been resolved, so this suggestion must be considered speculative. Although we feel that such speculation is along the right lines, it contains a difficulty which, to us, seems significant. According to Ruderman and Sutherland⁸ an initial seed magnetic field, B_s , of spatial scale R , is rigorously frozen into the fluid and stretched and tangled by the turbulence until it attains an equilibrium intensity, B_{eq} , such that the energy density of the magnetic field is equal to the energy density of the turbulence. A convective core with radius $R \sim 10^8$ cm, and with an average density of 10^9 g cm⁻³, produces an equipartition magnetic field with $B_{eq} \sim 10^9$ gauss. It follows, however, that in the absence of some specially contrived fluid velocity which is a function of the magnetic field, the final field will have a spatial scale, δ , given by

$$\delta \sim R \sqrt{(B_s/B_{eq})}$$

Using the discussed values, $\delta \sim 3 \times 10^3 \sqrt{B_s}$ cm; even with a seed field as large as 10^5 gauss, $\delta \sim 10^6$ cm. Collapse through two orders of magnitude in radius, to the dimensions of a neutron star, then results in the neutron star magnetic field having a scale of $\sim 10^4$ cm and an intensity of $\sim 10^{13}$ gauss. Therefore, the surface magnetic field of a neutron star consists of some 10^4 patches of magnetic field with intensities of 10^{13} gauss, with randomly varied polarities. Each patch has a magnetic flux of $\sim 10^{21}$ gauss cm². The mean net flux through a hemisphere of the neutron star is then expected to be $\sim \sqrt{(10^4) \times 10^{21}}$, which is $\sim 10^{23}$ gauss cm². This is about an order of magnitude below what is inferred to be characteristic of pulsars¹. Furthermore, this process of magnetic field amplification leads to a large statistical spread in the net magnetic moments of neutron stars. The uniformity of neutron star magnetic moments, as inferred from the observed properties of pulsars, seems to indicate that the magnetic fields are produced with large spatial scales as well as with uniform intensities.

We suggest that convection during the carbon-burning stage (before collapse to a neutron star) in a massive, rapidly and differentially rotating, degenerate stellar core, leads to the production of a large scale magnetic field within the core itself, through the action of a hydromagnetic dynamo. Solutions of the hydromagnetic dynamo equations⁹⁻¹¹ use a dimensionless number, N , known as the dynamo number^{12,13}:

$$N = \gamma \Gamma R^3 / \eta^2$$

where γ is the rate of nonuniform rotation, Γ is essentially the cyclonic component of the convection in a rotating body, R is the spatial scale of the dynamo region and η is the magnetic diffusivity. We have calculated (not yet published) the conditions which will produce a quadrupole like magnetic field confined to the convecting core of a star. A magnetic field will be generated if the fluid motions are such that N falls within the range of $\sim -3,400$ to $\sim -12,000$. In a turbulently convecting core, transport and diffusion of the magnetic field will be dominated by turbulent mixing, and so we use the magnetic diffusivity, $\eta \sim 0.1 \nu_t \lambda$, where ν_t is the turbulent fluid velocity and λ is the scale of the large turbulent eddies^{14,15} (which we take to be about a pressure scale height). Setting ν_t equal to the average differential fluid velocity¹⁵ in the convecting and differentially rotating core, a magnetic field is produced when the convection extends over 5–10 pressure scale heights, as is the case in the carbon-burning core. The e-folding growth time of the magnetic field intensity is calculated to be of the order of days ($\sim 10^5$ s) in the carbon-burning core. This is short compared with the time over which carbon burning occurs⁴, so the field has sufficient time during this stage of stellar evolution to reach a saturation value, which will be determined by the dynamics of the fluid flow. We have estimated the saturation value of the

mean magnetic field throughout the rapidly rotating degenerate core by equating the Coriolis force on the convection, to the magnetic field stress. We find the mean of the poloidal and toroidal components to be $\leq 10^9$ – 10^{10} gauss. This estimate should only be taken as an approximation. It has been calculated by neglecting density variations throughout the dynamo region and by using only the crudest hydrodynamic considerations. The final state of the magnetic field will depend on quantities not yet calculated, such as the fraction of the core which actually collapses to a neutron star. With this quantitative caution in mind, however, we suggest that such a dynamo magnetic field—with a spatial scale which is that of the core itself, and a poloidal intensity of the order of 10^{12} – 10^{13} gauss after collapse to neutron star dimensions—is the progenitor of pulsar magnetic fields.

We consider elsewhere¹⁶, further dynamical consequences of this hypothesis.

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- ¹ Ruderman, M., *A. Rev. Astron. Astrophys.*, **10**, 427 (1972).
- ² Rose, W. K., *Astrophys. J.*, **155**, 491 (1969).
- ³ Paczynski, B., *Acta. astr.*, **21**, 271 (1970).
- ⁴ Couch, R. G., and Arnett, W. D., *Astrophys. J. Lett.*, **180**, L101 (1973).
- ⁵ Paczynski, B., *Astrophys. Lett.*, **11**, 53 (1972).
- ⁶ Arnett, W. D., *Astrophys. space Sci.*, **5**, 180 (1969).
- ⁷ Bruenn, S. W., *Astrophys. J. Lett.*, **183**, L125 (1973).
- ⁸ Ruderman, M. A., and Sutherland, P. G., *Nature phys. Sci.*, **246**, 93 (1973).
- ⁹ Parker, E. N., *Astrophys. J.*, **122**, 293 (1955); and **162**, 665 (1970).
- ¹⁰ Braginskii, S. I., *Soviet Phys. JETP*, **20**, 726, 1462 (1964).
- ¹¹ Steenbeck, M., Krause, F., and Rädler, K.-H., *Z. N. Naturf.*, **21a**, 369 (1966).
- ¹² Parker, E. N., *Astrophys. J.*, **163**, 255 (1971).
- ¹³ Levy, E. H., *Astrophys. J.*, **171**, 621 (1972).
- ¹⁴ Radler, K.-H., *Z. Naturf.*, **23a**, 1851 (1968).
- ¹⁵ Parker, E. N., *Astrophys. J.*, **163**, 279 (1971).
- ¹⁶ Levy, E. H. and Rose, W. K., *Astrophys. J.* (in the press).

Lewisian age for the Scardroy Mass

Rocks of Lewisian aspect have long been recognised east of the Moine Thrust in Sutherland, Ross and Inverness. They were correlated with the Lewisian west of the Moine thrust on petrological grounds¹ and were originally considered to be unconformably overlain by Moine rocks². Sutton and Watson^{3,4} at first questioned this correlation, but later accepted the view that rocks derived from the Lewisian basement are exposed at

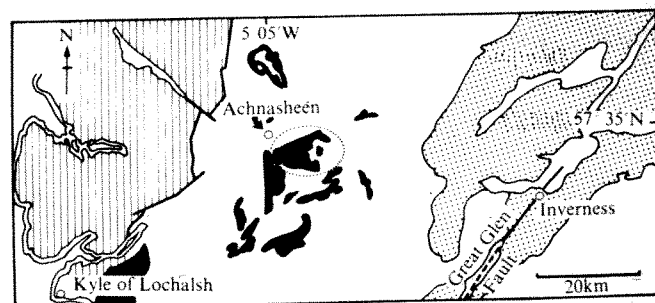


Fig. 1 Sketch map showing Lewisian 'inliers' (black) originally recognised by the Geological Survey within the area of Moine rocks (white). The Scardroy area, south-east of Achnasheen, is enclosed by a circle of dots. Vertical lines, Lewisian and younger rocks west of the Moine thrust; dots, Old Red Sandstone.

Table 1 Rb-Sr whole rock analytical data

Sample no.	Description* and locality	$^{87}\text{Rb}/^{86}\text{Sr}\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}\dagger$	Rb (p.p.m.) \ddagger	Sr (p.p.m.) \ddagger
MT-1	Leucocratic, strongly banded, fine-grained gneiss. Pl, Qz, Kf, Bi, Ap. North side of Loch Beannacharain: grid ref. NH229523.	0.859 ± 0.012	0.7383 ± 0.0002	84	285
MT-5	Mesocratic, strongly banded, medium-grained gneiss. Pl, Qz, Ep, Bi, Kf, ore. Ap. North side of Loch Beannacharain: grid ref. NH233520.	0.191 ± 0.006	0.7110 ± 0.0003	29	436
MT-9	Mesocratic, banded, medium-grained gneiss. Pl, Qz, Bi, Ep, Zo, Kf, Sph, Ap. Between Scardroy Lodge and Carn Mhartuin: grid ref. NH203526.	0.461 ± 0.006	0.7222 ± 0.0003	75	472
MT-10	Mesocratic strongly banded, fine-grained gneiss. Pl, Qz, Bi, Hb, Kf, Ep. North of Loch Beannacharain: grid ref. NH220520.	0.127 ± 0.006	0.7090 ± 0.004	19	427
MT-12	Mesocratic, weakly banded, coarse-grained, massive gneiss. Pl, Bi, Qz, Ep, Hb, Ap. North side of Loch Beannacharain: grid ref. NH223519.	0.055 ± 0.002	0.7069 ± 0.0005	19	988

* Kf, potash feldspar; Pl, plagioclase; Qz, quartz; Bi, biotite; Hb, hornblende; Ep, epidote; Zo, zoisite; Sph, sphene; Ap, apatite; ore, iron oxide minerals.

\dagger Errors quoted at 95% confidence level. $^{87}\text{Sr}/^{86}\text{Sr}$ normalised to $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$.

\ddagger Semiquantitative values to $\pm 10\%$.

Grid references refer to the 1-inch Ordnance Survey Map of Great Britain, Sheet 27, Strathpeffer.

Table 2 K-Ar analytical data

Sample no.	Description and locality	Mineral	K(%) \ast	Radiogenic ^{40}Ar ($\text{cm}^3 \text{g}^{-1} \text{STP} \times 10^{-4}$)	Radiogenic ^{40}Ar Total ^{40}Ar (%)	Age (Myr) \dagger
MT-2c	Melanocratic, banded, medium-grained, amphibolitic gneiss with feldspathic patches. Hb, Pl, Qz. North-east side of Meall Dubh: grid ref. NH232530.	Hornblende	0.61, 0.61, 0.62	0.1510	89.7	535 ± 26
MT-3	Melanocratic, massive, coarse-grained, patchy, amphibolitic gneiss. Hb, Pl, Gt, Bi, Qz. North side of Meall Dubh: Grid ref. NH227532.	Hornblende	0.74, 0.74, 0.74	0.1532	94.6	459 ± 22
MT-11a	Melanocratic, massive, coarse-grained, amphibolitic gneiss. Hb, Bi, Pl, Gt. North side of Loch Beannacharain: grid ref. NH222520.	Hornblende Biotite	0.55, 0.54, 0.55 7.26, 7.27, 7.26	0.1040 1.361	83.7 96.9	426 ± 20 420 ± 20
MT-11b	Massive, black, coarse-grained hornblende-biotite rock. Hb, Bi. Grid ref. NH222520.	Biotite	7.47, 7.48, 7.40	1.422	98.2	427 ± 10

\ast The mean of the individual K determinations is used for the age calculations.

\dagger Errors quoted at 95% confidence level.

Abbreviations as in Table 1; Gt, garnet. Grid references as in Table 1.

Scardroy and elsewhere in central Ross-shire (Fig. 1), and take the form of sheets tectonically intercalated in the Moine succession⁵.

In order to test this hypothesis, Rb-Sr whole rock analyses were carried out on a suite of typical gneisses from the Scardroy sheet (Table 1). $^{87}\text{Sr}/^{86}\text{Sr}$ ratios were obtained on a 12-inch mass spectrometer, and Rb/Sr ratios were accurately determined by X-ray fluorescence⁶. Rb and Sr contents were estimated semiquantitatively only. The decay constant of ^{87}Rb was taken as $1.39 \times 10^{-11} \text{yr}^{-1}$. K-Ar dates of separated minerals are presented in Table 2. K was determined by flame photometry and ^{40}Ar by isotope dilution using an AEI MS-10 mass spectrometer. The decay constants for ^{40}K are $\lambda_{\beta} = 4.72 \times 10^{-10} \text{yr}^{-1}$ and $\lambda_{\epsilon} = 0.584 \times 10^{-10} \text{yr}^{-1}$. Full analytical details have been given elsewhere^{6,7}.

The Rb-Sr whole rock data yield an isochron age of $2,810 \pm$

120 Myr, with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7039 ± 0.0007 (Fig. 2). The scatter about the least-squares fitted line is not accounted for by analytical error alone and an increase of errors by a factor of two is needed to obtain a valid fit.

The data prove beyond any doubt that the rocks of the Scardroy sheet are, indeed, Lewisian. The actual age obtained is well within the age range for early Scourian rocks west of the Moine thrust, dated by Rb/Sr, U/Pb and Pb/Pb methods⁸⁻¹⁰. This age has been interpreted as the time of Scourian metamorphism. The rather low initial $^{87}\text{Sr}/^{86}\text{Sr}$ value is in general accord with much published (and unpublished) isotopic work^{8,10,11} suggesting that Scourian gneisses are not produced by remobilisation of substantially older (> about 200 Myr) sialic rocks with average continental Rb/Sr ratios.

The K-Ar dates for hornblendes and biotites (Table 2) are mostly within the typical range of mineral dates found in the

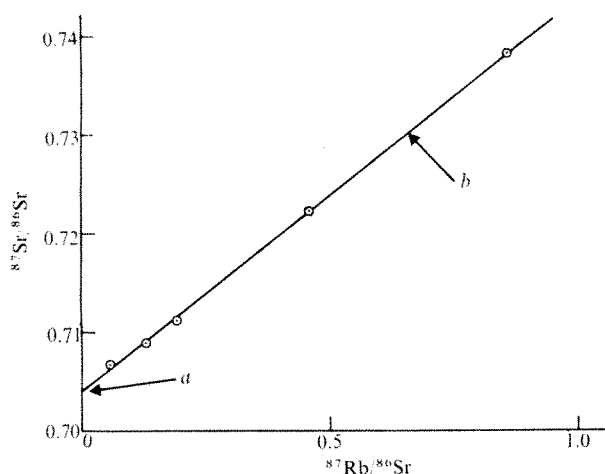


Fig. 2 Rb-Sr whole rock isochron plot for analysed samples. Errors are at 95% confidence level: a, 0.7039 ± 0.0004 ; b, $2,810 \pm 120$ Myr.

British Caledonides^{12,13}. Sample MT-2c yields a date of 535 Myr and may not have been completely degassed during the Caledonian metamorphism.

In the western Glenelg Lewisian inlier immediately to the east of the Moine thrust at Loch Duich, where Caledonian effects are not nearly as intense as at Scardroy, most hornblende and biotites still yield K-Ar dates of approximately 2,200–1,600 Myr, whereas slightly further east in the eastern Glenelg Lewisian inlier at Loch Duich biotites and hornblendes already yield typical Caledonian dates (Moorbath, unpublished work).

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- ¹ Flett, J. S., *Mem. geol. Surv. Summ. Prog.* (1905).
- ² Peach, B. N., *et al.*, *Mem. geol. Surv. UK* (1913).
- ³ Sutton, J., and Watson, J., *Q. Jl geol. Soc.*, **108**, 99–126 (1953).
- ⁴ Sutton, J., and Watson, J., *Q. Jl geol. Soc.*, **110**, 21–54 (1955).
- ⁵ Sutton, J., and Watson, J., *Geol. Mag.*, **99**, 527–541 (1962).
- ⁶ Pankhurst, R. J., and O'Nions, R. K., *Chem. Geol.*, **12**, 127–136 (1973).
- ⁷ Moorbath, S., and Park, R. G., *Scott. J. Geol.*, **8**, 51–74 (1971).
- ⁸ Moorbath, S., Welke, H., and Gale, N. H., *Earth planet. Sci. Lett.*, **6**, 245–256 (1969).
- ⁹ Pidgeon, R. T., and Bowes, D. R., *Geol. Mag.*, **109**, 247–258 (1972).
- ¹⁰ Lyon, T. D. B., Pidgeon, R. T., Bowes, D. R., and Hopgood, A. M., *J. geol. Soc.*, **129**, 389–404 (1973).
- ¹¹ Evans, C. R., *Nature*, **207**, 54–56 (1965).
- ¹² Dewey, J. F., and Pankhurst, R. J., *Trans. R. Soc. Edinb.*, **68**, 361–389 (1970).
- ¹³ Dunning, F. W., *Scott. J. Geol.*, **8**, 179–192 (1972).

Solid tides recorded with 1 m interval mechanical strainmeter

A TENSIONED-QUARTZ strainmeter, 1 m in length has successfully recorded solid tidal strain variations in the Cooney Observatory. The designed is based on the 10-m quartz catenary strainmeter already in use at Cooney¹ Vitreosil quartz rods (2-mm diameter) linked with 4-mm diameter

Vitreosil hooks comprise the standard. This is tensioned by a brass beam-balance mounted to the hornfels by two $\frac{3}{8}$ inch expanding bolts. A single $\frac{3}{8}$ inch expanding bolt supports the other end of the quartz standard. Mounted within the fixed body of the beam balance is the coil housing of a Tesa GT10 inductive displacement transducer. The sensor armature is connected to the balance arm, (rotating on flexure strips), entering the transducer coil without contact (see ref. 2 for details).

A low impedance output (sensitivity $500 \text{ mV } \mu\text{m}^{-1}$) is provided from the Tesa electronic unit by a buffer amplifier. A Multiscript Model 3 chopper-bar recorder was used in these tests, sensitivity corresponding to 200 nm full scale deflection. The trace can be resolved visually to 2 nm (4% of maximum tidal amplitude). Zeroing is achieved with the visual readout and the electrical output facility provided in the Tesa unit.

The chopper-bar recorder was used for long period signals, sampling at 6 s intervals. Short period records of noise level were taken on an analogue pen recorder. (The use of a shorter interval of quartz results in a considerably higher resonant frequency than for the 10-m instrument³.)

Installation involves drilling three holes in the rock and setting the expanding bolts which support the covers and

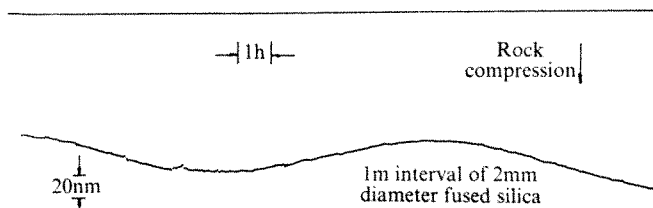


Fig. 1 Section of actual record obtained with the 1-m tensioned-quartz instrument.

instrument ends. The instrument is then mounted and set approximately. Fine adjustment of the length finally is made by varying the balance mass to obtain a zero reading on the electronic unit. The task takes no more than an hour; no special skills are needed.

We selected a site to make best use of available conditions. The Lower Cooney tunnel, (other strainmeters reported are placed in an upper complex) is 316 m below the topographical surface. A 12-m long, narrow chamber, situated 300 m into and parallel with this drive has been sealed off at both ends, entry being through a sealed door at one end. In addition, a 10 cm thick expanded polystyrene box surrounds the entire meter. Temperature variations of 4 mK occur within the insulated cover with a noticeable regularity of 20 to 30 min cycles. (Measurements were made with a thermistor bridge, resolution of $100 \mu\text{K}$.)

Figure 1 shows part of the record obtained with the 1 m interval strainmeter, taken 6 d after completion of the installation. The drift over this interval is 2 parts in 10^9 per hour. A drift curve for the same meter, installed on the thermally controlled measuring base⁴, is given in Fig. 2, showing that the wall-mounted unit is stabilising in an expected manner.

The 10 Hz bandwidth noise level is no greater than 0.4 nm peak-to-peak, giving a signal to noise ratio of better than 40 dB when the amplitude of the tide is large.

The amplitude of the tide rises to about 50 nm. This is greater than expected at this site for a 1 m interval. Records from 10 m interval instruments in the Upper Cooney complex indicate that a maximum strain of 3 parts in 10^8 is normal. Comparison between this 1 m instrument and a 10 m quartz catenary are also situated in the same chamber, will enable us to investigate this departure in due course.

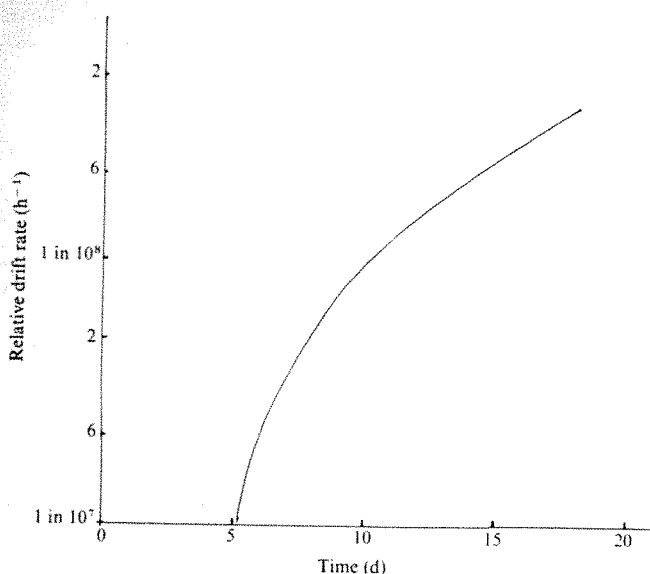


Fig. 2 Drift curve of 1-m instrument mounted in test base³ (relative to stabilised steel).

Also under development, in the same chamber, is a 1-m interval floor-mounted strainmeter that is coupled to the Earth by brass plates resting on a thin bed of sand. One plate carries a modified Tesa GT10 probe which has its armature supported by two thin phosphor-bronze diaphragms. Clamped onto the other plate, in a horizontal attitude, is a 1 m length of Vitreosil tube (outside diameter 16 mm). The tube is connected to the transducer by a quartz wobble-pin, completing the measurement loop. So far we have been unable to achieve satisfactory continuous records with this instrument because of excessive instability. But because of the advantages of plates on sand in terms of rapid mount stabilities⁴ and ease of installation, we will continue to investigate this design. Separate studies of mounts⁵ and experience with a 10-m quartz-tube instrument using bolted rock joints¹ suggests the method should perform satisfactorily.

Initial drift being a function of mounting rather than the standard, has an absolute value independent of gauge length. A 1-m instrument will, therefore, require a longer settling time than a 10-m unit.

Future research should be aimed at obtaining a closer thermal coupling between the instrument and rock and that it be located in deep undisturbed rock. Records obtained from a 1-m corehole meter (possibly using a rock core as a standard) may bear a closer relationship to the phenomena being studied than any records obtained to date.

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¹ Sydenham, P. H., *Geophys. J. R. astr. Soc.* (in the press).

² Sydenham, P. H., *Geophys. J. R. astr. Soc.*, **29**, 219 (1972).

³ Sydenham, P. H., *Meas. and Control Lond.*, **2**, 572 (1972).

⁴ Sydenham, P. H., *J. Phys. E. Sci. Instru.*, **6**, 572 (1972).

⁵ Jeffery, G. J., and Sydenham, P. H., *Geophys. J. R. astr. Soc.*, **33**, 185-193 (1973).

Obsidian hydration profile measurements using a nuclear reaction technique

AMBIENT water diffuses into the exposed surfaces of obsidian, forming a hydration layer which increases in thickness with time to a maximum depth of 20-40 μm (ref. 1), this layer being the basic foundation of obsidian dating^{2,3}.

We have used the resonance at a ^{19}F energy of 16.45 MeV (0.83 MeV centre-of-mass energy) (ref. 4) in the nuclear reaction $^1\text{H}(^{19}\text{F}, \alpha\gamma)^{18}\text{O}$ to measure directly the hydration profiles of obsidian samples. This technique has already been used to measure the hydrogen concentration profiles in lunar samples and other solids to depths up to 0.4 μm with a resolution of 0.02 μm (refs 5 and 6). A second strong resonance at 17.64 MeV is encountered in extending these measurements to greater depths⁴, but its contribution can be unfolded from the data.

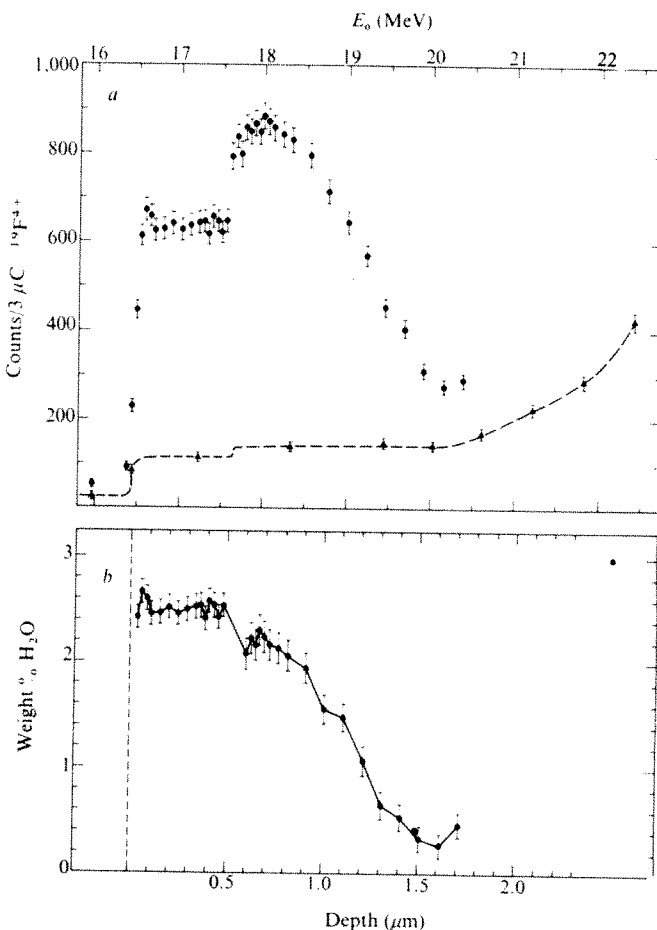


Fig. 1 Raw data for sample 5 (●) and for an unhydrated (0.3% H_2O) (▲) sample. Error bars indicate uncertainties arising from counting statistics. The background problem for $E_0 > 20$ MeV is illustrated by the profile for the unhydrated sample; a corresponding increase in counting rate occurs for all samples at $E_0 > 20$ MeV. To minimise this background, the interpolated data for the unhydrated sample were subtracted point-by-point from the data for sample 5. Then, for data points at energies $E_0 > E_R = 17.64$ MeV, the effects of the second resonance at E_{R2} were unfolded in accordance with equation (3) in the text: K_2 multiplied by the number of counts at $E_0 - \Delta E$ was subtracted from $Y_1(E_0)$ to obtain the unfolded hydration profile (b). The error bars in b indicate the propagation of errors contributed by uncertainties arising from counting statistics in the data for sample 5 and the unhydrated sample, and the unfolding of the second resonance. Using a calibration sample of known H content, the counting rate was converted to a weight % H_2O scale: using tabulated stopping powers⁷, the E_0 scale was converted to a depth scale. The segment of maximum H_2O gradient (for b, for example, at $1.2 \pm 0.2 \mu\text{m}$) was interpreted as corresponding to the thickness of the optically observed hydration band.

Table 1 Summary of the quantitative data derived from the measured hydration profiles (see Fig. 1)

Sample no.	Source, identification	% H ₂ O by weight	Thickness of hydration band (μm) ¹ H(¹⁹ F, αγ) ¹⁶ O measured profile	Thin-section technique
1	Big Obsidian Flow, Newberry Craters, Oregon. Artificially hydrated at 75° C for 1 d	2.11 ± 0.14	0.089 ± 0.020	—
2	Big Obsidian Flow, Newberry Craters, Oregon. Artificially hydrated at 75° C for 2 d	2.08 ± 0.13	0.113 ± 0.020	—
3	Big Obsidian Flow, Newberry Craters, Oregon. Artificially hydrated at 75° C for 4 d	2.45 ± 0.14	0.14 ± 0.02	—
4	Big Glass Mountain, Medicine Lake Highlands, California. Collected August 1963—fresh surface exposed then	2.16 ± 0.12	0.19 ± 0.02	0.39*
5 (Fig. 1)	Big Obsidian Flow, Newberry Craters, Oregon. Exposed surface	2.5 ± 0.1	1.2 ± 0.2	1.2 ± 0.2
6	Amapa, Nayarit, Mexico. Artefact	1.9 ± 0.5	1.55 ± 0.5	1.4 ± 0.2
7	Amapa, Nayarit, Mexico. Artefact	2.15 ± 0.15 1.56 ± 0.05	0.9 ± 0.2 1.77 ± 0.27	1.7 ± 0.2
8	Amapa, Nayarit, Mexico. Artefact	2.3 ± 0.1	1.8 ± 0.3	1.8 ± 0.2
9	Amapa, Nayarit, Mexico. Artefact	2.65 ± 0.10 2.1 ± 0.1	0.53 ± 0.1 1.82 ± 0.25	2.1 ± 0.2
10	Borax Lake, California. Chipping waste	2.8 ± 0.1	1.8 ± 0.5	0.7 ± 0.5†

* Thickness of hydration layer was not measured directly using the thin-section technique but was estimated from the known time of exposure of the fresh surface.

† Large uncertainty because of poor sample preparation.

The '% H₂O by weight' was assigned to the plateau value of the H₂O concentration profile for the sample, with uncertainties determined by finding the highest and lowest horizontal lines that could be construed to comprise the plateau. The thicknesses of the hydration bands given by the ¹H(¹⁹F, αγ)¹⁶O measured profile were obtained by taking the midpoint of the segment of maximum H₂O gradient on the sample profiles. The uncertainty is determined by the depths corresponding to the endpoints of the segment of maximum concentration gradient. The two values of H₂O content and hydration-layer thickness, as measured by the ¹H(¹⁹F, αγ)¹⁶O technique, for samples 7 and 9 may reflect the existence of two plateaux in their profiles. A possible systematic error introduced by our calculated stopping powers into the measurements of both hydration-band thickness and H₂O content has not been taken into consideration; the calculated stopping power has roughly a 5% uncertainty. Thicknesses of hydration bands, optically determined by microscopic examination of thin sections from the same obsidian samples, are given for comparison.

If a monoenergetic beam of ¹⁹F⁺ ions with energy E_0 slightly above the resonance energy E_R is focused on an obsidian sample, the ions gradually slow down because of electronic collisions (the rate of energy loss being characterised by the stopping power dE/dx of the obsidian) until at a depth X_R the resonance energy E_R is reached. At resonance, the nuclear reaction—in particular the γ-ray production—proceeds at a rate proportional to the hydrogen concentration in a thin layer at X_R . Assuming that all hydrogen present in the hydration layer is incorporated as H₂O, the reaction therefore proceeds at a rate proportional to the water concentration at X_R .

As the beam penetrates still deeper into the sample than X_R , the ¹⁹F energy decreases below the resonance energy. Because the nonresonant cross section on either side of the resonance energy is negligible compared with the cross section at resonance, the hydrogen outside the layer at X_R contributes negligibly to the total reaction yield. Since the stopping power dE/dx is very nearly constant in the relevant range of ¹⁹F energies (16 to 22 MeV) (ref. 7), the depth X_R is related to the incident beam energy E_0 by

$$X_R = (E_0 - E_R)/(-dE/dx) \quad (1)$$

(dE/dx is a negative quantity). Thus, measuring the γ-ray production rate as E_0 is varied gives a direct indication of water concentration as a function of depth in the target.

Since there actually are two resonances (at $E_{R1} = 16.45$ MeV and $E_{R2} = 17.64$ MeV), three situations are possible: $E_0 < E_{R1}$, $E_{R1} < E_0 < E_{R2}$ and $E_{R2} < E_0$.

If $E_0 < E_{R1}$, the ¹⁹F ions are gradually slowed down in the obsidian and the ion energy never reaches either resonance. Hence, the γ-ray production for $E_0 < E_{R1}$ is very small and defines the background level below the resonance.

If $E_{R1} < E_0 < E_{R2}$, the ¹⁹F ions will lose energy in the obsidian, and will eventually reach E_{R1} at a depth X_{R1} and

produce γ-rays at a rate proportional to the hydrogen concentration at X_{R1} . That is:

$$Y_1(E_0) = Y_{R1}(E_0) = K_1 W(X_{R1}) \quad (2)$$

for $X_{R1} < \Delta E/(-dE/dx)$, where K_1 is a measurable proportionality constant determined by the width and cross section of the resonance and the stopping power dE/dx , $Y_1(E_0)$ is the total γ-ray production rate, $Y_{R1}(E_0)$ is the rate of γ-ray production due to the resonance at energy E_{R1} as a function of E_0 , $W(X_{R1})$ is the water concentration at depth X_{R1} , and $\Delta E = E_{R2} - E_{R1} = 1.19$ MeV.

If $E_{R2} < E_0$, the ¹⁹F ions will, after losing some energy in penetrating the obsidian, reach both resonance energies, but at different depths X_{R1} and X_{R2} . So,

$$Y_1(E_0) = Y_{R1}(E_0) + Y_{R2}(E_0) \\ = K_1 W(X_{R1}) + K_2 W(X_{R2})$$

where K_2 is a second proportionality constant differing from K_1 because of different resonance parameters. But since $X_{R2} = X_{R1} - \Delta E/(-dE/dx)$,

$$Y_1(E_0) = K_1 W(X_{R1}) + K_2 W(X_{R1} - \Delta E/[-dE/dx]) \quad (3)$$

for $X_{R1} > \Delta E/(-dE/dx)$. So for a given E_0 , the contribution of γ-ray counts related to the resonance at E_{R2} is proportional to the H₂O content at a shallower depth than X_{R1} , the depth corresponding to the contribution due to E_{R1} . At this shallower depth, however, ($X_{R2} = X_{R1} - \Delta E/[-dE/dx]$), the H₂O concentration has already been measured by the first resonance at E_{R1} using a lower ¹⁹F beam energy ($E_0 - \Delta E$). Thus, the contribution $K_2 W(X_{R1} - \Delta E/[-dE/dx])$ from the second resonance can be calculated from other data (the constant K_2 having been independently determined) and may be subtracted from $Y_1(E_0)$, leaving only $K_1 W(X_{R1})$, the contribution from the first resonance. In this way $W(X_{R1})$, the water concentration as a function of depth in the obsidian target, may be determined by measuring $Y_1(E_0)$ and applying this simple un-

Table 2 Comparison of water content (in percentage by weight) of hydrated and unhydrated obsidian samples from the same source

Sample location	Intrinsic unhydrated % H ₂ O	Hydrated saturation level % H ₂ O
Bodie Hills, California	0.21 ± 0.04	2.31 ± 0.20
Coso, California	0.23 ± 0.07	2.68 ± 0.25
Borax Lake, California	0.35 ± 0.09	3.32 ± 0.32
East Dago Valley, California	0.66 ± 0.06	3.47 ± 0.25

The unhydrated surfaces were freshly chipped from the interiors of obsidian samples, and thus had no significant exposure to ambient water. The hydrated samples all had hydration profiles whose saturation plateaux extended deeper than the 2 μm limit of our depth range. There is a monotonic relationship between intrinsic and hydrated water concentration levels; the higher the intrinsic concentration of water, the higher the final saturation level. This suggests a possible relationship between the factor which determines the intrinsic water content and the mechanism by which ambient water diffuses into obsidian.

folding procedure. With the present experimental configuration^{5,6}, γ rays are detected with an efficiency of 0.022, using a 7.6 cm \times 7.6 cm NaI (TI) scintillation detector.

We made hydration profile measurements as follows on a number of obsidian samples with hydration bands less than 2 μm thick. Raw data (γ -ray counts per 3 μC of $^{19}\text{F}^{4+}$ against E_0) were taken over an energy range of 16 to 22 MeV. The beam current was sufficiently low that the hydrogen concentration was not perturbed, that is, subsequent measurements on the same samples gave virtually identical results. The counting rate (Y) was converted to water concentration by comparison with a standard chlorite sample of known H content, and other mineral standards.

A value of 2.4 g cm⁻³ for the density of hydrated obsidian, and values of stopping powers taken from Northcliffe⁷, were used to compute dE/dx for ^{19}F ions in the obsidian (~ -2.3 MeV μm^{-1}). Using this value of dE/dx in equation (1), the ^{19}F energy scale was converted into a depth scale. (The depth resolution is determined by the ratio of the resonance half width to the stopping power and is about 0.02 μm at the surface; energy straggling gradually reduces the resolution to about 0.04 μm at a depth of 2 μm . Because of background problems at higher energies, this technique is limited to depths of $\lesssim 2$ μm .)

The hydration layers of samples 1–4 (Table 1) were sufficiently narrow that the second resonance was not encountered and unfolding was unnecessary, so the raw data were plotted directly. But since the contribution of the natural linewidth of the resonance to the observed energy width of the hydration layer is significant for such thin layers, correction for this effect was made in order to obtain the measured values for the thickness of the hydration layer.

For samples 5–10, data from an unhydrated obsidian sample (water content uniformly 0.3%) were subtracted point-by-point from the raw data to minimise background problems for $E_0 > 20$ MeV. (For this reason, the zero point of the water concentration scale is displaced by 0.3%.) The profile was then unfolded (equation 3) to leave only the contribution from the resonance at 16.45 MeV. These reduced data were then plotted and were fitted with the calculated water content and depth scales. This procedure is illustrated for sample 5 in Fig. 1.

The detailed hydration profiles can be used to obtain information about the mechanism of water diffusion into obsidian and the factors which influence hydration. First, the general shape of the diffusion profiles agrees qualitatively with that espoused by Friedman and others^{1–3}, characterised by a saturated hydration plateau followed by a steep diffusion front, rather than the more conventional exponential profile suggested by Marshall⁸. Occasionally, however, the data suggest either a double plateau level or that the top of the hydration plateau has an appreciable slope, as in samples 7 and 9, which may indicate the existence of more than one

mechanism of water diffusion and binding as proposed by Ericson, MacKenzie, and Berger⁹.

Table 1 shows that the thickness of the hydration layer corresponding to the maximum gradient of water concentration obtained from our measurements agrees reasonably well with the layer thicknesses obtained from the optically measured thin sections. This seems plausible on theoretical grounds since the hydration band is visible under cross-polarised light because of stress birefringence, with the border between hydrated and unhydrated regions corresponding to the line of maximum stress⁹. (Under ordinary light, the demarcation between hydrated and unhydrated glass is given by a dark line which is the line of maximum gradient of the water concentration⁹.) Indeed, a set of tektite samples with measured H₂O distributions characterised by gently sloping exponential diffusion profiles rather than the steep diffusion fronts observed in hydrated obsidians, did not have visible hydration bands in thin section. (The tektites we measured all had exposure times of roughly 700,000 yr, according to K–Ar and fission track dates, and were recovered from laterite soils in South-east Asia. Two samples, of the splash-form type, were recovered in the early 1960s; a third sample, recovered in the later 1960s, is from the Muong Nong site (J. O'Keefe, private communication).)

Our resolution of 0.02 μm represents a significant improvement over the resolution of 0.1 or 0.2 μm achieved with optical techniques². The uncertainty in our measurements of the thickness of the hydration layer is limited by the finite slope of the concentration decrease for the layers 1–2 μm thick; it is not a limitation imposed by our technique (except where the spacing of data points has been wider than optimum), but by the actual profile itself.

Second, as a result of our measurements of H₂O concentrations in hydrated and unhydrated samples from the same source, it would seem that the higher the intrinsic (unhydrated) concentration of water in a given obsidian sample, the higher the final saturation level (Table 2). (Tektites have extremely low intrinsic water content compared with obsidians¹⁰; this possibly indicates differences in their internal structure, as proposed by Berger and Ericson¹⁰, which might explain the qualitatively different hydration profiles.) This correlation supports previous findings by Kimberlin¹¹.

Third, it seems that, for a set of samples from the same obsidian source exposed for equal times¹², the saturation concentration of water is weakly correlated with the thickness of the hydration layer (Table 1, samples 6–9).

Fourth, we observed a progressive increase in hydration depth with hydration time for the artificially hydrated samples (Table 1, samples 1–3). This type of experiment should prove particularly useful in investigating variations of hydration rate with chemical composition, since the hydration can be carried out under controlled conditions.

Using a similar technique with the resonance $^{23}\text{Na}(p, \gamma)^{24}\text{Mg}$ at 1.318 MeV (ref. 13), we have measured the sodium depth distributions to a depth of 1 μm in obsidian samples 1 and 4 to check a hypothesised ionic-exchange diffusion mechanism which predicts a sodium depletion in the hydration layer⁹. We found no significant variation of sodium content between the layer and the intrinsic unhydrated obsidian; however, we were limited by counting statistics to a detection threshold of about 10% variation in the Na content.

Resonant nuclear reaction techniques of this type may be extended to many other diffusion problems. What is required is a strong, isolated resonance which can be associated unambiguously with the diffusing agent under investigation. The technique provides a relatively nondestructive method of measuring diffusion profiles directly on a microscopic scale.

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- ¹ Friedman, I., Smith, R. L., and Long, W. D., *Geol. Soc. Am. Bull.*, **77**, 323 (1966).
- ² Friedman, I., and Smith, R. L., *Am. Antiq.*, **25**, 476 (1960).
- ³ Haller, W., *Phys. Chem. Glasses*, **4**, 217 (1963).
- ⁴ Ajzenberg-Selove, F., *Nucl. Phys.*, **A190**, 1 (1972).
- ⁵ Leich, D. A., and Tombrello, T. A., *Nucl. Instr. Meth.*, **108**, 67 (1973).
- ⁶ Leich, D. A., Tombrello, T. A., and Burnett, D. S., *Earth planet Sci. Lett.*, **19**, 305 (1973).
- ⁷ Northcliffe, L. C., *A. Rev. nucl. Sci.*, **13**, 67 (1963).
- ⁸ Marshall, R. R., *Geol. Soc. Am. Bull.*, **72**, 1493 (1961).
- ⁹ Ericson, J. E., MacKenzie, J. D., and Berger, R., in *Advances in Obsidian Glass Studies: Archaeological and Geochemical Perspectives* (edit. by Taylor, R. E.), (Noyes Press, Park Ridge, New Jersey, in the press).
- ¹⁰ Berger, R., and Ericson, J. E., *Proc. second Cairo Solid State Conference*, April 21-26, 1973 (The American University in Cairo-Ein Shams University, Egypt, in the press).
- ¹¹ Kimberlin, J., in *Advances in Obsidian Glass Studies: Archaeological and Geochemical Perspectives* (edit. by Taylor, R. E.), (Noyes Press, Park Ridge, New Jersey, in the press).
- ¹² Ericson, J. E., and Berger, R., in *Advances in Obsidian Glass Studies: Archaeological and Geochemical Perspectives* (edit. by Taylor, R. E.), (Noyes Press, Park Ridge, New Jersey, in the press).
- ¹³ Endt, P. M., and Van der Leun, C., *Nucl. Phys.*, **A105**, 1 (1967).

Using artificial thermoluminescence to reassemble statues from fragments

THE thermoluminescence (TL) of marbles and limestones¹⁻³ from various quarries in Greece⁴ has been described. It had been hoped that features characteristic of each quarry would be found. The glow curves of samples as taken from the quarry are called natural thermoluminescence curves (NTL). These curves did not allow any determination of origin, because in nearly every case only a single peak was obtained.

Glow curves of artificial thermoluminescence (ATL) provide considerably more information. ATL glow curves were obtained by heating samples until the NTL was erased, then exposing them to X rays at room temperature, and subsequently heating them at a constant rate.

Another type of glow curve, the curve of mixed thermoluminescence (MTL), was obtained by exposing untreated samples to X rays at room temperature and subsequently heating them up at a constant rate. With this kind of glow curve the ATL curve is superimposed on the NTL curve. Most MTL glow curves displayed three peaks, and thus supplied five parameters for the identification of each sample:

(temperatures T_1 , T_2 , T_3 ; and intensity ratios I_1/I_2 and I_2/I_3). A table of all five parameters in material from 36 quarries has been compiled. In some of the quarries, significant differences could be detected between samples from widely separated blocks. The differences were more pronounced between blocks of different colours.

Because of the differences in the parameters of samples from any one quarry, it is not possible to identify unambiguously the origin of any piece of marble. The differences between samples from any one block are, however, small, suggesting that there could be a good possibility of determining whether separate fragments of marble are from the same statue or slab. Only small quantities of powder are available for investigations of archaeological fragments. A comparison of powders (~ 0.1 g) from adjacent holes showed that the MTL glow curves give identical peak temperatures, although the intensity ratios were sometimes different. This could be because of differences in the degree of heating of the material during the drilling process, which depends on the pressure exerted.

The results are consistent if the comparison is made from the ATL glow curves (Fig. 1a and b) which are not affected by any heating during drilling. Although these usually have only three independent parameters: T_1 , T_2 and I_1/I_2 they differ very little (about 5° C for the temperature and 5% for the ratios on powders from adjacent drilling holes).

From a consideration of several glow curves of the same sample mean values of the parameters can be obtained, together with their standard deviations (Table 1).

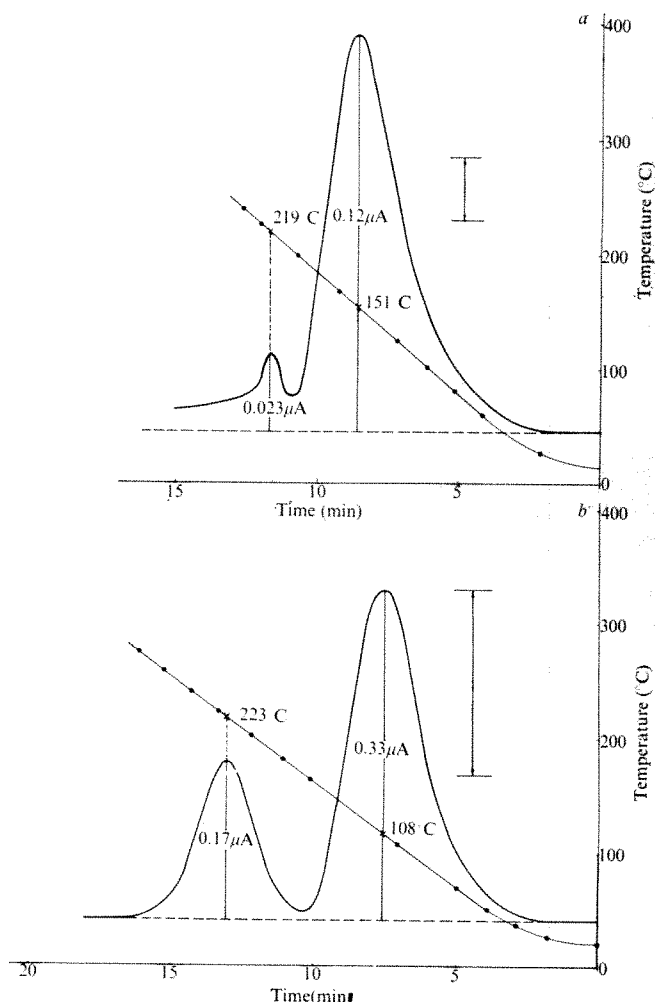


Fig. 1 ATL glow curves from two different blocks. The results were obtained on a chart recorder. Scale bars: a, 0.02 μ A; b, 0.2 μ A. The dependence upon temperature is shown on both graphs.

Table 1 ATL curve parameters

	T_1 °C	T_2 °C	I_1/I_2
Fig. 1a	108 ± 5	223 ± 4	1.9 ± 0.1
Fig. 1b	151 ± 5	219 ± 7	5.1 ± 0.6

If the parameters are the same, it does not necessarily follow that the fragments belong to the same block, as they could simply have originated in the same quarry. If, however, two fragments have different parameters it can be said with a high degree of certainty that they do not belong to the same block.

This method was of great assistance in the restoration of statues from fragments found in a shipwreck near the island of Antikythera.

An extensive description of the method will appear, in English, in the *Praktika* of the Athens Academy.

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¹ McDougall, D., *Thermoluminescence of Geological Materials* (Academic Press, 1968).

² Goldberg, P., *Luminescence of Inorganic Solids* (Academic Press, 1966).

³ Curie, D., *Luminescence Cristalline* (Dunod, Paris, 1960).

⁴ Afordakos, G., thesis, Univ. Athens (1972).

BIOLOGICAL SCIENCES

Mutant of bacteriophage T4D affecting expression of many early genes

IN T4 infections the mechanisms determining the time and rates of expression of early genes are not yet clearly understood. Although mutants which do not make late gene products or which are defective in DNA synthesis generally fail to turn off the synthesis of most early gene products, no mutations have been described which affect either the time of appearance or the initial rate of synthesis of proteins from more than a single early gene. This preliminary report describes the isolation and some of the properties of such a mutation. This mutation which maps between genes 52 and *t* defines a new regulatory gene.

When a lambda lysogen is singly infected at 37° C with a phage carrying temperature-sensitive mutations in both *rIIA* and in gene 42 (dCMP-hydroxymethylase¹), the infected cells quickly lose their ability to form a plaque at 30° C. After 25 min at the restrictive temperature only about 2% of the infected cells can still form a plaque at 30° C. It seemed possible that the survival of the *rIIA*-gene 42- infected cells might be increased by an additional mutation whose effect would be to prevent the expression of many early genes. That is, if the normal development of the phage-infected cell was suspended at an early stage then the early absence of functional *rIIA* product might not be lethal². On shifting such infected cells to the

permissive temperature, normal development might then proceed as active *rIIA* protein was synthesised. According to this rationale, the survivors of the heat treatment (25 min at 37° C) would include phage which were genetically less sensitive to this treatment. Among mutants thus selected there might therefore also be mutants controlling the expression of early T4 genes.

The selection was done by singly infecting *E. coli* K112-12 (λ h) #3/S at 37° C with the non-mutagenised double temperature-sensitive mutant *tsDG12* (*rIIA*³)-*tsL13* (gene 42). After 25 min at 37° C the infected cells were filtered and resuspended in fresh media at 30° C. The infected cells were lysed at 50 min by the addition of chloroform. This enrichment procedure was repeated several times using the progeny phage from one cycle of infection as the input phage for the next cycle. The infected cells in the fifth cycle of enrichment were significantly more resistant to the temperature treatment than cells infected with the parental phage. Stocks were made from isolated plaques of several of these surviving phage and one such isolate was thoroughly examined. Although this phage still contained both of the parental markers, about 20% of the K112-12 (λ h) #3/S cells infected with it (by comparison with only about 2% for the parental phage) could still make plaques on *E. coli* B (su⁻) at 30° C after 25 min of infection at 37° C. This phage contained a new mutation in addition to the two parental markers. This mutation, *tsG1*, when separated from the two parental markers is itself a temperature-sensitive mutation. It seems to reduce the lethal effects caused by the initial absence of functional *rIIA* protein, but not the lethal effects due to the absence of functional gene 42 product (P42) (manuscript in preparation).

Figure 1 presents the results of two-factor crosses which show that *tsG1* maps in the region between *amH17* (gene 52) and *amtA3* (gene *t*). The mapping of *tsG1* to the right of *amH17* is consistent with the results of a three-factor cross between *tsG1* and the double mutant *amH17-ac*³. Most of the *ts*⁺-*am*⁺ recombinants (78 out of 103) examined were sensitive to acridine.

Reversion studies suggest that *tsG1* is a point mutation. In three isolated plaques of *tsG1* grown at 30° C revertants appeared at an average frequency of 1×10^{-6} . Experiments with one of these *tsG1* revertants showed that it was like wild type at both 30° C and 42° C in its growth in liquid medium and in its pattern of protein synthesis as seen on SDS-polyacrylamide gels (data not shown). This revertant was crossed to wild type and 870 progeny phage tested for their temperature sensitivity. No *tsG1* segregants were found. On this basis it is concluded that *tsG1* is a point mutation and that reversion can occur at the site of the original lesion or very close to it.

The mutants *tsG1* and *amH17* (gene 52) complement one another in a mixed infection of *E. coli* B^E at 42° C in the sense that the mixed infection begins to produce progeny at the same time

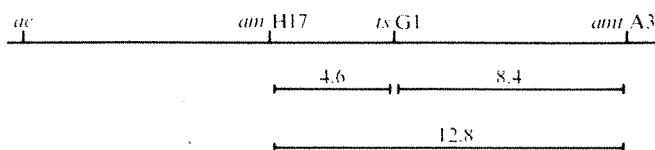


Fig. 1 Mapping by two-factor crosses. Log phase *E. coli* B^E grown at 37° C to 10^8 ml⁻¹ in M9S medium¹⁰ containing 0.2% casamino acids (Difco) were centrifuged and resuspended at 2×10^8 ml⁻¹ in fresh medium. Crosses were done at 30° C using a multiplicity of infection of five for each parent. After 3 min of adsorption the infected cells were diluted 10^4 into fresh medium and aerated by bubbling. Chloroform was added at 50 min to ensure complete lysis. Progeny were assayed on plates made of EHA bottom and top agar¹¹. Total progeny were assayed at 30° C using *E. coli* B₄₀ sul⁻ as indicator. Wild type recombinants from all crosses were selectively assayed on plates seeded with *E. coli* B^E and incubated at 42° C. The map distances given are 200 times the frequency of wild type recombinants scored in crosses between the markers indicated. *tsG1* and *amH17* each recombine with the terminal *rIIB* marker, *r73*, to about the same extent, giving a map distance of approximately 15 units.

as a wild type infection and not with the delay characteristic of either *amH17* or *tsG1* (data not shown). The pattern of protein synthesis seen in *amH17* infections (see below) is also quite different from that observed in *tsG1* infections. It is therefore very unlikely that *tsG1* is in gene 52.

In addition *tsG1* is not in the *t* gene for the following reasons. The *t* mutants specifically affect late events in T4 development (lysis and the shutoff of macromolecular synthesis³) but the early events seem to be entirely normal (ref. 3 and H. Krisch, personal communication). In mixed infections at 42° C *tsG1* complements the late lysis phenotype of *amtA3* mutation and progeny phage are produced at the same time as in wild type infections (data not shown). As there are no other known genes in the gene 52 to *t* region, the *tsG1* mutation defines a new gene which is called *mot* for modifier of transcription.

SDS-polyacrylamide gel analysis of the proteins synthesised after infection of *Escherichia coli* B^F (*su*⁻) at 42° C with *amH17* or with *tsG1* is presented in Fig. 2. *amH17* was used as the control in this experiment because it maps close to *tsG1* and because it⁴ as well as *tsG1* has a DNA synthesis delayed phenotype. The initial rates of synthesis of the proteins as seen on these gels are identical in *amH17* and in wild-type infections (unpublished observations). Many differences between the *amH17* and *tsG1* infections can be seen. Since nearly the same amount of radioactivity precipitable by trichloroacetic acid was layered on each gel, differences in band intensities for individual proteins reflect differences in their relative rates of synthesis. In *amH17* infections the band corresponding to the *rIIA* protein is quite dense in the 2–6 min labelling, decreases in intensity during the 6–10 min labelling and is even less dense in the 10–14 min pulse. But in *tsG1* infections the *rIIA* band is at least as dense in the 6–10 min labelling as in the 2–6 min labelling and a considerable amount of it is still being synthesised in the 10–14 min pulse although its rate of synthesis is then slightly less than in the 6–10 min pulse. Other experiments show that in *tsG1* infections detectable amounts of *rIIA* protein are still synthesised as late as 35–40 min after infection at 42° C. Thus in *tsG1* infections the *rIIA* protein is synthesised for a considerably longer time than in either *amH17* or wild-type infections.

There are many mutants of T4 which affect the normal turnoff of early gene functions: maturation-defective genes (33 and 55)^{5,6}; the DNA-delayed mutants⁷ and all of the DNA-negative mutants^{1,7}, have this property to varying extents although synthesis of the *rIIA* protein is not affected by any of these mutants to the extent that it is affected by *tsG1*. But *tsG1* is different in a much more important respect from all other mutants having an effect on early gene turnoff. After infection with all these mutants the initial rates of synthesis are normal for all the early proteins which have been studied. Though most of the phage induced proteins do appear at the normal time in *tsG1* infected cells, the initial rates of synthesis for some of them are considerably reduced (see Fig. 2). The products of genes 43, 45 and *rIIB* are detectable during the first labelling period but their rates of synthesis are significantly reduced. P43 and P45 seem to be more severely affected than *rIIB*. Whereas most of the proteins from *tsG1* infections as seen on these gels appear at the normal time a few are not detectable during the first or even the second labelling period. For example, the band migrating at the position of the gene 32 protein is not visible until the 6–10 min labelling period.

The effect of *tsG1* on the expression of the *rIIB* protein is of special interest because it suggests an hypothesis for the mode of action of the *mot* gene product. Schmidt *et al.*⁸ have shown that the *rIIB* cistron is transcribed as part of a polycistronic message which includes the *rIIA* species and that it is also transcribed from a promoter located between the *rIIA* and *rIIB* cistrons. Preliminary evidence indicates that early after infection there is less *rIIB* transcription relative to *rIIA* transcription in *tsG1* infected cells than in cells infected with wild type. Since at early times in *tsG1*-infected cells there is a relative lack of *rIIB* message and protein but a normal amount of *rIIA* protein we hypothesise that in *tsG1* infected cells transcription from the

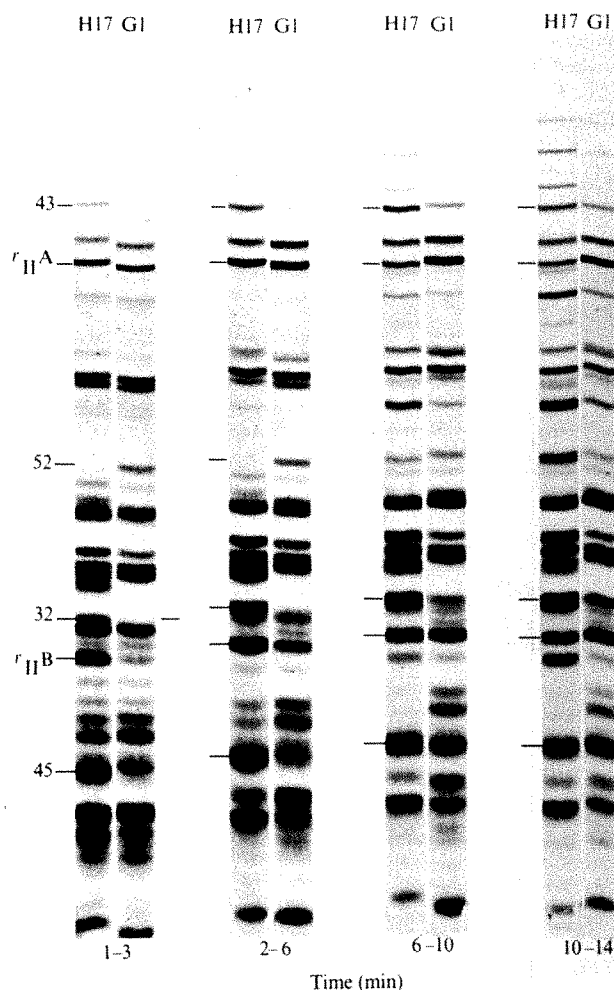


Fig. 2 SDS-polyacrylamide gel analysis. *E. coli* B^F (*su*⁻) grown in M9S containing 0.2% casamino acids to 10^8 ml⁻¹ was centrifuged and resuspended at 5×10^8 ml⁻¹ in M9S* (M9S containing only 10% of the normal amount of casamino acids). One millilitre of cells warmed for 2 min at 42° C were added to 24 ml of M9S* at 42° C containing either *tsG1* or *amH17* at 2×10^8 ml⁻¹. At 1, 2, 6, and 10 min after infection 5 ml aliquots of the infected cells were transferred to tubes at 42° C containing 5 μ Ci of a mixture of 15 ¹⁴C-labelled amino acids (NEN 445). The pulses 1–3, 2–6, 6–10 and 10–14 min were terminated by the addition of a 20-fold excess of unlabelled casamino acids. After a 3 min chase the samples were chilled by addition of ice and then centrifuged. The pellets were resuspended in 0.25 ml of 0.01 M Tris pH 7.5 and 1.0 ml of sample buffer (10% glycerol, 5% 2-mercaptoethanol, and 3% sodium dodecyl sulphate in 0.0625 M Tris pH 6.8) and then placed in boiling water for 3 min. Disc 10% SDS-polyacrylamide gels were prepared as described by Laemmli¹². The gels were run at a constant current of 1–2 mA per tube and were stained, sliced, dried and exposed to X-ray film as described by Fairbanks *et al.*¹³. About 30,000 trichloroacetic acid precipitable counts were layered on each gel. The numbers on the left margin of the figure identify the genes coding for the proteins indicated. The band migrating just slightly faster than P32 is P44 (ref. 14). The absence of the gene 52 band in the early labellings with *amH17* is expected because *amH17* is in gene 52. The band appearing at the position of P52 in the last two labelling periods is a cleavage product of the main structural protein of the phage head. In the gels from the 1–3 min labelling, the migration of the proteins was not identical. The gels were matched in the P32–*rIIB* region and as a consequence the positions of P43 and *rIIA* in the gel from the *tsG1* infection are slightly lower than the corresponding bands in the gel from the *amH17* infection.

promoter located between the *rIIA* and *rIIB* cistrons is defective. Thus the normal *mot* gene product may have a positive role in transcription related to the recognition of promoters like the one between the *rIIA* and *rIIB* cistrons. The *mot* gene itself might code for this regulatory element or it may merely control

its expression. In either case the postulated positive transcriptional element could function similarly to the sigma-like factor described by Travers⁹.

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- ¹ Wiberg, J. S., Dirksen, M. L., Epstein, R. H., Luria, S. E., and Buchanan, J. M., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 293–302 (1962).
- ² Mattson, T., and Russel, M., *Virology*, **49**, 160–167 (1972).
- ³ Josselin, R., *Virology*, **44**, 101–107 (1971).
- ⁴ Warner, H. R., and Hobbs, M. D., *Virology*, **33**, 376–384 (1967).
- ⁵ Young, E. T., II, *Cold Spring Harbor Symp. quant. Biol.*, **35**, 189–195 (1970).
- ⁶ Hosoda, J., and Levinthal, C., *Virology*, **34**, 709–727 (1968).
- ⁷ Warner, H. R., and Lewis, N., *Virology*, **29**, 172–175 (1966).
- ⁸ Schmidt, D. A., Mazaitis, A. J., Kasai, T., and Bautz, E. H. F., *Nature*, **225**, 1012–1016 (1970).
- ⁹ Travers, A. A., *Nature*, **225**, 1009–1012 (1970).
- ¹⁰ Bolle, A., Epstein, R. H., Salzer, W., and Geiduschek, E. P., *J. molec. Biol.*, **31**, 325–348 (1968).
- ¹¹ Steinberg, C., and Edgar, R. S., *Genetics*, **47**, 187–208 (1962).
- ¹² Laemmli, U. K., *Nature*, **227**, 680–685 (1970).
- ¹³ Fairbanks, G., jun., Levinthal, C., and Reeder, R. H., *Biochem. biophys. Res. Commun.*, **20**, 393–399 (1965).
- ¹⁴ O'Farrell, P. Z., Gold, L. M., and Huang, W. M., *J. biol. Chem.*, **248**, 5549–5501 (1973).

Immunotherapeutic suppression in transplantable solid tumours

α -FOETOPROTEIN (AFP) is a glycoprotein, of unknown function, synthesised by the foetal liver and yolk sac of mammals¹. Increased serum levels of AFP in adults are indicative of either pregnancy or a pathological state, that is primary hepatocellular carcinoma, regenerating liver and teratoblastomas of the ovary and testis^{2,3}. Recently, abnormal levels of AFP in amniotic fluids have been correlated with foetal distress⁴. While attempting to study the physiological role of AFP during ontogenic and oncogenic growth, we have observed an interaction between AFP and its homologous antiserum that may have immunotherapeutic value. We report here the suppression of mouse hepatoma growth *in vivo* and *in vitro* using passively administered antiserum to AFP.

Amniotic fluid (AF) containing AFP was obtained from C57BL mice as described by Sell⁵. AFP was isolated from the AF by upward flow gel filtration on Sephadex G-75, followed by preparative polyacrylamide gel (PAAG) column electrophoresis using a modification of Gussev's method⁶. A single band could be demonstrated subsequently by disc PAAG electrophoresis. The AFP isolate was emulsified with equal amounts of Freund's complete adjuvant for use as an immunogen. New Zealand white rabbits were immunised twice weekly for 2.5 weeks by subcutaneous flank injections. The rabbit antiserum, collected 2 weeks after the last injection was

immunoreactive against mouse AFP, but not albumin, as determined by microimmunodiffusion. A single band in the α_1 position was demonstrated after immunoelectrophoresis. The antiserum was absorbed further with nonimmune mouse serum (NMS) to insure monospecificity. Using the absorbed anti-AFP serum, AFP was quantitated in mouse sera using radial immunodiffusion (unpublished results of G. J. Mancini and S. R. Young).

Male and female mice of the C57L/J inbred strain bearing the AFP-secreting hepatoma BW7756 (Jackson Laboratory) were used as the animal tumour model. A standard tumour growth curve was obtained during 30 d (after transplantation) using mice weighing 20–28 g. The transplanted tumours usually exhibited 100% takes. The anti-AFP antisera were tested against the hepatoma during this period by three *in vivo* and two *in vitro* methods. The *in vivo* testing included (1) administration of graded doses during tumour growth; (2) a large, single dose administered 2 d after implantation; and (3) incubation of the tumour inocula in test serum before implantation. The *in vitro* methods encompassed (4) the cytotoxic antibody assay, and (5) tissue culture cytopathic testing. The tissue culture experiments, however, will be described in detail elsewhere⁷.

The graded doses included alternate daily intraperitoneal injections (a total of 2.60 ml) of absorbed anti-AFP serum given in small divided doses during 28 d. Using method (2), a single large dose of absorbed antiserum (2.0 ml) was injected intraperitoneally into the mice 2 d after transplantation. Method (3) was performed by incubating the tumour inocula (1.0 mm² ml⁻¹ whole antiserum) for 30 min at 25° C immediately before implantation. The incubated tumour inocula were subsequently washed twice in Hanks balanced salt solution before implantation. All animals were observed and inspected daily for tumour growth over the post-transplantation period. At autopsy, 28 d later, all animals were bled and tumour weight and size were determined. For each experiment described above, nonimmune rabbit serum (NRS) absorbed with NMS was administered as control serum to an age-matched and weight-matched group of mice. The cytotoxic antibody assay was performed *in vitro* according to the method of Gorer and O'Gorman⁸. Fresh guinea pig serum was titred and used as a complement source. In some studies, only deactivated anti-AFP and NRS were used. For each determination, 3×10^6 – 4×10^6 screen-tested tumour cells were counted in a haemocytometer by trypan blue exclusion.

The *in vivo* methods of tumour growth suppression are compared in Table 1, which shows that antiserum administered gradually throughout incubation resulted in a 52% reduction in tumour size as compared with untreated controls (5% significance level). However, the NRS control group displayed a 20% reduction; thereby subtracting out to a final 32% reduction in the experimental group. Similarly, the animals

Table 1 The *in vivo* methods of immunotherapeutic tumour growth suppression are compared in C57L/J mice administered anti-AFP serum

Treatment groups	Mean	s.e.m.	Growth (%) reduction*	Significance level†
Graded dose (11) A·AFP	2.84	0.74	32.0	< 0.05
NRS	4.70	0.47		
Single dose (9) A·AFP	3.52	0.52	33.0	< 0.10
NRS	5.44	1.05		
Pre-incubated A·AFP	0.52	0.18	67.0	< 0.01
inocula (11) NRS	4.42	0.64		
Untreated (13) Control	5.87	0.50	0.0	—

The numbers of animals in the sample are given in parentheses.

* Treated control — Experimental
tumour weight — tumour weight
× 100.

Untreated control
tumour weight

† Student's *t* test was used.

subjected to a single, large dose also underwent a 33% reduction in tumour growth even though the statistical significance of this latter experiment differed considerably from treatment with graded doses (10% compared with 5% probability level). Finally, treatment with incubated inocula demonstrated not only a high statistical significance (1% level) but also a 67% reduction in tumour weight. Thus, direct contact of the tumour cells in the test reagents before implantation resulted in cytotoxicity which was twice as effective as the other modes of treatment.

The results of the cytotoxic antibody assay *in vitro* are presented in Fig. 1. An innate cytotoxicity of nonimmune rabbit and guinea pig serum against mouse cells was observed which could be eliminated by a 1:10 to 1:16 serum dilution. Nonimmune rabbit serum control levels maintained a 70% tumour cell viability throughout a serum dilution range of 1:32 to 1:128. In contrast, the anti-AFP serum had a 100% cell killing effect at 1:16 and a 66% effect at 1:32 dilution. Unlike the NRS, which remained stable, the cytotoxicity of the AFP antisera could be eliminated uniformly by extension to a 1:128 dilution as evidenced by the cytotoxic index in Fig. 1. Thus, the complete cell cytotoxicity (CI = 1.0) could be titred out over three two-fold dilutions. If the innate cell cytotoxicity of NRS for mouse cells is subtracted out (as above), it could still be observed that anti-AFP exerted a 30–33% killing effect on hepatoma tumour cells *in vitro* above that of NRS. Further cytotoxic testing using anti-AFP serum indicated that complement (host or foreign) was not an absolute requirement for cell killing but its presence intensified the killing effect. Finally, the cytotoxicity of the anti-AFP serum could be abrogated by absorption with purified AFP.

The gross pathology observed during the 30 d included fibroid accumulation, serous exudation, loss of connective tissue capsule and massive haemorrhaging at the tumour site. By 28 d, much of the tumour mass presented as nonviable tissue in mice treated with anti-AFP serum. In contrast, the mice administered NRS displayed only occasional haemorrhaging about the tumour mass. The corresponding histopathology revealed the presence of mononuclear infiltrates which invaded the tumour tissue of the animals treated with AFP antiserum. The cellular infiltrate populations increased uniformly from the 14th to the 21st day, and by day 28 much of the tumour mass had been replaced by inflammatory tissue. However, the animals treated with NRS displayed only focal infiltrates with occasional ischaemic necrosis. The histopathological results, which were confirmed by Dr J. J. McCoy jun. of the Veteran's Administration Hospital, Columbia, South Carolina, will be reported in more detail in a forthcoming publication.

The presence of AFP in serum, determined quantitatively by radial immunodiffusion, was suppressed in the antibody-treated mice, while those subjected to NRS exhibited a steady increase in AFP serum concentrations. The mice treated with NRS displayed a nine-fold increase in serum AFP from the 14th to the 28th day after transplantation (final AFP concentration = 9.75 mg ml⁻¹). In contrast, the mice treated with anti-AFP serum showed a slight increase in AFP production from the 14th to the 21st day with a drastic decrease thereafter to the 28th (final AFP concentration = 0.38 mg ml⁻¹). Thus, the presence of AFP in the serum seems to correlate well with tumour status.

Our results indicate that humoral immunotherapy was effective against a progressively growing solid tumour. The passive administration of heterologous antiserum to AFP in tumour-bearing mice resulted in a suppression of hepatoma growth and a decrease in AFP production over 30 d. The greatest reduction in tumour size (67%) occurred when the tumour inocula were incubated directly in the test serum. When the antiserum was administered by two methods of intraperitoneal injection, tumour size was reduced 32%. Extrinsic complement was not used or added to the serum; however, the serum was not heat deactivated and complement

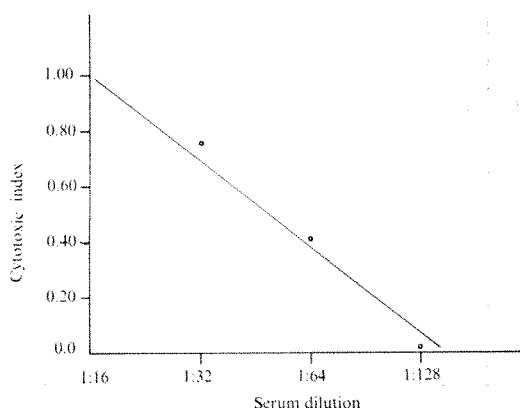


Fig. 1 Cytotoxic index for anti-AFP serum (exp) versus nonimmune rabbit serum (control) tested against mouse hepatoma target cells. Cytotoxic index is obtained from:

$$\frac{\text{Proportion of dead cells in control} - \text{Proportion of dead cells in exp}}{\text{Proportion of dead cells in control}}$$

may have been present in either the rabbit or mouse serum. In comparison with the *in vivo* test, the use of guinea pig complement *in vitro* also displayed a 30–33% cell cytotoxicity with direct incubation of teased tumour cells with various antibody dilutions. Neither native nor heterologous complement was required for *in vitro* cytotoxicity. Finally, the specificity of the cytotoxic reaction was demonstrated by absorption studies using purified antigen.

These experiments signify that some humoral factor or group of factors (anti-AFP) present in the sera of rabbits immunised to AFP induced growth retardation in progressively growing mouse hepatomas. One cannot rule out the possibility that soluble antigen–antibody complexes, present in the serum after absorption, were not involved in the observed suppression. Thus, the host defences may be responding to the presence of immune complexes in and about the tumour. In any case, the killing effect of the antiserum *in vivo* was probably due to (1) tumour cell-surface destruction, and (or) (2) vascular damage within and around the tumour mass. In either case, immune complexes would be formed which can act with or without complement. Since this immunotherapeutic approach is effective in tissue culture, the absolute requirement of active factors from the host mouse can be ruled out. The gross and histopathological evidence suggests that an Arthus-type tissue destruction occurred at the tumour site which, in effect, starved the tumour and retarded overall growth.

Since the function of AFP has not been established, it is unclear whether the actual blocking of AFP function played a role in the retardation of hepatoma growth. Some investigators have suggested that AFP functions in a growth factor capacity being essential for foetal liver and (or) hepatoma growth^{9,11}. Recently, indirect evidence has implicated AFP as a steroid (oestrogen) binding protein¹⁰. It is tempting to speculate that the tumour growth suppression reported here may have occurred at the level of hormonal induction of protein synthesis.

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¹ Gitlin, D., and Boesman, M., *J. clin. Invest.*, **46**, 1010 (1967).

- ² Abelev, G. I., *Adv. Cancer Res.*, **14**, 295 (1971).
- ³ Smith, J. B., and O'Neil, R., *Am. J. Med.*, **51**, 767 (1971).
- ⁴ Campbell, S., Coltart, T. M., and Singer, J. D., *Lancet*, **ii**, 73 (1973).
- ⁵ Sell, S., Jalowayski, I., Bellone, C., and Wepsic, H. T., *Cancer Res.*, **32**, 1184 (1972).
- ⁶ Gushev, A. I., and Yazova, I., *Biokhimiya* (USSR), **35**, 1972 (1970).
- ⁷ Mancini, G., Carbonara, A. O., and Heremans, J. F., *Immunochemistry*, **2**, 235 (1965).
- ⁸ Gorer, P. A., and O'Gorman, P., *Transplant. Bull.*, **3**, 142 (1956).
- ⁹ Abelev, G. I., Perova, S. D., Khramkova, N. I., Postnikova, Z. A., and Irlin, I. S., *Transplantation*, **1**, 174 (1963).
- ¹⁰ Uriel, J., Aussel, C., Bouillon, D., and DeNechaud, B., *Nature new Biol.*, **244**, 190 (1973).
- ¹¹ Smith, J. B., *Med. clin. N. America*, **54**, 797 (1970).

Inhibition of cell-dependent cytotoxicity as an assay for mouse alloantibody

MANY assays for alloantibody depend on functional activities of the Fc portion of the immunoglobulin molecule. These functional activities are acquired when the antibody binds to its target cell. Complement binding, as demonstrated by lysis or complement fixation, has been the most widely used of these activities. In man, alloantibody can also be detected by its ability to mediate cell-dependent lysis of target cells¹⁻³. In this system, effector cells (human peripheral blood leukocytes) lyse allogeneic chromium ⁵¹Cr-labelled target cells (lymphocytes) in the presence of the appropriate alloantibody, providing a sensitive, complement-independent assay for alloantibody. In contrast, we have found that mouse alloantisera do not usually mediate lysis of mouse target cells (lymph node lymphocytes, phytohaemagglutinin (PHA) blast cells, and tumour cells) by mouse effector cells (spleen cells). Data supporting this conclusion will be presented elsewhere.

In man, alloantibody has a second activity in this cell-dependent assay: namely, that when the antibody is directed against the effector cell it inhibits cell-dependent killing of antibody-coated target cells². We have found that mouse alloantisera have a similar effector-cell inhibiting capacity. Mouse spleen cells are excellent effectors for lysing antibody-coated xenogeneic target cells (in contrast to their inability to lyse alloantibody-coated allogeneic cells). When alloantibody directed against H-2 specificities of the effector cell is added, the lysis is abolished. This assay, which we refer to as the cytotoxicity inhibition assay (CIA) is the subject of this report.

The cytotoxic system used is similar to that of Perlmann⁴, using mouse spleen cells (effector cells) to lyse chicken red cells (CRBC) (target cells) in the presence of rabbit anti-chicken red cell antibody (RACA). Single-cell suspensions of mouse spleen cells in Eagles' minimal essential medium (MEM) with 5% foetal calf serum were placed in plastic culture tubes. The alloantiserum to be tested was added to these, with 10⁴ radio-active ⁵¹Cr-labelled CRBC and RACA (1/25,000) in a final volume of 250 μ l. All experiments are performed in triplicate. Sheep red cells were added at 10⁷ ml⁻¹ to lower background ⁵¹Cr release⁵. The mixture was then incubated at 37°C for 3 h in a 10% oxygen, 5% carbon dioxide atmosphere in a dessicator. After 3 h, 500 μ l of cold MEM was added to each sample; the cells were spun down and the supernatant removed. Cells and supernatant were then counted separately on a Packard Autogamma scintillation spectrometer, and the results expressed as % ⁵¹Cr release.

Excellent ⁵¹Cr release with good sensitivity to inhibition by antibody occurred at spleen cell to CRBC ratios of 200:1 and RACA concentrations of 1/25,000, and these concentrations were used in all studies discussed here. Maximum killing usually ranged between 30% and 60% ⁵¹Cr release, with background ⁵¹Cr release of 0.5–3.0%. The alloantisera tested are conventional anti H-2 antisera, with good titres in complement

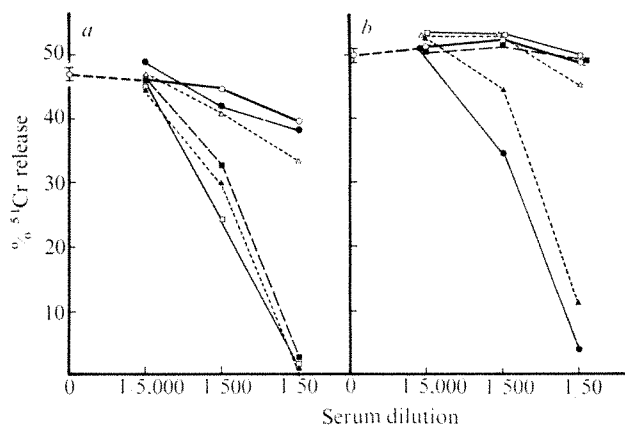


Fig. 1 The specificity of the CIA test. Various sera were incubated with BALB/c (H-2^d) or CBA/H (H-2^k) spleen cells (2×10^6) plus RACA (1/25,000) and CRBC (10^4) for 3 h. The reactions of the three anti H-2^d sera against BALB/c are very strong, whereas the anti H-2^k and anti H-2^b sera do not inhibit. The reaction of the anti H-2^k serum with CBA/H is similarly very strong. Serum 'b anti d', C57Bl6 anti BALB/c, reacts with CBA/H as would be predicted by public specificities shared by H-2^k and H-2^d. *a*, BALB/c effectors; *b*, CBA/H effectors. ○ 'NMS': Normal BALB/c serum; ● 'anti k': BALB/c anti CBA/H; △ 'anti b': (CBA/H × BALB/c) F₁ anti C57 Bl6; ▲ 'b anti d': C57Bl6 anti BALB/c; □ 'k anti d' No. 1: CBA/H anti P815Y; ■ 'k anti d' No. 2: CBA/H anti BALB/c.

mediated microcytotoxicity (up to 1/1000).

Many mouse alloantisera show a powerful specific inhibitory effect on cell-dependent antibody-mediated lysis of CRBC by spleen cells (Fig. 1). The effect of normal mouse serum, irrelevant antiserum or antiserum directed against specificities of the effector cell on ⁵¹Cr release are shown in Fig. 1. The killing by BALB/c spleen cells (H-2^d) is virtually eliminated by anti H-2^d antisera, but is only weakly inhibited by normal mouse serum, anti H-2^k serum, or anti H-2^b serum. Similarly, killing by CBA/H spleen cells (H-2^k) is strongly inhibited by anti H-2^k serum, but only weakly inhibited by anti H-2^d and anti H-2^b serum or by normal mouse serum. The exception, 'b anti d', anti H-2^d raised in C57Bl6 mice (H-2^d), reacts with CBA/H spleen cells in a manner predictable on the basis of public specificities shared between H-2^d and H-2^k (ref. 6). When allowance is made for this predictable cross-reaction, and when compared with the weak inhibitory activity of any mouse serum, the CIA is thus highly specific. The weak inhibitory activity of normal mouse serum and of irrelevant antiserum is similar to that noted in other systems⁷.

The specific inhibitory activity can be absorbed out (Fig. 2). Anti CBA/H serum which had been absorbed $1 \times$, $2 \times$, or $3 \times$ against 3×10^7 CBA/H spleen cells was assayed for its ability to inhibit killing by CBA/H spleen cells (Fig. 2a). One absorption produced a dramatic decline in the inhibitory activity, with further decline after each subsequent absorption. The cells used for absorption were resuspended and washed thoroughly and then assayed for their activity as effector cells (Fig. 2b). The first absorption leaves the cells used for this purpose with greatly reduced killing capacity, and each successive absorption reduces the killing capacity of the absorbing cells less. Thus, the inhibitory activity of the alloantiserum is absorbed out, and the absorbing cells become inhibited in their killing capacity. Additional experiments indicate that this inhibition of killing capacity lasts for at least 24 h when the antibody-coated effector cells are washed and incubated at 37°C.

We therefore feel that the CIA detects a specific, absorbable activity which is probably alloantibody in nature. We investigated whether this antibody is directed towards the H-2D and H-2K 'serologically-defined' specificities, or towards other H-2 or non H-2 antigens by testing an anti H-2^d serum and an anti H-2^k serum against various congenic mouse

strains, with a C57Bl/10 Sc Sn (B10) background, differing only in all or part of the H-2 region (Fig. 3). Here the specificity controls, BALB/c and CBA/H, show that the reaction is completely specific. The anti H-2^k serum causes strong inhibition of B10.BR, B10.AKM and B10.A, each of which shares part or all of its H-2 region specificities with H-2^k. Similarly, anti H-2^d serum inhibits killing by B10.A and B10.AKM cells which have part of their H-2 regions from H-2^d (B10.A) or from H-2^a (B10.AKM), which has many specificities in common with H-2^d. The weak reaction of both anti H-2^d and anti H-2^k sera with B10 cells is expected on the basis of shared public specificities.

While complete understanding of the specificities involved remains to be established, we believe that 'serologically-defined' H-2 specificities can explain most of the inhibitory activity of these alloantisera.

The mechanism of this inhibition is under study. The absence of complement from the medium, the high titres of inhibitory activity in the sera (1/3,500 or more) and cell counts made after many hours of incubation seem to make cell death or agglutination unlikely mechanisms. Damage to a small sub-population of cells, the actual effector cells, is impossible to rule out at present because these cells are morphologically unidentifiable. On the basis of current knowledge of the killing systems mediated by antibody and effector cells^{8,9}, the most likely mechanism is that 7S antibody attached to a surface antigen on either the effector cell or an adjacent cell, blocks the receptor by means of the Fc portion of the antibody, perhaps in the form of an immune complex. This would be in agreement with present concepts of the binding of immune complexes by the Fc receptor^{10,11}.

We cannot explain the dichotomy between the ability of mouse spleen cells to lyse antibody-coated CRBC and their apparent inability to lyse alloantibody-coated allogeneic cells. One report¹² documents the cell-dependent lysis of YAC and P815Y tumour cells coated with antibody raised against YAC tumour cells in CBA mice but the possibility that this is antitumour activity, and not anti H-2 activity, which is mediating this lysis has not been investigated*. If most alloantisera in mice cannot mediate cell-dependent target cell destruction, then what is the importance of the interaction of these antibodies

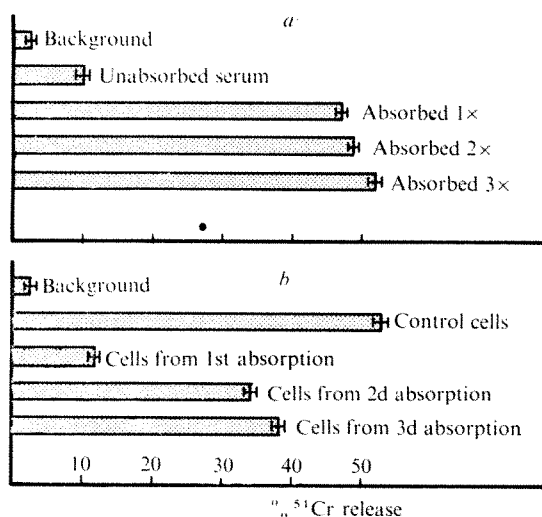


Fig. 2 The effect of absorption of serum with specific cells. 1 ml of BALB/c anti CBA/H (1/50) was absorbed for 30 min at room temperature with 3×10^7 CBA/H spleen cells, once, twice, or three times. The inhibitory activity was then assayed against fresh CBA/H spleen cells at serum concentration 1/125. Similarly, the cells used for the absorption were resuspended and their cytotoxic activity was assayed to see if they had been inhibited. *a*, The inhibitory activity is greatly reduced after only one absorption; *b*, the cytotoxic activity of the cells used for the absorption is greatly reduced. All studies done for 3 h at 37° C at effector: CRBC ratios of 200:1 and RACA concentration 1/25,000. Absorption with BALB/c cells (not shown) has no effect.

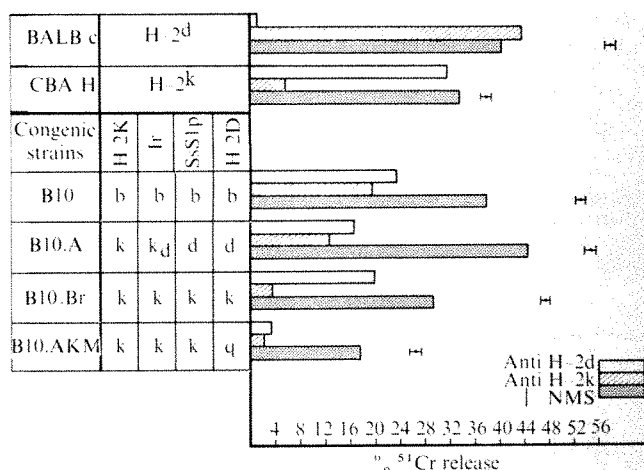


Fig. 3 Typical study of the association of inhibitory activity in alloantisera with H-2 antigens. Spleen cells (2×10^6) were incubated with RACA (1/25,000) and chromium labelled CRBC (10^4), in the presence of 1/50 normal mouse serum, anti H-2^d serum (CBA/H anti BALB/c) or anti H-2^k serum (BALB/c anti CBA/H). The controls with no added normal mouse serum are indicated (—●—). Complete specificity is shown on the basis of the reactions with BALB/c and CBA/H. The strong reactions of both sera with B10.A, and of anti H-2^k with B10.BR and B10.AKM are attributable to private and public specificities. The reactions of both sera with B10, and of anti H-2^d with B10.AKM, can be attributed to public specificities alone. The table indicates the source of the genetic material for this region in the H-2 complex. 'd', Derived from H-2^d; 'k', derived from H-2^k; 'b', derived from H-2^b; and 'q', derived from H-2^a (ref. 13).

with the Fc receptor of the effector cell? Perhaps the lack of killing of the target cell in such interaction in mice permits enhancement phenomena to occur more readily.

Finally, the CIA is negative with some sera that are weakly positive in complement-dependent lysis. This could be explained by differences in antibody classes: complement-dependent lysis will detect 7S and 19S antibody, whereas it seems likely the Fc receptor will be blocked only by 7S antibody. The sera which are positive in the CIA usually have titres higher than their complement-dependent cytotoxic titre. The CIA thus provides an important new alloantibody assay, independent of complement, for mouse immunogenetic studies, and also, as we shall report elsewhere, for immunogenetic studies in man.

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Note added in proof:* Similar arguments apply to other recent reports (Zigheboim, J., Bonavida, B., and Fahey, J. L., *J. Immunol.*, **111, 1737; 1973; Britton, S., and Forman, J., *Transplantation*, **17**, 180; 1974).

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- Wunderlich, J. R., Rosenberg, E. B., and Connolly, J. M., in *Progress in Immunology* (edit. by Amos, B.), **1**, 473 (Academic Press, New York, 1971).
- Hersey, P., Cullen, P., and MacLennan, I. C. M., *Transplantation*, **16**, 9 (1973).
- McConnachie, P. R., and Dossetor, J. B., *Tissue Antigens*, **3**, 303 (1973).
- Perlmann, P., and Perlmann, H., *Cell. Immunol.*, **1**, 300 (1970).
- Van Boxel, J. A., Stobo, J. D., Paul, W. E., and Green, I., *Science*, **175**, 194 (1972).

- ⁶ Snell, G. D., Cherry, M., and Demant, P., *Transpln Rev.*, **15**, 3 (1973).
⁷ Chia, E., and Festenstein, H., *Eur. J. Immun.*, **3**, 438 (1973).
⁸ MacLennan, I. C. M., *Transpln Rev.*, **13**, 67 (1972).
⁹ Perlmann, P., Perlmann, H., and Wigzell, H., *Transpln Rev.*, **13**, 91 (1972).
¹⁰ Perlmann, P., Perlmann, H., and Biberfeld, P., *J. Immun.*, **108**, 558 (1972).
¹¹ Greenberg, A. H., and Shen, L., *Nature new Biol.*, **145**, 282 (1973).
¹² Kiessling, R., and Klein, E., *J. exp. Med.*, **137**, 527 (1973).
¹³ Festenstein, H., and Demant, P., *Transpln Proc.*, **5**, 1321 (1973).

Inhibition and reversal of capping by cytochalasin B, vinblastine and colchicine

THE phenomenon of capping in lymphocytes¹⁻⁴ consists of the segregation of membrane components, specifically cross-linked by antibody or other ligand, at one pole of the cell, typically that surrounding the centrosome^{1,2}. Capping has been interpreted as resulting from a countercurrent flow of membrane components, in which the cross-linked patches are transported towards the centrosome region by interaction with cytoplasmic structures, while the unaffected parts of the membrane flow in the opposite direction^{1,2,5}.

This interpretation indicated a possible role in capping of contractile microfilaments and microtubules. With the exception of a negative report⁶, cytochalasin B, which apparently impairs microfilament function⁷, was found to inhibit consistently, although in most cases only partially, capping of surface immunoglobulin (Ig) (refs 1, 3, and 8). Microtubule-affecting drugs, like colcemid and vinblastine, had no inhibitory effect^{1,8}. It has been suggested, however, that microtubules could be involved in the control of surface Ig movement, as the inhibition by con A of capping of surface Ig-anti-Ig complexes could be reversed by high doses (10^{-4} M) of vinblastine and colchicine^{8,9}. By studying the combined effect of vinblastine or colchicine and cytochalasin B on capping of surface Ig and con A receptors on mouse spleen lymphocytes, I have now found evidence indicating that both microfilaments and microtubules can play a direct role in capping.

Rhodamine-labelled rabbit anti-mouse immunoglobulin (rh-RaMIg), fluorescein-labelled sheep anti-mouse immunoglobulin (fl-SaMIg) and rhodamine-labelled rabbit anti-sheep immunoglobulin (rh-RaSIg) were prepared according to Cebra and Goldstein¹⁰. Con A (Miles-Yeda) similarly was conjugated to fluorescein¹⁰ (fl-con A) in the presence of 0.1 M glucose, 5×10^{-4} M MnCl₂ and 5×10^{-4} M CaCl₂ and purified by chromatography on Sephadex G-50. Only that fraction which strongly bound to Sephadex at pH 7.2., that is, which in a stepwise elution could be eluted between 0.01 M and 0.10 M glucose, was used. BALB/c spleen cells were incubated at 37° C in Leibovitz medium (L15) (Flow) containing 0.2% bovine serum albumin. This medium does not contain glucose, reducing the possibility of secondary effects due to inhibition of glucose transport by cytochalasin^{11,12}.

Vinblastine (5×10^{-5} M or less) had no effect on, or slightly enhanced the percentage of cells capped by rh-RaMIg, while cytochalasin B (10 or 20 μ g ml⁻¹) was partially inhibitory (Table 1). However, when cytochalasin B and vinblastine were used together, capping was virtually completely inhibited (Table 1), even after several hours. The same effect was obtained substituting colchicine for vinblastine (Table 2). The inhibition occurred with these drugs in a dose range of 10^{-4} – 10^{-6} M, at which they are known to affect microtubules^{13,14}. The antibody was distributed as random spots all over the inhibited round cells (Fig. 1a). Using a second label (rh-RaSIg) to stain the first fluorescent antibody (fl-SaMIg), it was found that several, but not all, of these spots were intracellular. The combined use

Table 1 Capping of surface Ig in the presence of vinblastine and cytochalasin B

	Sample	% caps
Experiment 1	1 Control	94
	2 Vinblastine 2.5×10^{-5} M	92
	3 Cytochalasin B 10 μ g ml ⁻¹	66*
Experiment 2	Cytochalasin B (18 μ g ml ⁻¹) present in all samples:	
	4 No vinblastine	84†
	5 Vinblastine, 5×10^{-5} M	8
	6 Vinblastine, 1×10^{-5} M	8
	7 Vinblastine, 5×10^{-6} M	13
	8 Vinblastine, 1×10^{-6} M	18
	Recovery (≤ 0.1 μ g ml ⁻¹ cytochalasin B):	
	5a Recovery from 5 (vinblastine, 5×10^{-5} M)	27†
	6a Recovery from 6 (vinblastine, 1×10^{-5} M)	78†
	7a Recovery from 7 (vinblastine, 5×10^{-6} M)	88†

Washed BALB/c spleen cells (2×10^7 cell ml⁻¹) filtered through glass wool, were preincubated 35 min (Experiment 1) or 90 min (Experiment 2) at 37° C in vinblastine or cytochalasin B as indicated. Each sample (20–40 μ l) was incubated with 20–40 μ l of rh-RaMIg, 15 min at 37° C and washed once by centrifugation (always in the presence of the drugs). Part of samples 5, 6, 7 were washed with medium containing vinblastine, but not cytochalasin B, and incubated for 15 to 30 min at 37° C, before examination. Samples with cytochalasin B in this and other experiments contained 0.25% dimethylsulphoxide which had no effect on capping. Cells were scored as 'caps' if the stain was detectable over no more than one half of the cell, irrespective of whether it was on the surface or pinocytosed.

* In other experiments the inhibition varied from 20 to 70%.

† Mainly pinocytosed label.

of cytochalasin and vinblastine therefore also blocked the polar accumulation of this pinocytosed material, which was not completely prevented by either drug alone. The cooperative inhibitory effect of these drugs on capping was even more apparent when the inhibited cells were resuspended in fresh medium containing vinblastine or colchicine, but no cytochalasin (< 0.2 μ g ml⁻¹, final concentration). The inhibition was reversed (Tables 1 and 2) and in less than 15 min all the label accumulated at one pole of the cell. Most of the label seemed, however, to be intracellular, that is, pinocytosed. The recovery was essentially complete in the presence of 10^{-4} M colchicine or 10^{-5} M vinblastine, incomplete at higher doses of vinblastine (Tables 1 and 2). If colchicine was removed and cytochalasin left in the medium, the recovery was incomplete (Table 2).

Cytochalasin B was even more effective on capping of con A receptors by fl-con A. At doses of 20–100 μ g ml⁻¹ this fl-con A

Table 2 Inhibition of capping of surface Ig by cytochalasin B and colchicine

	Sample	% caps
	1 Control	85
	2 Colchicine (10^{-4} M)	98
	3 Cytochalasin B 20 μ g ml ⁻¹	71
	4 Cytochalasin B (20 μ g ml ⁻¹) + 10^{-4} M colchicine	5
	5 Cytochalasin B (20 μ g ml ⁻¹) + 10^{-5} M colchicine	8
	6 Cytochalasin B (20 μ g ml ⁻¹) + 10^{-6} M colchicine	17
	Recovery (< 0.2 μ g ml ⁻¹ cytochalasin B):	
	4a Recovery from 4 (colchicine, 10^{-4} M)	83
	5a Recovery from 5 (colchicine, 10^{-5} M)	82
	Recovery (15 μ g ml ⁻¹ cytochalasin B):	
	4b Recovery from 4 (colchicine, $< 10^{-8}$ M)	30
	5b Recovery from 5 (colchicine, $< 10^{-9}$ M)	36

4×10^7 cells ml⁻¹ were preincubated at 37° C for 75 min in the presence of colchicine and cytochalasin B, as indicated, and then incubated with rh-RaMIg for 15 min at 37° C. Part of samples 4 and 5 were washed to remove either colchicine or cytochalasin B from the samples, and incubated for 15 min at 37° C (recovery).

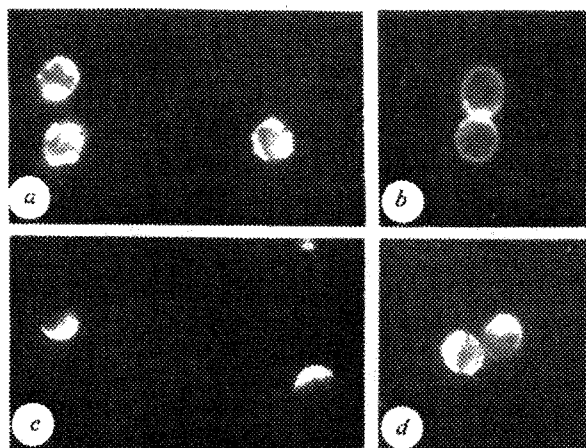


Fig. 1 Inhibition and reversion of caps. The photographs were taken using a Leitz Ortoplan microscope equipped with Opak-Fluor vertical illumination (Leitz GmbH-Wetzlar). *a*, Cells stained with rh-RaMlg at 37° C in the presence of 20 $\mu\text{g ml}^{-1}$ cytochalasin B and 10^{-6} M colchicine (see Table 2); *b*, cells stained with 50 $\mu\text{g ml}^{-1}$ of fl-con A at 37° C in the presence of 20 $\mu\text{g ml}^{-1}$ cytochalasin B; *c*, cells stained for 10 min at 23° C with rh-RaMlg in the presence of 5×10^{-5} M vinblastine. Typical caps; *d*, the same, but incubated further for 35 min at 37° C with 20 $\mu\text{g ml}^{-1}$ cytochalasin B. Spotty rings, with some accumulation of label on one side of the cell.

preparation inhibited surface Ig capping^{3,8} only by 30–40% and formed con A caps in about 50% of the spleen cells in 10 min at 37° C. This capping was completely and reversibly inhibited by 10 min exposure to 10 $\mu\text{g ml}^{-1}$ of cytochalasin B, either in the presence or in the absence of vinblastine (Table 3; Fig. 1*b*). The change in cell shape from round to variably elongated which accompanied con A capping was also inhibited by cytochalasin.

Table 3 Inhibition of capping of con A receptors by cytochalasin B and vinblastine

Sample	% caps
Experiment 1	
1 Control	32
2 Vinblastine, 10^{-4} M	51
3 Cytochalasin B (10 $\mu\text{g ml}^{-1}$)	< 1
4 Vinblastine, 10^{-4} M + cytochalasin B (10 $\mu\text{g ml}^{-1}$)	0
Recovery (<0.15 $\mu\text{g ml}^{-1}$ cytochalasin B):	
3a Recovery from 3 (no vinblastine)	45
4a Recovery from 4 (vinblastine, 10^{-4} M)	36
Experiment 2	
5 Vinblastine, 10^{-5} M + cytochalasin B (10 $\mu\text{g ml}^{-1}$)	0
5a Recovery (cytochalasin B <0.1 $\mu\text{g ml}^{-1}$, vinblastine, 10^{-5} M)	52
5b Recovery (cytochalasin B 10 $\mu\text{g ml}^{-1}$, vinblastine $\leq 5 \times 10^{-5}$ M)	0

4×10^7 spleen cells were preincubated for 30 or 60 min at 37° C in vinblastine or cytochalasin B or in medium alone, then 90 μl of each sample were incubated with 10 μl of fl-con A (50 $\mu\text{g ml}^{-1}$ final concentration) for 15 min at 37° C. Part of samples 3, 4 and 5 were washed with medium free of cytochalasin or, respectively, vinblastine, as indicated, and incubated for further 30 min at 23° C (samples 3a, 4a) or 20 min at 37° C (samples 5a, b) before examination.

These findings demonstrate that cytochalasin-sensitive structures play an essential role in cap formation, and that microtubules are also somehow involved. The nature of the interaction between these structures is unknown. It is likely that the former corresponds to a cortical layer of microfilaments which interact directly with membrane elements⁷. The latter may interact indirectly with the membrane, perhaps through the mediation of microfilaments, and probably constitutes at least part of the internal cellular frame with respect to which mem-

brane movement occurs^{2,5}. As capping is not blocked by microtubule disruption, it seems likely that part of this frame and its polarity are preserved by other cellular structures. The different degree of inhibition by cytochalasin B on con A receptors and surface Ig capping could reflect differences in their interaction with cytoplasmic structures. It is also conceivable, however, that the function of these structures is affected only incompletely by cytochalasin B, to an extent sufficient to block cell movement, but not partial membrane displacements, which can occur also in the absence of cell movement^{2,5}. Differences in the extent of segregation achieved could then be quantitative and depend on the degree of cross-linking, size and number of the patches formed by a particular ligand.

Further evidence for the role of cytochalasin-sensitive structures, which indicates that they are involved in holding together the capped membrane, was provided by experiments in which preformed caps were 'reversed' into rings using cytochalasin. Cytochalasin B (10 $\mu\text{g ml}^{-1}$), either in the presence or the absence of vinblastine, reversed almost 90% of con A caps in 45 min at 37° C (Table 4), whereas little or no reversion was observed in rh-RaMlg caps. A large part of rh-RaMlg, however, was already pinocytosed within 10 min before the addition of cytochalasin, whereas, on the contrary, most of the capped con A remained on the surface. Repeating the experiment with

Table 4 Reversal of caps by cytochalasin B

	Time (min)	% caps
Experiment 1		
fl-con A caps (37° C, no vinblastine)	0	45
	45	6
Experiment 2		
rh-RaMlg caps (23° C, 5×10^{-5} M vinblastine)	0	91
	35	34

In Experiment 1, fl-con A caps were induced in 10 min at 37° C as described in Table 3 ($t=0$), then 10 $\mu\text{g ml}^{-1}$ of cytochalasin B were added and incubation continued, without washing, for 45 min at 37° C. In Experiment 2, spleen cells, preincubated for 2 h at 37° C with 5×10^{-5} M vinblastine, were incubated with rh-RaMlg at 23° C for 20 min. After washing, part of the sample was examined ($t=0$) and the rest incubated for 35 min at 23° C in the presence of 20 $\mu\text{g ml}^{-1}$ of cytochalasin B.

rh-RaMlg at 23° C and in the presence of vinblastine, which favours the formation of a true surface cap before the onset of appreciable pinocytosis, rh-RaMlg caps could be reversed by cytochalasin into spotty rings in about 60% of the cells (Fig. 1*b, c*; Table 4). Other (unpublished) data indicate that con A 'reversions' can also be induced by metabolic inhibitors, in agreement with an earlier report of Sällstrom and Alm¹⁵. These findings indicate that cytochalasin-sensitive structures are responsible for holding together the labelled patches into a single cap, even when these are not completely cross-linked by the ligand into a single complex; microtubules seem to be less important. On adding cytochalasin, the labelled patches are released and can mix again with the unlabelled membrane.

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¹ Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225 (1971).

² de Petris, S., and Raff, M. C., *Eur. J. Immun.*, **2**, 523 (1972).

³ Loo, F., Forni, L., and Pernis, B., *Eur. J. Immun.*, **2**, 203 (1972).

⁴ Unanue, E. R., Perkins, W. D., and Karnovsky, M. J., *J. exp. Med.*, **136**, 885 (1972).

⁵ de Petris, S. and Raff, M. C., in *Locomotion of Tissue Cells*, Ciba Foundation Symposium 14 (new series), 27, (Elsevier-North Holland, Amsterdam, 1973).

- ⁶ Unanue, E. R., Karnovsky, M. J., and Engers, H. D., *J. exp. Med.*, **137**, 675 (1973).
- ⁷ Wessels, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, T. T., and Yamada, K. M., *Science*, **171**, 135 (1971).
- ⁸ Edelman, G. M., Yahara, I., and Wang, J. L., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1442 (1973).
- ⁹ Yahara, I., and Edelman, G. M., *Nature*, **236**, 152 (1973).
- ¹⁰ Cebra, J. J., and Goldstein, G., *J. Immun.*, **95**, 230 (1965).
- ¹¹ Kletzien, R. F., Perdue, J. F., and Springer, A., *J. biol. Chem.*, **247**, 2964 (1972).
- ¹² Cohn, R. H., Banerjee, S. D., Shelton, E. R., and Bernfield, M. R., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 2865 (1972).
- ¹³ Bishey, A. N., and Freed, J. J., *Expl Cell Res.*, **64**, 419 (1971).
- ¹⁴ Borisy, G. G., and Taylor, E. W., *J. Cell Biol.*, **34**, 525 (1967).
- ¹⁵ Sällström, J. F., and Alm, G. V., *Expl Cell Res.*, **75**, 63 (1972).

Inhibition of surface capping of macromolecules by local anaesthetics and tranquillisers

IMMUNOGLOBULIN (Ig) molecules on the surface of B lymphocytes aggregate into a 'cap' when the cell is treated with anti-Ig¹⁻³. A similar process occurs with concanavalin A (con A) on neutrophil polymorphonuclear leukocytes (PMN)⁴. Capping occurs in two stages in both of these cell types^{4,5}: first, the ligand-receptor complexes aggregate into multiple clusters of variable size, leaving intervening bare membrane; and second, the clusters are swept into a single mass. Both stages are temperature-dependent (that is, they do not occur in the cold), but only the latter is energy-dependent (that is, clustering occurs but capping is inhibited on cells treated with metabolic inhibitors)⁴⁻⁶. The formation of clusters can be attributed to the ability of the ligand to cross-link receptors⁵; some form of membrane activity then sweeps the clusters into the cap. Capping can occur in the absence of cell locomotion^{4,7}, but if locomotion does occur the cap is found at the trailing end of the cell⁴. These aspects of capping have recently been analysed in detail^{4,7}.

We have now found that capping of con A on PMN, and of anti-Ig-Ig complexes on lymphocytes, is reversibly inhibited by local anaesthetics and tranquillisers which act as membrane stabilisers, characteristically able to inhibit membrane depolarisation⁸⁻¹¹.

Human PMN attached to glass coverslips⁴ were exposed to fluorescein-conjugated con A (100 $\mu\text{g ml}^{-1}$) in Hanks balanced salt solution (BSS) for 10 min at 4° C, washed in BSS, incubated (in the presence or absence of drugs) in BSS containing 10% foetal calf serum for 10 min at 4° C and then warmed to 37° C. In agreement with our previous observations⁴, fluorescence microscopy showed that, in the absence of drugs, approximately 95% of such cells developed caps of con A within 5 min; the PMN were motile and the caps were situated at the trailing end of the cell. Capping was inhibited in the presence of the local anaesthetics and tranquillisers; in addition, cell locomotion was suppressed. Testing with a range of doses (from 10^{-6} M to 4×10^{-2} M) established that complete inhibition of capping and locomotion occurred with 4×10^{-2} M Xylocaine, 10^{-3} M Nupercaine, 2×10^{-4} M chlorpromazine and 10^{-4} M trifluoperazine. Reversibility of this phenomenon was tested by washing away the drug just before warming to 37° C: capping was restored on approximately 95% of cells after treatment with Xylocaine, on 85% after Nupercaine, on 75% after chlorpromazine and on 50% after trifluoperazine.

The effect of Xylocaine on the redistribution of con A receptors on PMN was examined by electron microscopy of

shadow-cast surface replicas (using *Busycon canaliculatum* haemocyanin as a marker for con A¹²). Glass-attached PMN were exposed to con A (100 $\mu\text{g ml}^{-1}$) in BSS for 10 min at 4° C, washed in BSS, treated with haemocyanin (500 $\mu\text{g ml}^{-1}$) in BSS for 10 min at 4° C, washed in BSS and incubated (in the presence or absence of Xylocaine) in BSS containing 10% foetal calf serum for 10 min at 4° C. The cells were then warmed to 37° C for 10 min before fixation in glutaraldehyde and preparation of surface replicas⁴. As previously described⁴, electron microscopy of such specimens showed a random, dispersed distribution of con A on control cells labelled and fixed at 4° C; after warming to 37° C, the cells showed active locomotion and the con A quickly formed a single aggregate at the tail; if cell motility was impaired (for example, with cytochalasin B) capping occurred, but over the central region of the cell⁴. On PMN treated with Xylocaine, the con A aggregated into multiple, irregular, scattered patches at 37° C, but did not cap. Thus, capping was inhibited but not the temperature-dependent formation of patches.

We then studied the effect of these drugs on the capping of anti-Ig on lymphocytes. Mouse spleen cells were incubated with fluorescein-conjugated rabbit anti-mouse Ig (RAMG)⁵ for 15, 30 or 45 min at room temperature in the presence or absence of the drugs. After fixation in paraformaldehyde, the pattern of labelling was assessed by fluorescence microscopy. Capping was inhibited to different degrees by each drug (using the doses, mentioned above, which caused complete inhibition of con A capping on PMN) (Fig. 1). The inhibition by Xylocaine was complete. On the uncapped cells, label was distributed in multiple patches over the surface; such patches were small, in contrast to the large aggregates seen on metabolically inhibited lymphocytes⁵. Reversibility of this drug inhibition was tested in two ways: cells were exposed to the drug for 30 min, washed, and treated with RAMG for 30 min without the drug; or cells were treated with RAMG in the presence of the drug for 30 min, washed and incubated without RAMG or the drug for 30 min. Figure 2 shows that removal of the drug restored capping ability. This reversibility was particularly striking with Xylocaine.

Local anaesthetics inhibit membrane depolarisation by reducing sodium and potassium conductances⁹. This seems to follow displacement of calcium ions from binding sites in the membrane^{9,11,13,14}. In erythrocytes, such treatment leads

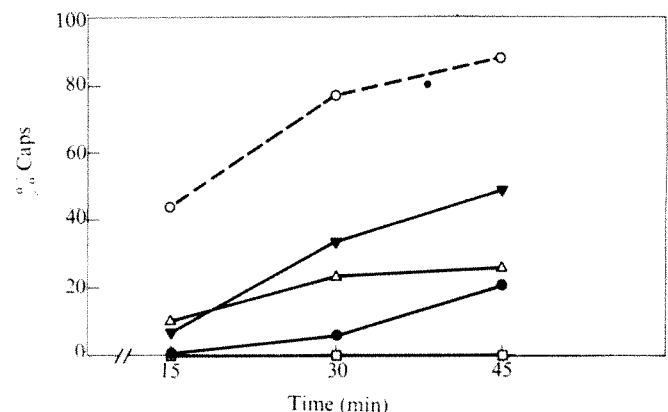


Fig. 1 Lymphocytes were collected from mouse spleens and purified by centrifuging on a Ficoll-Hypaque gradient; 5×10^6 cells (in 0.5 ml of Hanks BSS containing 1% foetal calf serum and the appropriate drug) were placed on tissue culture dishes; 50 μg of fluorescent RAMG was added and the suspension was incubated for 15, 30 or 45 min at room temperature, at which time an equal volume of 2.5% paraformaldehyde was added to stop the reaction. O, Control; □, 4×10^{-2} M Xylocaine; ▼, 10^{-3} M Nupercaine; ●, 2×10^{-4} M chlorpromazine; △, 10^{-4} M trifluoperazine.

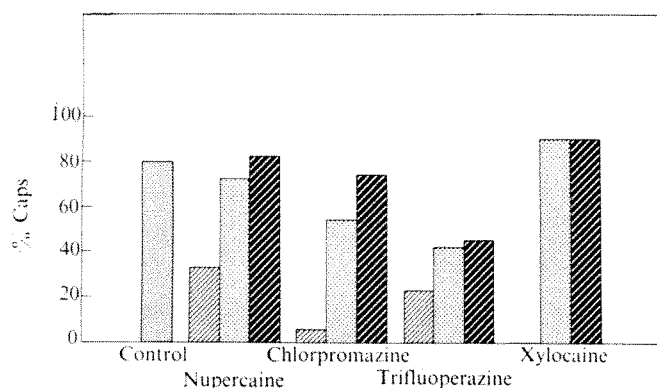


Fig. 2 Reversibility of the inhibition produced by the drugs. Light hatching, Lymphocytes (5×10^6 in 0.5 ml) were incubated with the drug for 30 min in the presence of 50 μ g of fluorescent RAMG. (Note complete inhibition by Xylocaine). Coarse dots, Lymphocytes were incubated with the drug for 30 min, washed once and then incubated in fresh media containing RAMG for 30 min. Heavy hatching, Lymphocytes were incubated with the drug and RAMG for 30 min, washed once, and then resuspended in media free of drug and RAMG for 30 min.

to expansion^{15,16} and physical stabilisation¹⁰ of the membrane, providing protection against osmotic lysis¹⁷. Other effects of such drugs include the inhibition of several kinds of cell-to-cell interaction (platelet adhesiveness^{18,19}, cell fusion²⁰, leukocyte adherence to endothelium²¹), as well as inhibition of phytohaemagglutinin-induced lymphocytic transformation²². Our experiments indicate that they also affect the redistribution of membrane-bound ligands. Whether this is due to 'molecular packing' induced by the burying of drug molecules within the membrane⁸, or distortion and expansion of membrane protein¹⁵, or some other intrinsic membrane derangement is not clear. Because the movement of patches into caps may depend on cytoplasmic influences (such as an intact metabolism, and a colchicine-sensitive system and (or) cell locomotion)⁴⁻⁷, another possibility is that the drugs affect cytoplasmic constituents. Thus, the immobilisation of PMN during drug exposure (also reported for procaine-treated fibroblasts²³) may be due to an inhibition of release of internal calcium causing an inability to activate the contractile process²⁴. In addition, Xylocaine has been reported to cause a reversible disappearance of microtubules in rabbit vagus nerve²⁵. This may have relevance to recent data from this laboratory showing that capping can occur with a cross-linking ligand in the absence of cell movement, but only if a colchicine-sensitive system is intact^{4,7}.

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- Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225-229 (1971).
- Unanue, E. R., Perkins, W. D., and Karnovsky, M. J., *J. exp. Med.*, **136**, 885-906 (1972).
- Karnovsky, M. J., Unanue, E. R., and Leventhal, M., *J. exp. Med.*, **136**, 907-930 (1972).
- Ryan, G. B., Borysenko, J. Z., and Karnovsky, M. J., *J. Cell Biol.* (in the press).
- Unanue, E. R., Karnovsky, M. J., and Engers, H. D., *J. exp. Med.*, **137**, 675-689 (1973).
- Loor, F., Forni, L., and Pernis, B., *Eur. J. Immun.*, **2**, 203-212 (1972).
- Unanue, E. R., Ault, K. A., and Karnovsky, M. J., *J. exp. Med.*, **139**, 295-312 (1974).

- Shanes, A. M., *Nature*, **188**, 1209-1210 (1960).
- Blaustein, M. P., and Goldman, D. E., *Science*, **153**, 429-432 (1966).
- Seeman, P. M., *Int. Rev. Neurobiol.*, **9**, 145-221 (1966).
- Covino, B. G., *New Engl. J. Med.*, **286**, 975-983 (1972).
- Smith, S. B., and Revel, J.-P., *Dev. Biol.*, **27**, 434-441 (1972).
- Feinstein, M. B., *J. gen. Physiol.*, **48**, 357-374 (1964).
- Papahadjopoulos, D., *Biochim. biophys. Acta*, **265**, 169-186 (1972).
- Seeman, P., Kwant, W. O., Sauks, T., and Argent, W., *Biochim. biophys. Acta*, **183**, 490-498 (1969).
- Seeman, P., Kwant, W. O., and Sauks, T., *Biochim. biophys. Acta*, **183**, 499-511 (1969).
- Roth, S., and Seeman, P., *Nature new Biol.*, **231**, 284-285 (1971).
- O'Brien, J. R., *J. clin. Path.*, **14**, 140-149 (1961).
- O'Brien, J. R., *J. clin. Path.*, **15**, 446-455 (1962).
- Poste, G., and Reeve, P., *Expl Cell Res.*, **72**, 556-560 (1972).
- Giddons, D. B., and Lindhe, J., *Am. J. Pathol.*, **68**, 327-338 (1972).
- Cullen, B. F., Chretien, P. B., and Leventhal, B. G., *Brit. J. Anaesth.*, **44**, 1247-1251 (1972).
- Gail, M. H., and Boone, C. W., *Expl Cell Res.*, **73**, 252-255 (1972).
- Feinstein, M. B., and Paimre, M., *Fedn Proc.*, **28**, 1643-1648 (1969).
- Byers, M. R., Fink, B. R., Kennedy, R. D., Middaugh, M. E., and Hendrickson, A. E., *J. Neurobiol.*, **4**, 125-143 (1973).

Increased sensitivity of cell-free protein synthesis to double-stranded RNA after interferon treatment

NATURALLY occurring and synthetic double-stranded RNAs (dsRNAs) are interferon inducers¹. They inhibit protein synthesis in animal cells² and cell-free systems³⁻⁵. It is intriguing, therefore, that interferon treatment renders cells more sensitive to the toxic effects of dsRNA⁶, particularly as viral dsRNA may be involved in the replication of RNA viruses and has been isolated from DNA virus-infected cells⁷. Accordingly, the production of viral dsRNA in response to infection could bring about a general inhibition of protein synthesis in the interferon-treated cell, as is indeed observed in interferon-treated, vaccinia virus-infected mouse fibroblast L cells^{8,9}. The cell dies but little virus is produced: an effective way of limiting a natural infection.

Here we report an interferon-induced increase in the sensitivity of virus protein synthesis to inhibition by dsRNA in cell-free systems from interferon-treated L cells. Previously, we showed a similar enhanced inhibition of protein synthesis in such systems in response to infection with vaccinia or encephalomyocarditis (EMC) virus^{10,11}. The possibility that, in accord with the above hypothesis, this enhanced interferon-mediated inhibition of protein synthesis seen after infection is triggered by the synthesis of very small amounts of viral dsRNA is discussed.

The cell-free extracts used throughout were crude post-mitochondrial supernatant fractions from uninfected L cells. They were neither preincubated to lower endogenous incorporation nor passed through Sephadex to reduce the pool of cold amino acids. With such systems, a two to five-fold stimulation of amino acid incorporation in response to added EMC RNA as message is routinely observed and this stimulation is normally only slightly inhibited ($\leq 30\%$) by treatment of the cells with low doses (≤ 300 reference or 30 effective units ml^{-1} , see legend to Fig. 1) of interferon¹⁰. It is known that the added EMC RNA is translated correctly in these systems to yield EMC-specific polypeptides, initiation occurring at a single major site as in the intact EMC-infected cell¹²⁻¹⁶.

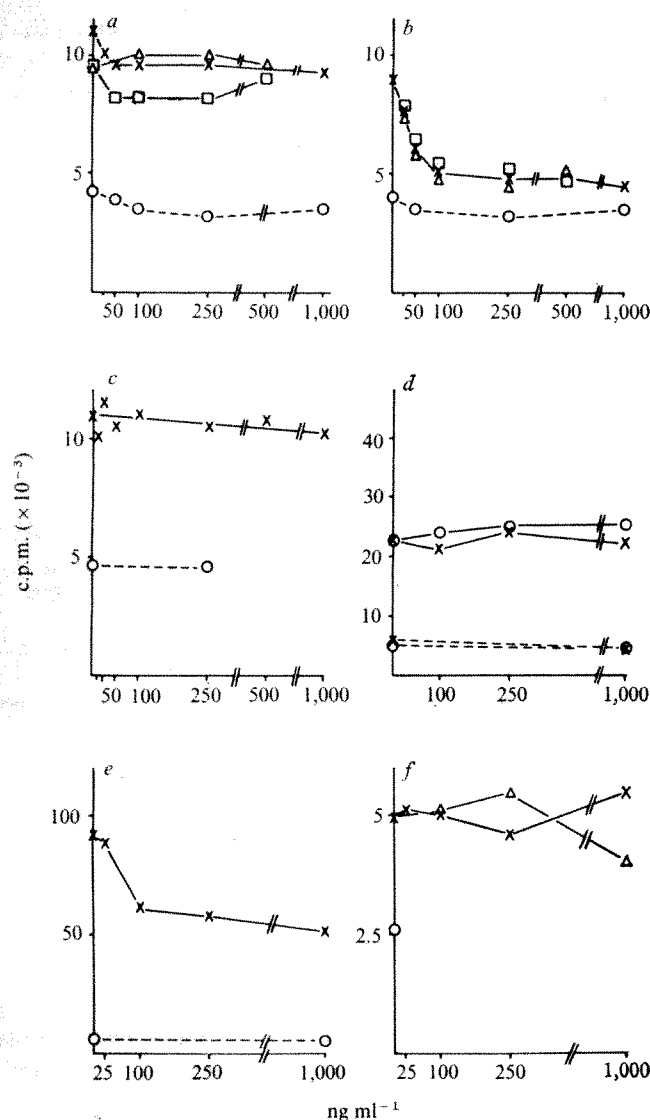


Fig. 1 Sensitivity of various cell-free systems to dsRNA and DNA. Crude postmitochondrial supernatant fractions were used throughout. The extracts in *a*, *b*, *c*, *d* and *f* were from L cells, that in *e* from Krebs cells. Interferon pretreatment of the cells was with 200 (*b*) or 300 (*d* and *f*) units ml^{-1} . The interferon concentrations throughout are in International Reference units based on the NIH standard. Ten reference units were equivalent to approximately one effective unit in reducing the yield of EMC by 50%. The heat-inactivated interferon (*c*) was at a concentration equivalent to 300 units ml^{-1} before inactivation: the heat treatment (60°C for 20 min) was just sufficient to destroy its antiviral activity. *a*, Control; *b*, endogenous amino acid incorporation \pm increasing amounts of reovirus dsRNA (O); EMC RNA-stimulated incorporation \pm reovirus (\times) and *P. chrysogenum* (\square) dsRNAs or poly(I)·poly(C) (Δ). The abscissa in all cases gives the range of final concentrations of the dsRNA, poly(I)·poly(C), or (in *f*) DNA in the assay. *c*, Heat-inactivated interferon; endogenous (O) and EMC RNA-stimulated (\times) amino acid incorporation \pm reovirus dsRNA. *d*, Endogenous (---) and poly U-stimulated (—) ^3H -phenylalanine incorporation \pm poly(I)·poly(C) with extracts from interferon-treated (\times) and control (O) cells. *e*, Krebs cell-free system; endogenous (O) and EMC RNA-stimulated incorporation (\times) \pm reovirus dsRNA. *f*, Endogenous (O) and EMC RNA-stimulated incorporation with extracts from interferon-treated L cells \pm calf thymus (Δ) and adenovirus (\times) DNA. The interferon pretreatment of the cells, the preparation of the cell-free extracts and their assay \pm EMC RNA or poly(U) have already been described¹³. The ordinates give the ^{14}C -amino acid (mixture) or ^3H -phenylalanine incorporation (in c.p.m. $\times 10^{-3}$ per 50 μl assay)¹³. Mouse L cell interferon purified by affinity chromatography²¹ ($\geq 5 \times 10^7$ units per mg protein) from Dr K. Paucker was used in most experiments. Electrophoretically purified interferon ($> 10^7$ units per mg protein) was from

MRE Porton, England. To check the effectiveness of the interferon treatment, samples of the cells used to prepare the extracts in these experiments were plated as monolayers, infected with 4–10 plaque-forming units per cell of EMC and the virus yield after 17 h at 37°C was assayed by haemagglutination. Reovirus dsRNA was from Dr J. J. Skehel, *P. chrysogenum* virus dsRNA²² from Dr R. A. Cox and adenovirus DNA from Dr W. C. Russell. Poly(I)·poly(C) lot 8, Control No. 11-8-321) and poly(U) were from Miles-Seravac (Pty) Ltd, Maidenhead, Berkshire, England.

In such cell-free systems from interferon-treated L cells the translation of added EMC RNA was markedly inhibited (50–90%) by reovirus and *Penicillium chrysogenum* phage dsRNAs and by poly(I)·poly(C) (Fig. 1*b*). These dsRNAs had much less effect on the corresponding untreated systems (Fig. 1*a*), on endogenous incorporation (Fig. 1*a* and *b*) or on the translation of poly(U) (Fig. 1*d*). The insensitivity of the untreated L cell system could be analogous to that of the chick fibroblast cell-free system¹⁷, but is in contrast to the rabbit reticulocyte and, to a lesser extent, the Krebs cell-free systems (Fig. 1*e*) (refs 3–5). The basis for this is not known.

On occasion, particularly on pretreatment of L cells with higher concentrations of interferon, we have observed some inhibition of the translation of EMC RNA in the cell-free system in the absence of infection or dsRNA. Such systems, however, still showed an enhanced sensitivity to dsRNA. The addition of dsRNA to a 50% inhibited system from cells pretreated with 500 reference units ml^{-1} of interferon for example, resulted in a virtually complete inhibition of the translation of the added EMC RNA.

The minimum concentration of dsRNA required for inhibition in cell-free systems from interferon-treated L cells (10–50 ng ml^{-1} , Fig. 1*b*) was higher than that required in the reticulocyte cell-free system (0.1–1 ng ml^{-1}) but was of the same order as that observed for the Krebs system¹ (Fig. 1*e*). No inhibition of incorporation was observed with similar concentrations (10–1,000 ng ml^{-1}) of adenovirus, calf thymus or SV40 DNA, rRNA, poly(I), poly(C) or alkali-digested poly(I)·poly(C) all of which gave data essentially identical to those shown in Fig. 1*f*. These results indicate that the inhibitory agent is indeed dsRNA and that the major inhibitory effect is likely to be on the initiation of polypeptide chains^{3–5} rather than on chain elongation as represented by the majority of endogenous incorporation in these systems. The rate and extent of EMC RNA-stimulated incorporation were both inhibited by poly(I)·poly(C) in cell-free systems from interferon-treated L cells (Fig. 2).

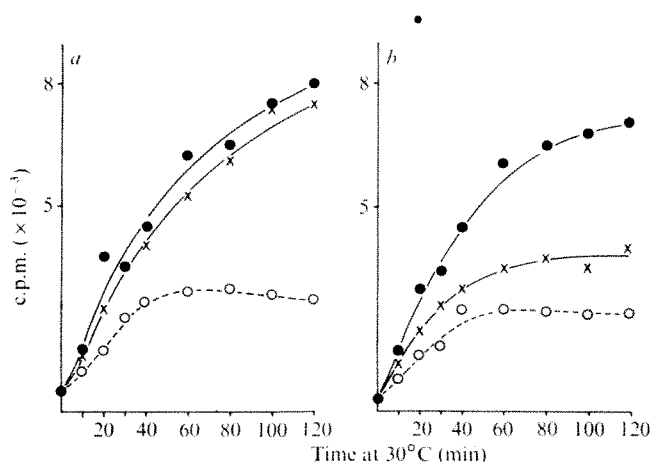


Fig. 2 Time course of amino acid incorporation in L cell-free systems from *a*, Control and *b*, interferon-treated (30 reference units ml^{-1}) cells. Endogenous (O) and EMC RNA-stimulated ^{35}S -methionine incorporation in the presence (\times) and absence (\bullet) of poly(I)·poly(C) (250 ng ml^{-1}).

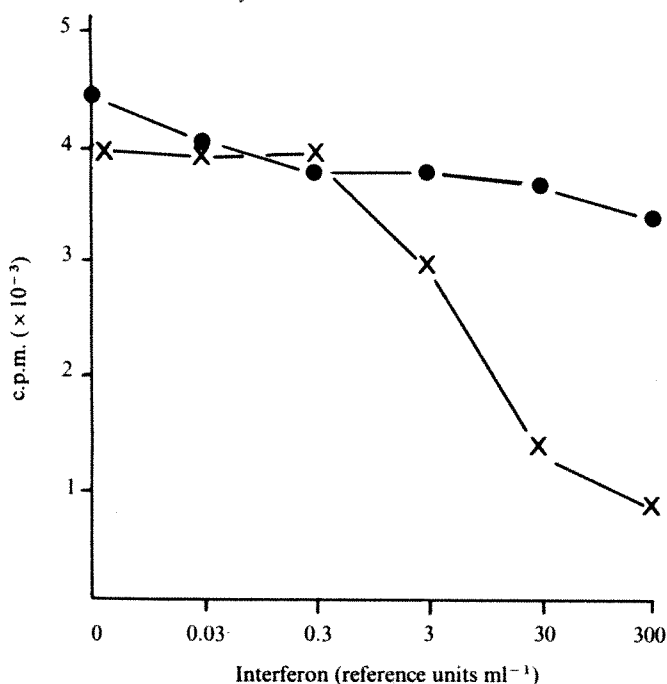


Fig. 3 Dependence on interferon concentration of the enhanced sensitivity to dsRNA of EMC RNA-programmed cell-free protein synthesis. Ordinate: EMC RNA-stimulated amino acid incorporation in the L cell-free system in the absence (●) and presence (x) of 250 ng ml⁻¹ of poly(I):poly(C). Abscissa: interferon concentration used in treatment of the L cells¹³ before isolation of the cell-free extracts. Ten reference units (legend Fig. 1) of interferon were equivalent to approximately 1 effective unit. Accordingly, here EMC virus growth was inhibited by 30, ≥97 and ≥97% when assayed (legend Fig. 1) in samples of these cells exposed to 3, 30 and 300 reference (0.3, 3 and 30 effective) units ml⁻¹, respectively, of interferon.

The inhibition was relatively independent of extract concentration and was observed at all concentrations of EMC RNA tested.

An important consequence of these results is that in any experiment involving a comparison of the translation of different viral and host mRNAs in cell-free systems from interferon-treated cells, it will be necessary to consider the possible effect of even very small amounts of intrinsic or contaminating dsRNA structures.

The enhanced sensitivity to dsRNA has been seen with many cell-free extracts from eight different batches of L cells exposed to 10–500 reference (1–50 effective) units ml⁻¹ of four different preparations of highly-purified mouse interferon (>10⁷ reference units per mg protein) from two different laboratories. A dose response curve is shown in Fig. 3. Cell-free systems from L cells pretreated with interferon inactivated with respect to antiviral activity by heat (Fig. 1c) or trypsin treatment were insensitive to dsRNA. In addition, interferon treatment of Krebs cells of the line in use in this laboratory, which are very insensitive to interferon, did not enhance the sensitivity of EMC RNA translation in the cell-free system to dsRNA (data not shown).

The increased sensitivity to dsRNA reported here for cell-free systems from interferon-treated L cells raises the possibility that the enhanced inhibition of translation seen in cell-free systems from interferon-treated, vaccinia¹⁰, EMC¹¹ and mengo¹⁸ virus-infected L cells is triggered by the synthesis of small amounts of viral dsRNA. The events observed in the intact interferon-treated, vaccinia-infected L cell are consistent with this^{8,9}: both host and viral protein synthesis are inhibited (although whether by the same or different mechanisms is not yet clear), the cell dies and little virus is produced. A similar sequence of events occurs although more slowly in the interferon-treated EMC-infected L cell¹¹ (M. Esteban, D. R. Tovell, and I. M. K.,

unpublished). Double-stranded RNA has been detected in extracts from vaccinia-infected cells⁷ and may play a part in the replication of RNA viruses such as EMC. It is still not clear, however, that dsRNA as such exists in the intact cell¹⁹ and our initial attempts to detect a dsRNA fraction inhibitory to protein synthesis in cell-free systems from interferon-treated, vaccinia-infected L cells have been unsuccessful (L. A. B. and T. Hunt, unpublished). A direct connection between the effect of dsRNA and of infection on protein synthesis in the interferon-treated cell, therefore, remains to be established, but the copurification of the antiviral and dsRNA toxicity-enhancing properties of interferon preparations²⁰ strongly suggests that they are related.

Our results demonstrate an enhanced sensitivity of protein synthesis to dsRNA which could play a part in determining the fate of interferon-treated cells after infection with a variety of different viruses, possibly reflecting a mechanism basic to interferon action. If nothing more, they indicate a basis for the enhanced toxicity of dsRNA in the interferon-treated cell and provide further direct evidence for an interferon-mediated change in the protein synthetic apparatus of the cell.

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- Colby, C., and Morgan, M. J., *A Rev. Microbiol.*, **25**, 333–360 (1971).
- Cordell-Stewart, B., and Taylor, M. W., *J. Virol.*, **11**, 232–242 (1973).
- Ehrenfeld, E., and Hunt, T., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1075–1078 (1971).
- Robertson, H. D., and Mathews, M. B., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 225–229 (1973).
- Kaempfer, R., and Kaufman, J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1222–1226 (1973).
- Stewart, W. E., de Clercq, E., and de Somer, J., *J. gen. Virol.*, **18**, 237–246 (1973).
- Duesberg, P. H., and Colby, C., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 396–403 (1969).
- Joklik, W. K., and Merigan, T. C., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 558–565 (1966).
- Metz, D. H., and Esteban, M., *Nature*, **238**, 385–388 (1972).
- Friedman, R. M., Metz, D. H., Esteban, M., Tovell, D. R., Ball, L. A., and Kerr, I. M., *J. Virol.*, **10**, 1184–1198 (1972).
- Kerr, I. M. *et al. Advances in the Biosciences* (edit. by Habermehl, K. O., and Diefenthal, W.) **11**, 109–123 (Pergamon Press, Vieweg, 1973).
- Dobos, P., Kerr, I. M., and Martin, E. M., *J. Virol.*, **8**, 491–499 (1971).
- Kerr, I. M., Friedman, R. M., Brown, R. E., Ball, L. A., and Brown, J. C., *J. Virol.*, **13**, 9–21 (1974).
- Boime, I., Aviv, H., and Leder, P., *Biochem. biophys. Res. Commun.*, **45**, 788–795 (1971).
- Eggen, K. L., and Shatkin, A. J., *J. Virol.*, **9**, 636–645 (1972).
- Smith, A. E., *Eur. J. Biochem.*, **33**, 301–313 (1973).
- Shenk, T. E., and Stollar, V., *Biochim. biophys. Acta*, **287**, 501–513 (1972).
- Falcoff, E., Falcoff, R., Lebleu, B., and Revel, M., *J. Virol.*, **12**, 421–430 (1973).
- Thach, S. S., and Thach, R. E., *J. molec. Biol.*, **81**, 367–380 (1973).
- Stewart, W. E., de Clercq, E., de Somer, P., Berg, K., Ogburn, C. A., and Paucker, K., *Nature new Biol.*, **246** 141–143 (1973).
- Ogburn, C. A., Berg, K., and Paucker, K., *J. Immun.*, **111**, 1206–1217 (1973).
- Cox, R. A., Kanagalingam, K., and Sutherland, E. S., *Biochem. J.*, **120**, 549–558 (1970).

Excess males among siblings of Australian antigen carriers

REPEATED studies have shown that Australia antigen carriers tend to group in families and there is considerable evidence that susceptibility to chronic asymptomatic infection producing Australia antigen (Au, hepatitis B antigen) is inherited as an autosomal recessive trait¹⁻³. The occurrence of asymptomatic carriers of Au varies in different populations, ranging from 0.1% in the United States and northern Europe to 24% in the Melanesian population of the Lau people on Malaita, as measured by immunodiffusion⁴. The frequency of Au carriers is relatively high in Oceania and particularly high in Melanesian populations^{5,6}. If this trait is genetically determined, there would be a greater number of individuals who are either homozygous or heterozygous for the proposed recessive gene, *Au*¹, in populations where the trait is common. Blumberg has suggested that these individuals may possess a selective advantage in certain environments⁷.

In 1960, Dr William Davenport confirmed earlier observations concerning an excess of males on the island of Santa Cruz in the British Solomon Islands Protectorate. He suggested that this alteration in sex ratio might be the result of selection by an infectious disease (personal communication). Blumberg⁸ reported that there was a deficiency of male offspring of certain matings when one of the parents had Au, that is, that Au is related to alteration in sex ratio. Here we report finding an excess of males among siblings of Au carriers in a Melanesian population.

During July, August and September of 1973, the population of the 15 villages in Graciosa Bay, Santa Cruz Island, Eastern District, British Solomon Islands Protectorate (BSIP) was tested for Australia antigen by immunodiffusion. Eight hundred and fifty-two people, comprising more than 95% of the total resident population of all ages were bled from a finger or toe prick into capillary tubes. None of these people had overt hepatitis. The serum was separated from the red cells and tested in a seven hole pattern using rabbit anti-Australia antigen (A5 and 6) in the centre well and control serum, from an asymptomatic blood donor which contained the antigen, in the top and bottom wells. The plates consisted of 1.1% agarose in 8.2 veronal buffer and were read at 24, 48 and 72 h (ref. 9). A census of the people living in Graciosa Bay and their immediate families was compiled. This included the names and present location of the mother, father and live-born siblings of all residents.

The frequency of Au carriers was 4.8%. Forty-one individuals with Au were distributed in 35 groups of siblings. Three groups contained more than one carrier. The sex ratio of the siblings of Au positive individuals and matched negative controls are shown in Table 1. The controls were matched by sex and within one year of age and had no first degree relatives who were Au carriers. The first appropriate individual listed in the census after the Au carrier to be matched was accepted as the control. The analysis includes all live-born siblings regardless of subsequent death or absence from the village. The ratio of males to females among the Au carriers approaches 2:1. This excess of males among carriers has been observed previously¹⁰ and presumably is not dependent on any special conditions existing on Santa Cruz. The number of males per 100 females among the siblings of the control group is 98. The ratio of males to females among the siblings of Au carriers is 161:100. When these data are tested by Fisher's exact test (Table 2), the result indicates that the observed distribution is not likely to occur by chance. Thus, the observed sex ratio represents a significant excess of males among the siblings of Au carriers. This observation has important genetic, cultural and biological implications.

The report on the census of the population, 1970 by the Western Pacific High Commission, (BSIP) (ref. 11) contains

Table 1 Sex ratio of siblings of Au carriers and negative controls

	Total	Female	Male	No. of males per 100 females
Au carriers	41	14	27	193
Siblings of Au carriers	146	56	90	161
Siblings of Au negative controls	180	91	89	98

Table 2 Fisher's exact test (two-tailed)

	Female	Male	
Siblings of Au positives	56	90	$P = 0.0159$ this occurrence
			$P = 0.0364$ this occurrence or more extreme
Siblings of Au negatives	91	89	

considerable comment on the high incidence of males compared with females in the Solomon Islands, particularly in Melanesian populations. In 1970 there were 530 males per 1,000 Melanesians. There were 526 males per 1,000 in the 0-14 yr age group. The mechanism of this selection is obscure. It is reasonable to assume however, that males have had a reproductive advantage in Santa Cruz. Although there is an excess of males living on Santa Cruz, there are excess women available for marriage in the Reef Islands; these women are brought to Santa Cruz as brides. The keeping of concubines¹² has been practised until recently and there has been a high mortality among women of childbearing age in the Solomons¹¹, resulting in men frequently having more than one wife. In the Santa Cruz population, in matings which can produce Au carriers, there is apparently a sex related effect which reduces the number of live born female offspring. We suggest that because Australia antigen families have a higher proportion of males who have had a reproductive advantage and may be capable of transmitting the Au trait, the Au trait would tend to increase in the population resulting in the observed high frequency of both Au carriers and males in the Santa Cruz and possibly other Melanesian populations. The question of whether the mechanism of the transmission of the Au trait which results in family clustering is genetic or not is irrelevant to this model for Au carrier amplification.

Davenport has described trade relationships which have existed between the Outer Eastern Islands¹³. The one-way travel of women from the Reef Islands to Santa Cruz in exchange for bride prices consisting of red feather money and food, was basic to the economy and trade relationships of the entire island group. The shortage of women on Santa Cruz, due in part to the excess masculinity in Au families, must have enhanced this trade. The removal of women to Santa Cruz also reduced the population expansion in the Reef Islands which have limited resources for the support of a growing population. Thus, the economy of an entire island group may to some degree be affected by selective pressures which are apparently related to the interaction between genetic factors and the epidemiology of an infectious agent.

The mechanism of prenatal selection among siblings of Au carriers is not clear. It may be directly related to foetal mortality resulting from infections with Au or the infectious agent associated with Au, or it may be due to maternal rejection of infected foetuses. It could also be the result of developmental or maternal-foetal interactions which are only indirectly associated with the production of Au carriers.

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- ¹ Blumberg, B. S., and Melartin, L., *Int. J. Lepr.*, **34**, 60 (1966).
- ² Blumberg, B. S., Friedlaender, J. S., Woodside, A., Sutnick, A. I., and London, W. T., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 1108 (1969).
- ³ Ceppellini, R., Bedarida, J., Carbonara, A. O., Trimchieri, J., and Filippi, G., *Atti Convegni Farmitalia Antigine Australia ed Epatite Virale* (Minerva Medica, Torino, 1970).
- ⁴ Mazzur, S., Vanstory, L., London, W. T., Sutnick, A. I., and Blumberg, R. S., *Res. Comm. chem. Path. Pharm.*, **3**, 435 (1972).
- ⁵ Blumberg, B. S., Sutnick, A. I., London, W. T., and Millman, I., *CRC, Crit. Rev. clin. Lab. Sci.*, **2**, 473 (1971).
- ⁶ Blumberg, B. S., Mazzur, S., Hertzog, K., Millman, J., Bloom, J., and Damon, A., *Human Biology*, (in the press).
- ⁷ Blumberg, B. S., *Am. J. phys. Anthropol.*, **32**, 305 (1970).
- ⁸ Blumberg, B. S., *Isr. J. med. Sci.*, **9**, 1437 (1973).
- ⁹ Mazzur, S., *Am. J. med. Tech.*, **38**, 343 (1972).
- ¹⁰ Blumberg, B. S., Sutnick, A. I., London, W. T., and Melartin, L., *Arch. Int. Med.*, **130**, 227 (1972).
- ¹¹ Groenewegen, K., *Report on the census of the population* (1970 Western Pacific Commission British Solomon Islands Protectorate, 1970).
- ¹² Davenport, W., *Scient. Am.*, **206**, 95 (1962).

Adenyl cyclase stimulation by aspirin in rat gastric mucosa

ASPIRIN consumption is associated with gastric mucosal erosions^{1,2} and upper gastrointestinal haemorrhage³⁻⁵, and has been postulated as a factor in the development of chronic peptic ulcer^{6,7}. It has been reported to cause chronic gastric ulcer in rats⁸. There is, however, no real understanding of the mechanism of these injuries. Menguy *et al.*⁹ suggested that the damage to the gastric mucosa results from lack of energy (ATP) for cell function. The reported antagonism of cholera enterotoxin by aspirin in the rat intestine¹⁰ might have been mediated through the inhibition of adenyl cyclase. We have now found, however, that aspirin can stimulate the activity of this enzyme in rat gastric mucosa.

Male Holtzman rats (200–300 g) were fasted overnight and then given aspirin by stomach tube (150–300 mg kg⁻¹ body weight, homogenised in 1% methyl cellulose). Control rats were given an equal volume of 1% methyl cellulose alone. After an appropriate time (5 min to 5 h) the rats were killed and mucosa was removed with a glass microscope slide; this and all subsequent manipulations were done at 2°–4° C. A 10% w/v homogenate of the mucosal scrapings was prepared in Tris–Mg²⁺ buffer, pH 7.5, in an all-glass homogeniser. This was centrifuged at 1,000g, and the precipitate was used for all assays.

Adenyl cyclase activity was determined from the rate of formation of 3',5'-cyclic AMP from ¹⁴C-ATP, (Amersham/Searle) specific activity 1.03 mCi mmol⁻¹, according to a

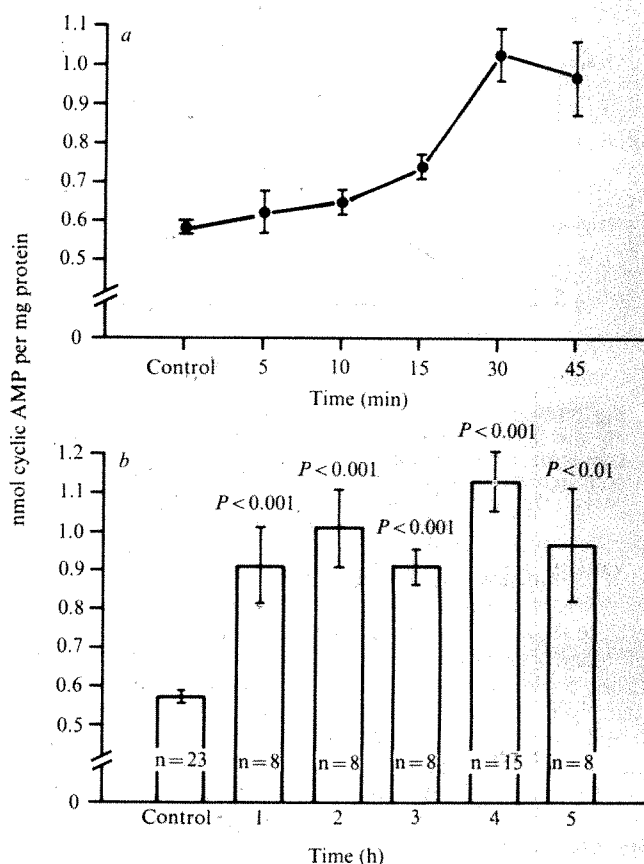


Fig. 1 *a*, Short term (5–45 min) effect of oral aspirin (300 mg kg⁻¹ body weight) on rat gastric mucosal adenyl cyclase. Each point represents a mean \pm s.e.m. for three rats. Stimulation of adenyl cyclase becoming apparent within 5 min after oral administration. *b*, Long term (between 1–5 h) results. Adenyl cyclase activity is expressed as nmol cyclic AMP per mg protein, represented as bars. Each bar shows mean value of adenyl cyclase \pm s.e.m. (*n* is the number of rats). All values are highly significantly different from the control. Various dose schedules (75–300 mg kg⁻¹ body weight) of aspirin were tried and a dose of 300 mg kg⁻¹ of body weight was selected as the optimal dose. 150 mg kg⁻¹ aspirin gave comparable results after 30 min of oral administration.

modification of the procedure of Krishna *et al.*¹¹. Enzyme activity was expressed as nmol of 3',5'-cyclic AMP produced per minute per mg protein as determined by the method of Lowry *et al.*¹².

Gastric mucosal homogenate (1,000g precipitate) as described above was prepared from 24 rats, in six groups of four. Aspirin in methyl cellulose was added to the gastric mucosa of each of four rats at concentrations of 0.3, 0.6, 0.9, 1.2 and 1.8 mg per 0.38 ml of reaction mixture. Four mucosae treated with equal volumes of methyl cellulose served as controls. The rest of the procedure for measuring adenyl cyclase activity was as described above.

The effect of aspirin (300 mg kg⁻¹) became apparent 5 min after the oral dose (Fig. 1). Stimulation of adenyl cyclase was maximum 4 h after the oral dose and was significantly different from the control value, as shown by Student's *t* test (*P* 0.001). Results were also significantly different from control after between 1 and 5 h, but not significantly different from each other.

Figure 2 shows that there is a direct correlation between aspirin concentration and the degree of adenyl cyclase activity. The activity seemed to increase linearly with aspirin concentration up to 1.8 mg of aspirin in 0.38 ml of reaction mixture.

The report that aspirin counteracted the effect of cholera toxin on the intestine of rat¹⁰ suggested a decrease in mucosal adenyl cyclase activity, and a similar decrease was expected

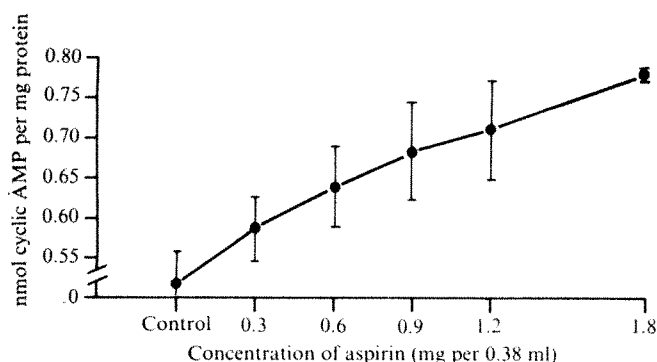


Fig. 2 Effect of aspirin *in vitro* at various concentrations (0.3–1.8 mg per 0.38 ml of reaction mixture) on rat gastric mucosal homogenate (1000g precipitate) adenylyl cyclase activity. Each point represents a mean \pm s.e.m. of mucosae of four rats. A linear relationship between aspirin concentration and adenylyl cyclase activity is evident.

after administration of aspirin. Since cyclic AMP was shown to decrease gastric acid secretion^{13–15}, the lowering of adenylyl cyclase activity after aspirin might have explained the mechanism of injury by aspirin. But our experiments refute this contention. If one could correlate these findings and if one believes reports that cyclic AMP stimulates gastric acid secretion^{16–19}, the stimulation of adenylyl cyclase by aspirin may explain the mucosal injury. It is also possible that the stimulation exhausts the ATP stores⁹ quickly, thus causing mucosal injury.

Davenport^{1,2} demonstrated that aspirin damages the gastric mucosal barrier and causes hydrogen ion back diffusion, as judged by the development of hypoacidity in a gastric pouch after administration of aspirin. If one postulates^{13–15} that cyclic AMP inhibits gastric acid secretion, this may be another explanation for the hypoacidity seen after injury by aspirin.

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- ¹ Davenport H. W., Cohen, B. J., Bref, M., and Davenport, V. D., *Gastroenterology*, **49**, 189–196 (1965).
- ² Davenport, H. W., *Gastroenterology*, **46**, 245–253 (1964).
- ³ Croft, D. N., and Wood, P. H. N., *Br. med. J.*, **1**, 137–141 (1967).
- ⁴ Parry, D. J., and Wood, P. H. N., *Gut*, **8**, 301–307 (1968).
- ⁵ Goulston, K., and Cooke, A. R., *Br. med. J.*, **4**, 664–665 (1968).
- ⁶ DuPlessis, D. J., *Lancet*, **i**, 974–978 (1965).
- ⁷ Allibone, A., and Flint, F. J., *Lancet*, **ii**, 179–182 (1958).
- ⁸ St John, D. J. B., Yeomans, N. D., and de Boer, W. G. R. M., *Gastroenterology*, **65**, 634–641 (1973).
- ⁹ Menguy, R., Desbaillets, L., and Masters, Y. F., *Seventy-fourth Ann. Meeting Am. Gastroenterol. Soc.*, A89 (1973).
- ¹⁰ Jacoby, H. I., and Marshall, C. H., *Nature*, **325**, 163–165 (1972).
- ¹¹ Krishna, G., Weiss, B., and Brodie, B. B., *J. pharmac. exp. Ther.*, **163**, 379–385 (1968).
- ¹² Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265–275 (1951).
- ¹³ Levine, R. A., Cafferata, E. P., and McNally, E. F., *The third world congress of gastroenterology*, **1**, 308–410 (1967).
- ¹⁴ Levine, R. A., and Wilson, D. E., *Ann. N.Y. Acad. Sci.*, **185**, 363–375 (1967).
- ¹⁵ Taft, R. C., and Sessions, jun., J. T., *Clin. Res.*, **20**, 43 (1972).
- ¹⁶ Harris, J. B., and Alonso, D., *Fedn Proc.*, **24**, 1368–1376 (1965).
- ¹⁷ Mertz, D. P., Mann, K., and Jahr, R., *Klin. Wschr.*, **49**, 936–939 (1971).
- ¹⁸ Shaw, J. E., and Ramwell, P. W., *Prostaglandins*, Symp. Worcester Foundation for Experimental Biology (Interscience, New York, (1968).
- ¹⁹ Shaw, J. E., and Ramwell, P. W., *J. Physiol. Lond.*, **195**, 34–36 (1968).

Cyclic AMP, ATP and cell contact

DENSITY-DEPENDENT¹ or contact-inhibited² cells stop dividing when they reach confluency; however, transformed cells do not exhibit this type of growth control³. Several experiments suggest that adenosine 3',5'-cyclic monophosphate (cyclic AMP) is involved in regulating the cell division of cultured fibroblasts. The dibutyl derivative of cyclic AMP inhibits proliferation of transformed cells⁴, and the steady state levels of cyclic AMP in normal density-dependent fibroblasts are higher than the corresponding virus and spontaneous transformed cell lines^{5,6}. There are, however, discrepancies between experiments which have attempted to compare the cyclic AMP levels of logarithmically growing with quiescent, density-inhibited 3T3 cells. Experiments in our laboratory showed that cyclic AMP levels of 3T3 cells do not change over the cell density of 1×10^4 – 6×10^4 cells per cm^2 (ref. 5). These results have been confirmed by others using the same cell system⁷. Otten *et al.*^{8,9}, however, reported that cyclic AMP levels of logarithmically growing 3T3 cells are lower than those of confluent, quiescent cells; Heidrick and Ryan⁹ reported similar results using L cells.

In an attempt to reconcile those contradictory data, we have thoroughly investigated the relationship between cell density and the cyclic AMP levels of mouse 3T3 cells. We have also studied deoxyglucose transport and cellular ATP levels as possible density-dependent phenomena. Measurements were made at sparse cell densities (no cell–cell contact), after cell contacts were established and again after growth to a confluent monolayer. A critical membrane-mediated event seems to occur at the cell–cell contact state (before confluency), resulting in decreased glucose transport, decreased ATP and increased cyclic AMP. Such changes may prime the normal cell for eventual density-dependent growth inhibition. Transformed cells which do not exhibit regulated inhibition of growth show little change in these biochemical parameters when compared at the three cellular densities.

Swiss 3T3, 3T6 and PY3T3 cells (a gift of Dr Howard Green) and BALB 3T3 cells and SV3T3 cells (a gift of Dr G. J. Todaro) were routinely grown in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (Colorado Serum Co.) and penicillin-streptomycin (100 U ml^{-1} and 100 $\mu\text{g ml}^{-1}$, respectively). Cyclic AMP was measured by Gilman's method¹⁰. Cells were prepared for assay by washing twice with phosphate-buffered saline (warmed to 37° C), then adding cold (4° C) 5% trichloroacetic acid (TCA). The TCA supernatant, obtained by centrifugation, was extracted five times with 5 volumes of water-saturated ether. Samples were then applied on small Dowex-1-formate columns (0.4 \times 3 cm) equilibrated with water. Columns were washed with 10 ml of water, and then cyclic AMP was eluted with 1 N formic acid. Eluates were lyophilised to dryness and redissolved in 0.2 or 0.4 ml of water. A sample of 0.05 ml was used for each cyclic AMP assay. Recovery of cyclic AMP, which was measured using ³H-cyclic AMP, was more than 90%, irrespective of total amounts of cyclic AMP (from 0.5 to 20 pmol). Another purification method using Dowex 50-H+ (ref. 8) was tested, but we found that the resin releases a substance which strongly inhibits cyclic AMP binding and so discarded this method. Internal standards and susceptibility to degradation by cyclic nucleotide phosphodiesterase were used routinely to verify that we were measuring cyclic AMP.

³H-deoxyglucose uptake was measured by the procedure of Martin *et al.*¹¹ after previous incubation at 37° C for 45 min in glucose-free Hanks salt solution. Cellular ATP was measured using a firefly lantern extract¹². Cells were washed three times with warm phosphate-buffered saline, and then cold 50% ethanol was added. The cells were scraped from the dish with a rubber policeman and mixed by a vortex. After at least 30 min in an ice bath, the suspension was directly used for determination of ATP. Protein was assayed according to Lowry *et al.*¹³.

Normal Swiss 3T3 cells grow in culture to 4×10^4 – 8×10^4 cells cm^{-2} at which density the cellular monolayer becomes quiescent as measured by DNA synthesis and cell division. The logarithmic phase of the growth cycle (under our laboratory conditions) extends to a density of 2×10^4 cells cm^{-2} ; at this density proliferation begins to plateau and eventually stops at 4×10^4 – 8×10^4 cells cm^{-2} . The relation between cell density and the intracellular cyclic AMP level is illustrated in Fig. 1. Since at least 10^6 cells were needed for each cyclic AMP assay, roller bottles were used for the lighter cultures and two to six 60- cm^2 plastic tissue culture dishes were used for the heavier cultures. Cells were plated at various concentrations (all other conditions being constant) and grown for 24 h. The cyclic AMP level and cell number were then determined. The basal level of cyclic AMP was low at sparse 3T3 cell density and began to increase at about 3×10^3 cells cm^{-2} (Fig. 1). Cyclic AMP then quickly increased to a stationary concentration that was twice the basal level of the sparse, noncontacted normal cells. This increase occurred during log phase growth at a density (3×10^3 cells cm^{-2}) that preceded confluency and the total cessation of cell division.

ATP levels of 3T3 cells at various densities were also investigated, and contrary to the results observed with the cyclic AMP levels, the amount of ATP per 10^6 cells decreased, reaching a minimum (about half of the maximum) after cell contact was established.

To preclude the possibility that cell volume changed as density increased, we measured the cell protein of 3T3 and transformed cell lines as they grew to confluency. Table 1 is a summary of that data and shows little effect of cell density on protein content of the cell lines studied. There were, however, large differences between mg protein per 10^6 cells of the various normal and transformed cell lines. Calculation of ATP concentrations using protein or cell number indicated differences in the ATP levels of normal and transformed cells.

PY3T3 and 3T6 cells had ATP levels similar to growing (precontact) Swiss 3T3 cells, but once the normal 3T3 cells touched and the ATP level decreased, there was a significant difference between the transformed and normal 3T3 cells. A similar trend was observed with the BALB mouse-derived normal and transformed cell lines. A previous report that growing 3T3 cells had slightly more ATP than quiescent 3T3 cells¹⁴ supports our present data, because the earlier comparison was made with cells that had established contact, and maximal differences between growing and confluent 3T3 cells were observed only with precontacted 3T3 cells.

Deoxyglucose transport followed a pattern similar to the ATP level in that uptake decreased after cell contact. Experiments using chick fibroblasts¹⁵, BALB 3T3 cells¹⁶, and mouse embryo cells¹⁷ have shown that deoxyglucose transport is inhibited at relatively low cell densities. Our experiments, using Swiss 3T3 cells, show that deoxyglucose transport is inhibited at a density as low as 2×10^3 cells cm^{-2} . Initial cell-cell contacts are established at 10^3 cells cm^{-2} . These data suggest that inhibited cellular transport is one of the first biochemical parameters affected by cell contact. Thus, the increase in cellular cyclic AMP may be a critical (although not necessarily primary) event and might, as previously suggested¹⁸, serve as a cellular signal which responds to the availability of crucial nutrients.

Increasing cyclic AMP levels could result from either a stimulated adenylate cyclase or an inhibited phosphodiesterase activity. The activity of adenylate cyclase, a plasma membrane enzyme, increases (both basal and adrenaline stimulated) as a function of 3T3 cell density¹⁹. But, Russell and Pastan²⁰ suggested that a membrane-associated cyclic AMP phosphodiesterase exerts a substantial effect on the cellular cyclic AMP level. The biological mechanism of the ATP change is unknown and any possible relationship between levels of ATP and cyclic AMP remains to be established.

These experiments settle the controversy concerning the role of cyclic AMP in the contact inhibition or density-dependency phenomenon. Otten *et al.*^{6,8} suggested that cyclic AMP mediates contact inhibition and that an increase of the basal level of cyclic AMP of the 3T3 cell occurred at confluency, but earlier we could detect no change in the basal level of cyclic AMP as 3T3 cells grew to confluence⁵. This controversy is now resolved by our finding that 3T3 cyclic AMP increases in response to cell contact which occurs at a density of 3×10^3 cells cm^{-2} . Little change in cyclic AMP level occurred at confluency. We missed the increase in cellular cyclic AMP before⁵ because the plating density of the 3T3 cells exceeded the cell density at which the cellular cyclic AMP increases from its initial level. Thus, we observed only a constant and increased cellular cyclic AMP level over the density of 10^4 cells per cm^2 to 6×10^4 cells per cm^2 . This change in the cyclic AMP level is critical in contact inhibition, as Otten *et al.*^{6,8} and Heidrick and Ryan⁹ concluded, although it does not, as they suggested, directly participate in or coincide with cessation of 3T3 cell division as cells become confluent. We suggest that this biochemical change is a prerequisite for contact inhibition of growth by showing that cyclic AMP

Table 1 ATP levels of several normal and transformed lines

Cell line	Protein (mg per 10 ⁶ cells)	Cell density (cells cm ⁻²)	ATP (nmol per 10 ⁶ cells)	ATP (nmol per mg protein)
Swiss-derived:				
3T3	0.48	1.5 × 10 ³ (precontact)	18.0 ± 1.8	37.5
	0.48	1.5 × 10 ⁴ (contact)	12.6 ± 1.7	26.3
	0.44	4.1 × 10 ⁴ (confluent)	10.9 ± 0.8	24.7
PY3T3	0.39	2.5 × 10 ³ (precontact)	14.5 ± 1.3	37.2
	0.43	2.0 × 10 ⁴ (contact)	14.4 ± 0.9	33.5
	0.41	5.0 × 10 ⁴ (confluent)	15.8 ± 0.8	38.5
3T6	0.37 ± 0.04	3.3 × 10 ³ (precontact)	11.6 ± 1.2	31.4
		4.4 × 10 ⁴ (contact)	12.0 ± 0.5	32.4
		1.1 × 10 ⁵ (confluent)	11.5 ± 0.5	31.1
BALB-derived:				
3T3	0.44 ± 0.04	1.4 × 10 ³ (precontact)	11.5 ± 0.5	26.1
		4.4 × 10 ⁴ (contact)	9.9 ± 0.2	22.5
		8.3 × 10 ⁴ (confluent)	8.7 ± 0.3	19.8
SV3T3	0.16 ± 0.02	3.0 × 10 ³ (precontact)	4.7 ± 0.2	29.4
		5.5 × 10 ⁴ (contact)	4.2 ± 0.3	26.3
		1.4 × 10 ⁵ (confluent)	4.3 ± 0.3	26.9

Protein determinations were performed on monolayer cultures at various densities after washing three times with phosphate-buffered saline. The cells were then solubilised in 1 N NaOH and protein was measured according to Lowry *et al.*¹⁴. ATP levels were measured by the method of Strehler and McElroy¹². Single points are the average of triplicate determinations. Variation is expressed as \pm s. d. when at least six separate determinations were done.

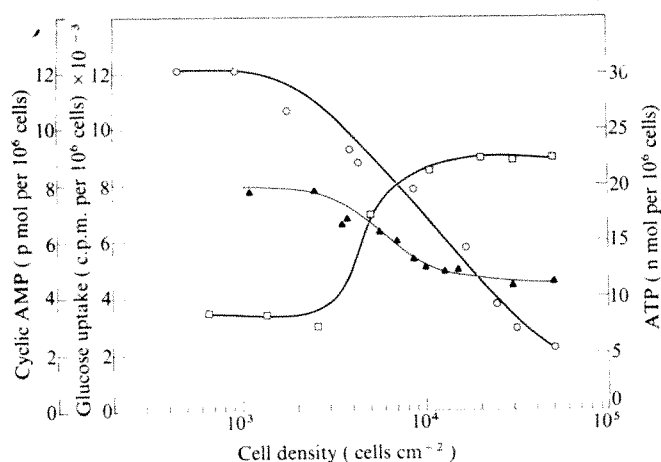


Fig. 1 Cyclic AMP levels (\square), ATP levels (\blacktriangle), and deoxyglucose transport (\circ) were measured as described in the text. These data points are the averages of triplicate determinations derived from one experiment.

increases at cell contact before the accumulation of 3T3 cells in a quiescent monolayer mechanisms.

Siefert and Paul²¹ found that cyclic AMP levels did not vary as a function of cell density; increased cyclic AMP was observed after 3T3 cells stopped dividing, regardless of density. They attributed the increase to the depletion of a serum factor necessary for continued proliferation. Our measurements were made 24 h after cell plating and were presumably not affected by serum or medium depletion. It is conceivable that both cell contact and serum factors could affect cyclic AMP levels, possibly through cell transport mechanisms.

These and other studies²²⁻²⁵ suggest that an important membrane-associated event in the contact inhibition or density-dependent inhibition of growth precedes the accumulation of normal cells in a confluent monolayer. Most likely, all membrane parameters that change as a function of normal cell contact—nutrient transport¹⁵⁻¹⁷, distribution of intramembranous particles²², membrane fluidity²³, plasma membrane enzyme activity²⁴ and cellular agglutinability²⁵—as well as the intracellular concentrations of cyclic AMP and ATP, signify important cellular changes culminating in inhibited cellular proliferation. But, each of these changes taken alone is probably not sufficient to induce true density-dependent inhibition of growth. Future studies should describe the membrane-associated signal which initiates these biochemical events and clarify relationships among the cellular processes that result in regulated cell division.

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- ¹ Stoker, M. G. P., and Rubin, H., *Nature*, **215**, 171 (1967).
- ² Abercrombie, M., and Ambrose, E. M., *Cancer Res.*, **22**, 525 (1962).
- ³ Dulbecco, R., *Science*, **166**, 962 (1969).
- ⁴ Sheppard, J. R., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1316 (1971).
- ⁵ Sheppard, J. R., *Nature new Biol.*, **236**, 14 (1972).
- ⁶ Otten, J., Johnson, G. S., and Pastan, I., *J. biol. Chem.*, **247**, 7082 (1972).

- ⁷ Oey, J., Vogel, A., and Pollack, R., *Proc. natn. Acad. Sci., U.S.A.*, **71**, 694, (1974).
- ⁸ Otten, J., Johnson, G. S., and Pastan, I., *Biochem. biophys. Res. Commun.*, **44**, 1192 (1971).
- ⁹ Heidrick, M. L., and Ryan, W. L., *Cancer Res.*, **31**, 1313 (1971).
- ¹⁰ Gilman, A. G., *Proc. natn. Acad. Sci., U.S.A.*, **67**, 305 (1970).
- ¹¹ Martin, G. S., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2739 (1971).
- ¹² Strehler, B. L., and McElroy, W. D., *Methods in enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **3**, 871 (Academic Press, New York, 1968).
- ¹³ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **188**, 265 (1951).
- ¹⁴ Weber, M. J., and Edlin, G., *J. biol. Chem.*, **246**, 1828 (1971).
- ¹⁵ Sefton, B., and Rubin, H., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 3154 (1971).
- ¹⁶ Bose, S. K., and Zlotnick, B. J., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2374 (1973).
- ¹⁷ Plagemann, P. G. W., *J. Cell Phys.*, **82**, 421 (1973).
- ¹⁸ Sheppard, J. R., in *Membranes and viruses in immunopathology* (edit. by Day, S., and Good, R. A.), 249 (Academic Press, New York, 1972).
- ¹⁹ Makman, M. H., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2127 (1971).
- ²⁰ Russell, T., and Pastan, I., *J. biol. Chem.*, **248**, 5835 (1973).
- ²¹ Seifert, W., and Paul, D., *Nature new Biol.*, **240**, 281 (1972).
- ²² Scott, R. E., Furcht, L. T., and Kersey, J. H., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 3631 (1973).
- ²³ Barnett, R. E., Furcht, L. T., and Scott, R. E., *Proc. natn. Acad. Sci., U.S.A.*, (in the press).
- ²⁴ Roth, S., and White, D., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 485 (1972).
- ²⁵ Nicolson, G. L., and Lacorbiere, M., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1672 (1973).

α - and β -Retinyl acetate reverse metaplasias of vitamin A deficiency in hamster trachea in organ culture

DEFICIENCY of vitamin A causes a well-defined lesion, namely keratinised squamous metaplasia, in tracheobronchial epithelium¹. In this lesion, the normal columnar ciliated and mucus cells of the epithelium, which depend on vitamin A for their formation, are totally replaced by squamous cells which produce keratin. The mechanism of action of vitamin A in controlling this normal differentiation of ciliated and mucus cells is still unknown. We report here an *in vitro* system for studying this process, using organ culture of hamster tracheas in a chemically defined, serum-free medium. Growth of tracheas in this medium without vitamin A causes keratinised squamous lesions. Addition of vitamin A to the organ cultures after development of such lesions causes reversal of the process of keratinisation and replacement of the squamous cells by columnar ciliated and mucus cells.

It has previously been noted² that cultivation of mouse prostate in chemically defined medium without vitamin A resulted in squamous metaplasia of the prostatic secretory epithelium and that inclusion of vitamin A in the defined medium completely prevented this metaplasia. The addition of the vitamin A antagonist, citral, to tracheal organ cultures has also been reported to cause squamous epithelial lesions, but simultaneous inclusion of vitamin A in the medium prevented their formation³. In the present study, we have used vitamin A acetate (β -retinyl acetate) to reverse lesions of vitamin A deficiency, rather than prevent their formation. Moreover, we have found that the α -retinyl isomer of vitamin A (Fig. 1), previously believed to be an essentially inactive form^{4,5}, is also active (as α -retinyl acetate) in our *in vitro* test system.

Syrian golden hamsters were maintained from birth on a diet deficient in vitamin A (ref. 6) (General Biochemicals, Chagrin Falls, Ohio). The animals were weaned at 21 d and killed at 30–33 d. At this time the hamsters were still gaining weight and did not yet show signs of severe vitamin A deficiency; the tracheal epithelium was generally low columnar

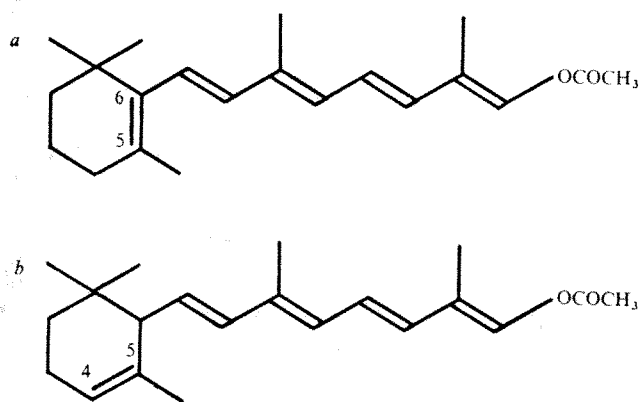


Fig. 1 Structures of (a) β and (b) α -retinyl acetate. The double bond in the 5-6 position of the cyclohexene ring of the naturally occurring β -isomer has been shifted to the 4-5 position in the α -isomer.

or cuboidal, with patches of squamous metaplasia only rarely found. The animals were randomly grouped for treatment, and their tracheas were removed by sterile technique. Each trachea was opened from the larynx to the carina along the membranous dorsal wall and placed into a 60×15 mm culture dish containing 2 ml of medium (CMRL-1066), (ref. 7); with crystalline bovine insulin, $1 \mu\text{g ml}^{-1}$; hydrocortisone hemisuccinate, $0.1 \mu\text{g ml}^{-1}$; glutamine, 2 mM; penicillin, 100 units ml^{-1} ; and streptomycin, 100 $\mu\text{g ml}^{-1}$, added. All tracheas were cultured at 37°C for 4 d in the above medium, which does not contain vitamin A, serum, or any added protein other than insulin. All cultures were gassed with 50% oxygen, 45% nitrogen, and 5% carbon dioxide in a sealed box, which was gently rocked approximately 12 times min^{-1} to allow the trachea contact with both gas and medium. After 4 d, some tracheas were collected and the rest were cultured for an additional 6 d (before collection) in the above medium, also containing either a, no added vitamin A; b, β -all-*trans*-retinyl acetate (0.25 – $1.0 \mu\text{g ml}^{-1}$); or c, α -all-*trans*-retinyl acetate (0.25 – $1.0 \mu\text{g ml}^{-1}$). Properties of α -retinyl acetate were as follows: melting point = 41 – 42.5 ; by ultraviolet analysis $\lambda_{\text{max}}^{\text{EtOH}} = 311 \text{ nm}$, $\epsilon = 64,900$. No β -isomer was detectable in the α -retinyl acetate by nuclear magnetic resonance (NMR) analysis, which is capable of detecting as little as 0.2% β -retinyl acetate impurity; details of these analyses will be published elsewhere. β and α -retinyl acetate were dissolved in dimethyl sulphoxide and added freshly to the cultures at the time of media change, which was done 4 times a week. The final concentration of dimethyl sulphoxide never exceeded 0.1%; an equivalent amount was added to all control cultures. At the time of collection, all tracheas were fixed in 10% buffered formalin and embedded in paraffin. Cross sections of $5 \mu\text{m}$ were made through the mid-portion, stained with haematoxylin and eosin, and evaluated independently by two observers. The status of the epithelium was graded with respect to both the extent of squamous metaplasia and the presence or absence of keratin.

The results of over 200 tracheal cultures are shown in Table 1 and demonstrate the progression of keratinising squamous metaplasia in culture, as well as its reversal on addition of β - or α -retinyl acetate to the medium. After 4 d *in vitro* in medium without vitamin A, 56% of cultures exhibited some squamous metaplasia and 52% had produced keratin. After 10 d *in vitro* without vitamin A, further progression resulted in 84% of the cultures showing squamous metaplasia and 76% showing keratinisation. Addition of β or α -retinyl acetate to the cultures from days 4–10 effected a reversal of squamous metaplasia, so that less than 25% showed squamous metaplasia at 10 d. In contrast to the marked or severe squamous metaplasia seen in 32% of the cultures on day 4 (before treatment with vitamin A), only 3% of the cultures had this extent of metaplasia after 6 d of treatment with β -retinyl acetate. Further, the keratinisation present in 52% of cultures at day 4 was reversed by β -retinyl acetate treatment in every case; keratinisation was reversed in all but two cultures treated with α -retinyl acetate.

This organ culture method will facilitate the study of vitamin A analogues for their capability to effect normal differentiation of tracheobronchial epithelium. Since the assay measures the reversal of lesions caused by the absence of vitamin A, as well as inhibition of their formation, it seems that the effect of an analogue in this assay could not be attributable solely to a sparing or stabilising effect that it might have on endogenous vitamin A metabolism. Such a sparing effect on endogenous hormone metabolism has been suggested to explain the wide range of chemical structures which show insect juvenile hormone activity^{8,9}.

This assay clearly demonstrates that the α -isomer of vitamin A, previously reported to be almost inactive (as the aldehyde or alcohol) in supporting growth in the rat, has intrinsic ability to maintain normal differentiation in hamster tracheobronchial epithelium. This finding has been extended (unpublished results) in *in vivo* growth studies in our laboratory, which have demonstrated the capacity of α -retinyl acetate to sustain growth and life if given intraperitoneally to hamsters that would otherwise lose weight and die on a vitamin A-deficient diet. The potency of the α -isomer, however, is markedly less than that of the β -isomer in the hamster growth assay. It has also been shown that both α -retinoic acid and α -retinyl acetate have definite ability to promote growth in rats maintained on vitamin A-deficient diets¹⁰.

Our organ culture system may also prove useful to determine whether vitamin A and its analogues are capable of reversing squamous metaplastic or preneoplastic lesions of tracheobronchial epithelium that are induced by chemical carcinogens. Addition of excess vitamin A to prostatic organ cultures previously treated with 3-methylcholanthrene can suppress the hyperplastic and metaplastic response caused by this carcinogen in prostatic epithelium¹¹. It has also been shown that simultaneous administration of vitamin A to hamster tracheal organ cultures treated with benzo[a]pyrene can inhibit the hyperplastic response caused by the carcinogen¹²; however, in this case the inhibitory effects of vitamin A may be caused by inhibition of activation of the carcinogen¹³. In view of the hypothesis that human tracheobronchial epithelium

Table 1 Response of tracheal organ cultures to β -retinyl acetate or α -retinyl acetate

Treatment		Cultures with respective amounts of squamous metaplasia (%)				Cultures with keratin (%)	Total cultures
Day 1–4	Day 4–10	None	Mild	Marked	Severe		
No vitamin A	Collected day 4	44	24	17	15	52	46
No vitamin A	No vitamin A	16	32	28	24	76	62
No vitamin A	β -retinyl acetate	83	14	3	0	0	63
No vitamin A	α -retinyl acetate	76	14	8	2	3	59

Cultures were graded as to the percentage of their total epithelium showing squamous metaplasia on 4–8 cross sections from the middle of each trachea; if between 1% and 10% of the total epithelial length was squamous, it was graded as having mild squamous metaplasia; between 10–40% was graded as marked; and greater than 40% was graded as severe.

lium undergoes a prolonged series of metaplastic and pre-neoplastic changes before development of bronchogenic squamous cell carcinoma¹⁴, it would be of definite interest to know whether vitamin A and its analogues are capable of arresting or reversing the development of these lesions in organ culture.

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- ¹ Wolbach, S., and Howe, P., *J. exp. Med.*, **42**, 753-769 (1925).
- ² Lasnitzki, I., *Expl Cell Res.*, **28**, 40-51 (1962).
- ³ Aydelotte, M. B., *J. Embryol. exp. Morph.*, **11**, 279-291 (1963).
- ⁴ Ames, S. R., Swanson, W. J., and Harris, P. L., *J. Am. Chem. Soc.*, **77**, 4136-4138 (1955).
- ⁵ Pitt, G. A., *Am. J. clin. Nutr.*, **22**, 1045-1046 (1969).
- ⁶ Harris, C. C., Sporn, M. B., Kaufman, D. G., Smith, J. M., Jackson, F. E., and Saffiotti, U., *J. natn. Cancer Inst.*, **48**, 743-761 (1972).
- ⁷ Parker, R. C., Castor, L. N., and McCulloch, E. A., *Spec. Publ. N.Y. Acad. Sci.*, **5**, 303-313 (1957).
- ⁸ Riddiford, L. M., Ajami, A. M., Corey, E. J., Yamamoto, H., and Anderson, J. E., *J. Am. Chem. Soc.*, **93**, 1815-1816 (1971).
- ⁹ Slade, M., and Wilkinson, C. F., *Science*, **181**, 672-674 (1973).
- ¹⁰ Goodman, D. S., Smith, J. E., Hembry, R. M., and Dingle, J. T., *J. Lipid Res.* (in the press).
- ¹¹ Lasnitzki, I., *Br. J. Cancer*, **9**, 434-441 (1955).
- ¹² Crocker, T. T., and Sanders, L. L., *Cancer Res.*, **30**, 1312-1318 (1970).
- ¹³ Hill, D. L., and Shih, T., *Cancer Res.*, **34**, 564-570 (1975).
- ¹⁴ Auerbach, O., Stout, A. P., Hammond, E. C., and Garfinkel, L., *New Engl. J. Med.*, **265**, 253-267 (1961).

Dynamics and function of vitamin A compounds in rat retina after a small bleach of rhodopsin

THE vertebrate visual pigment, rhodopsin, is located in the outer segment disks of the rod photoreceptor cells, and consists of a chromophore, 11-*cis* retinal, bound to a protein, opsin. During light adaptation, 11-*cis* retinal is isomerised to the all-*trans* configuration. All-*trans* retinal is then reduced to retinol by an oxido-reductase in the rod outer segments¹⁻⁴, and retinol diffuses to areas adjacent to the rod outer segments, where it is esterified to form retinyl esters⁵⁻¹². During dark adaptation rhodopsin is regenerated as essentially the reverse processes occur; however, it is not known in what form (retinyl ester, retinol, retinal) the chromophoric group is re-isomerised to the 11-*cis* configuration.

This understanding of the vertebrate visual cycle is based upon experiments in which high light intensities were used, and most or all of the rhodopsin was bleached. It is not known whether release, reduction and esterification of the chromophore occur after a smaller fraction of rhodopsin is bleached. Here, we report that these reactions also occur when 10% of the rhodopsin in a dark-adapted rat eye is bleached by a single light flash, and that some of the chromophore may be

re-isomerised to the 11-*cis* configuration without reduction to retinol.

Our technique for determining the *in vivo* proportions of vitamin A compounds consists of injecting radioactive vitamin A compounds into vitamin A-deficient rats, and then cochromatographing extracts of eye tissues with known marker compounds on thin layer chromatography plates¹³; the amount of radioactivity which cochromatographed with each marker compound was taken as an indirect measure of the amount of that compound in the extract. The method was used to study the distributions and proportions of vitamin A compounds in the eye during and after adaptation to high light intensities¹². In this study we determined the proportions of vitamin A compounds in rat eyes, one of which was kept in darkness, while the other was exposed to a light flash which bleached approximately 10% of the rhodopsin present.

One eye of a dark-adapted albino rat was exposed to a 20 ms flash of monochromatic light (504 nm) which had an intensity of 4.2 mW cm⁻². The flash apparatus consisted of a light source which focused light through an interference filter onto an optic fibre 7 mm in diameter; the other end of the optic fibre was mounted in front of a piece of doubly ground glass and an electronically-actuated mechanical shutter. At successive intervals after the light flash, both eyes were removed and the extracts of them were cochromatographed with marker vitamin A compounds (11-*cis* and all-*trans* retinal, retinol, retinyl ester) on silica gel thin-layer plates with a solvent of 8 parts ethyl acetate:92 parts hexane. The percentage of radioactivity in a vitamin A compound was obtained by dividing the amount of radioactivity which cochromatographed with that compound by the total amount of radioactivity which cochromatographed with all of the marker compounds¹². Subtraction of the percentage of radioactivity in a compound in the dark-adapted eye from the percentage of radioactivity in that same compound in the light-exposed eye yielded the results shown in Fig. 1.

By 5 to 10 min after the light flash, 11-*cis* retinal had decreased to its minimum, and *trans* retinal and retinol had increased. The 8% difference between the experimental and control eyes in the concentration of 11-*cis* retinal reflects a bleach of approximately 10% of the rhodopsin because 11-*cis* retinal, present only as the chromophore of rhodopsin, comprises 80% of the vitamin A compounds in the dark-adapted rat eye¹². Using the measurements of Cone¹³ for the attenuation

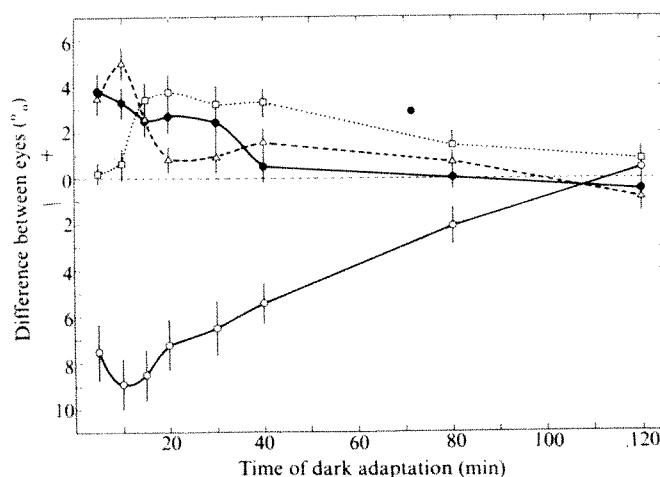


Fig. 1 Vitamin A compounds formed in the dark-adapted rat eye after a 20 ms flash of monochromatic light (504 nm). The ordinate scale is the difference between the percentage of radioactivity in a vitamin A compound in the flashed (experimental) eye and the percentage of radioactivity in that same compound in the dark-kept (control) eye. The abscissa scale is time after the light flash. Lines connect the mean values of four to six experiments; vertical lines indicate standard errors. □, Retinyl ester; △, retinol; ●, *trans* retinal; ○, 11-*cis* retinal.

and scattering of light by the rat cornea and lens and his measurements of the number of rods in the retina, we calculate that the rhodopsin of the entire retina should absorb 4.3×10^{13} light quanta. We can extract approximately 1 nmol of rhodopsin (6.0×10^{14} molecules) from the dark-adapted rat eye, and, assuming a quantum efficiency of bleaching of 0.65 (ref. 14), we should then expect to bleach about 5% of the rhodopsin by the light flash; considering the many sources of error in these measurements, this is in close agreement with our value of 10% inferred from the indirect detection technique.

By 10 min after the flash, *trans* retinal had started to decrease, retinol was at its peak concentration, and retinyl ester was increasing. As in adaptation to high light intensities, these results most probably reflect release of *trans* retinal from photolysed rhodopsin, reduction of retinal to retinol and esterification of retinol to fatty acids^{3,6-12}.

During 15 to 40 min after the flash there was regeneration of rhodopsin, as is evident from the increase in 11-*cis* retinal. During the same time, *trans* retinal decreased in concentration, but the concentration of retinyl ester remained stable. This result strongly suggests that some *trans* retinal was re-isomerised to the 11-*cis* configuration without being reduced to retinol. From 40 to 120 min after the flash, retinyl ester gradually decreased in concentration, while 11-*cis* retinal continued to increase; by 2 h after the flash, there was no longer any significant difference between the eyes in the proportions of vitamin A compounds. As in dark adaptation following extensive bleaching of rhodopsin, these results probably reflect hydrolysis of retinyl ester, diffusion of retinol into the rod outer segments, oxidation to retinal and isomerisation (at some point) to the 11-*cis* isomer^{3,7-12}.

In spite of the experimental variability, then, two important conclusions are suggested by these results: first, that release, reduction and esterification of the chromophore occur when 10% of the rhodopsin is bleached; and second, that isomerisation of the chromophore from the all-*trans* to the 11-*cis* isomer probably occurs as retinal. The latter conclusion is also supported by recent experiments with cattle retinae¹⁵ and rat retinae¹⁶.

As discussed elsewhere by one of us¹², the dynamics of vitamin A compounds in the eye (release, reduction and esterification of the chromophore and the reverse) may have one or some combination of three functions: (1) re-isomerisation of the chromophore; (2) minimising accumulation of reactive aldehyde groups and diffusional loss of retinol during light adaptation; and (3) confinement to and recycling of chromophore in the eye.

The present results argue against the first possibility—that re-isomerisation of the chromophore must occur in the retinol or retinyl ester form—since a 'short cycle' (release of all-*trans* retinal, re-isomerisation to the 11-*cis* form, and resynthesis of rhodopsin) seems to occur. To test the second possibility—that the 'long cycle' (reduction, esterification, hydrolysis, oxidation) is an overflow device which minimises accumulation of retinal and diffusional loss of retinol—would require determining whether a 10% bleach of rhodopsin is within the normal operating range of rod cells, and, if not, whether the long cycle occurs after even smaller bleaches of rhodopsin; the indications that some all-*trans* retinal is re-isomerised to the 11-*cis* form without being reduced to retinol might suggest that at still lower bleaching levels of rhodopsin, no reduction to retinol occurs at all. The third possible function—that of confinement and recycling of chromophore in the eye—seems especially important, not only during light adaptation, but also during the rod renewal process. In this renewal process, the rod disks (including rhodopsin) are synthesised *de novo* at the base of the outer segments, displaced apically, and finally digested by the pigment epithelium¹⁷⁻²⁰. Since the chromophore required for *de novo* synthesis of rhodopsin is not stored in the retina and much reach it through the pigment epithelium, the fraction of total vitamin A compounds in the eye which must be returned daily to the retina is $1/n$, where n is the number of

days required for complete rod renewal. Considering that rod renewal in the rat and monkey require, respectively, 9 and 12 d (refs 21, 22), the importance of a vitamin A transport mechanism is clear.

The chemical properties of vitamin A compounds and the locations of the enzymes mediating their interconversion seem especially well suited to such a function of confining the chromophore to the eye and transporting it within the eye. Thus, in addition to a reactive form, retinal, which forms visual pigment in the outer segments, there is a transport form, retinol, which is diffusible, penetrates cell membranes, and is formed in the outer segments; and there is a storage form, retinyl ester, which is nonreactive, nondiffusible and formed in areas adjacent to the outer segments.

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- ¹ Wald, G., *Science*, **162**, 230 (1968).
- ² Wald, G., and Hubbard, R., *J. gen. Physiol.*, **32**, 367 (1949).
- ³ Futterman, S., in *Biochemistry of the Retina* (edit. by Graymore, C. N.), 16 (Academic, New York, 1965).
- ⁴ de Pont, J. J. H. H., Daemen, F. J. M., and Bonting, S. L., *Archs Biochem. Biophys.*, **140**, 275 (1970).
- ⁵ Greenberg, R., and Popper, H., *Am. J. Physiol.*, **134**, 114 (1941).
- ⁶ Krinsky, N., *J. biol. Chem.*, **232**, 881 (1958).
- ⁷ Hubbard, R., and Colman, R. D., *Science*, **130**, 977 (1959).
- ⁸ Dowling, J., *Nature*, **188**, 144 (1960).
- ⁹ Hubbard, R., and Dowling, J., *Nature*, **193**, 341 (1962).
- ¹⁰ Andrews, J. A., and Futterman, S., *J. biol. Chem.*, **239**, 4073 (1964).
- ¹¹ Noell, W., and Delmelle, M. C., *Science*, **172**, 72 (1971).
- ¹² Zimmerman, W. F., *Vision Res.* (in the press).
- ¹³ Cone, R. A., *J. gen. Physiol.*, **46**, 1267 (1963).
- ¹⁴ Dartnall, H. J. A., in *Photochemistry of Vision*, vol. II/1 of *Handbook of Sensory Physiology* (edit. by Dartnall, H. J. A.), 122 (Springer, New York, 1972).
- ¹⁵ Daemen, F. J. M., Rotman, J. P., and Bonting, S. L., *Expl Eye Res.*, **18**, 97 (1974).
- ¹⁶ Amer, S., and Akhtar, M., *Nature new Biol.*, **245**, 221 (1973).
- ¹⁷ Droz, B., *Anat. Rec.*, **145**, 157 (1963).
- ¹⁸ Hall, M. O., Bok, D., and Bacharach, A. D. E., *J. molec. Biol.*, **45**, 397 (1969).
- ¹⁹ Young, R. W., and Bok, D., *J. Cell Biol.*, **42**, 392 (1969).
- ²⁰ Young, R. W., *Vision Res.*, **11**, 1 (1971).
- ²¹ Young, R. W., *J. Cell Biol.*, **33**, 61 (1967).
- ²² Young, R. W., *J. Cell Biol.*, **49**, 303 (1971).

Difference in the cellular cholesterol to phospholipid ratio in normal lymphocytes and lymphocytic leukaemic cells

FLUIDITY of the fatty acyl chains of phospholipids in cell membranes is required for essential cellular activities, and the degree of fluidity can be influenced by cholesterol. Changes in the ratio of cholesterol to phospholipids can, therefore, affect the internal viscosity and molecular motion of lipids within membranes and this may function as a regulator in cell behaviour¹⁻⁴. Our studies of the surface membrane of normal lymphocytes and

Table 1 Cholesterol and phospholipid content of normal lymphocytes and lymphocytic leukaemia cells

Cell type	Total lipid (g $\times 10^{-12}$ per cell)	Cholesterol		Phospholipids		Cholesterol: lipid - P molar ratio
		(mol $\times 10^{-13}$ per cell)	(g $\times 10^{-6}$ per mg lipid)	(mol $\times 10^{-13}$ per cell)	(g $\times 10^{-6}$ per mg lipid)*	
Rat lymphocytes	4.4 \pm 0.5	0.6 \pm 0.05	53.5 \pm 0.8	2.35 \pm 0.3	442 \pm 23	0.25 \pm 0.01
Mouse lymphocytes	3.9 \pm 0.5	0.55 \pm 0.1	54.7 \pm 2.7	2.1 \pm 0.3	403 \pm 33	0.26 \pm 0.03
YAC leukaemia	16.8 \pm 0.8	2.2 \pm 0.2	51.8 \pm 3.3	11.7 \pm 0.9	550 \pm 40	0.19 \pm 0.01
EL4 leukaemia	12.7 \pm 0.9	1.7 \pm 0.1	51.6 \pm 3.4	9.9 \pm 1.0	604 \pm 77	0.17 \pm 0.01

Standard deviations are given for the mean value of five or six determinations for each type of cell. Normal lymphocytes from the lymph nodes of 6-8-week-old animals were collected by teasing the tissue apart in a cold solution of tricine-buffered saline (pH 7.4) and allowing the pieces to sediment. The cells were then washed three times with cold tricine-buffered saline. Both ascites tumours were generally inoculated intraperitoneally at 10^5 cells per adult mouse, the cells collected 12-14 d later and washed three times in cold tricine-buffered saline. Lipids were extracted by chloroform-methanol (2:1, v/v), as described⁹. Stirring was continued overnight at 4° C and there was no significant increase in yield of lipid phosphorous by a second extraction of the residue obtained after filtration. The chloroform-methanol extracts were washed as described¹⁰ with 0.1 M KCl in the upper phase. Lipid phosphorous was determined by the method of Ames¹¹ after digestion of the sample with an ethanolic solution of Mg(NO₃)₂. Free cholesterol was separated from cholesterol ester by column chromatography on silicic acid, and the two components were then assayed separately by the FeCl₃ method¹². The cholesterol ester was only 0.5-1% of the total lipid.

* Assuming an average phospholipid molecular weight of 775.

lymphocytic leukaemic cells have shown that the surface membrane of the leukaemic cells has a lower rotational mobility of concanavalin A binding sites^{5,6}, and a higher fluidity of the lipid layer^{6,7}. We have now found that these differences in fluidity are associated with a difference in the cholesterol to phospholipid ratio in these cells.

Normal lymphocytes obtained from the lymph nodes of A and C57BL mice and CR/RAR rats all gave similar results. These cells contained 70-85% thymus-derived cells. Normal B lymphocytes were obtained from the lymph nodes of C57BL nude mice. The lymphocytic leukaemic cells used were from two thymus-derived mouse ascites tumours, one from A strain mice (YAC) used before⁵⁻⁷ and the second (EL4) from C57BL mice⁸.

The cellular content of total lipids, free cholesterol and phospholipids was determined in the different cells (Table 1). The leukaemic cells were larger than the normal lymphocytes, and contained a larger amount of total lipids, cholesterol and phospholipids per cell. In both types of cell, however, cholesterol was 5-6% of the total lipid, so that the normal lymphocytes and leukaemic cells had about the same amount of cholesterol per mg dry lipid. Phospholipids were 40-45% and 55-65% of the total lipids in normal and leukaemic cells, respectively, so that the normal lymphocytes contained less phospholipid. The proportion of phospholipids in the B lymphocytes from nude mice was about the same as that in the normal lymphocytes which contained 70-85% thymus-derived cells. The molar ratio of cholesterol to phospholipids (Table 1) was therefore significantly smaller ($P < 0.001$) in the thymus-derived leukaemic cells than in the thymus-derived or B normal lymphocytes.

Studies of leukaemic cells collected at different times after inoculation of 2.5×10^6 cells per animal have shown that cells with a decreased rate of cell division, 12-14 d after inoculation, had a similar cholesterol to phospholipid ratio to that of cells in the logarithmic phase of growth. The decreased cholesterol to phospholipid ratio that we have found in the mouse leukaemic cells that were larger than the normal lymphocytes, has also been observed with human lymphocytic leukaemias where the cells were smaller than the normal lymphocytes⁹. It is therefore unlikely that the difference in the ratio between normal and leukaemic cells is only due to a difference in cell size. Although there is a decrease in the cholesterol to phospholipid ratio, it is interesting that cholesterol is synthesised more rapidly in lymphocytic leukaemic cells than in normal lymphocytes¹³, presumably due to a loss of the feedback control that has been found with hepatomas¹⁴.

Our results indicate that the higher fluidity of the lipids in the surface membrane after malignant transformation of normal lymphocytes^{6,7} is associated with a decrease in the molar ratio of cholesterol to phospholipids, and not with a significant decrease in the amount of cholesterol per mg lipid. The larger cholesterol to phospholipid ratio in the normal lymphocytes can

explain the lower fluidity of their surface membrane lipids. We suggest that differences in this ratio may be involved in the regulation of growth and differentiation in normal and malignant cells.

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- Oldfield, E., and Chapman, D., *FEBS Lett.*, **23**, 285 (1972).
- Dekruyff, B., Denel, R. A., and Van Deenen, L. L. M., *Biochim. biophys. Acta*, **255**, 331 (1972).
- Rottem, S., Yashouv, J., Ne'eman, Z., and Razin, S., *Biochim. biophys. Acta*, **323**, 495 (1973).
- Papahadjopoulos, D., Cowden, M., and Kimelberg, H. K., *Biochim. biophys. Acta*, **330**, 8 (1973).
- Inbar, M., Shinitzky, M., and Sachs, L., *J. molec. Biol.*, **81**, 245 (1973).
- Sachs, L., *Harvey Lectures*, **68**, (Academic Press, New York, in the press).
- Inbar, M., Shinitzky, M., and Sachs, L., *FEBS Lett.*, **38**, 268 (1974).
- Shevach, E. M., Stobo, J. D., and Green, I., *J. Immun.*, **108**, 1146 (1972).
- Gottfried, E. L., *J. Lipid Res.*, **8**, 321 (1967).
- Folch, J., Lees, M., and Sloane-Stanley, G. H., *J. biol. Chem.*, **226**, 497 (1957).
- Ames, B. N., in *Methods in enzymology* (edit. by Neufeld, E. F., and Ginsburg, V.), **8**, 115 (Academic Press, New York, 1966).
- Wycoff, H. D., and Parsons, J., *Science*, **125**, 347 (1957).
- Chen, H. W., Kandutsch, A. A., Heimer, H. J., and Meier, H., *Cancer Res.*, **33**, 2774 (1973).
- Brown, S. M., Goldstein, J. L., and Siperstein, M. D., *Fedn Proc.*, **32**, 2168 (1973).

Quantum conductance changes in lipid bilayer membranes associated with incorporation of acetylcholine receptors

CONSIDERABLE attention has focused on the isolation and characterisation of the nicotinic acetylcholine (ACh) receptor from electroplax¹⁻⁴. This receptor is identified by its ability to bind cholinergic ligands, and its molecular weight and amino acid content have been estimated^{5,6}. Similar extraction procedures have also been applied to nicotinic receptors from mammalian brain⁷ but it is not known whether the coupling of ACh to the receptor results in the opening of transmembrane channels, the activation of ionic carrier mechanisms or some other mechanism that modifies mem-

brane permeability. Little is known about the ionic permeability changes associated with the function of the single receptor. It has been reported⁸ that crude acetylcholinesterase (AChE) preparations from electroplax contain material capable of inducing transient membrane permeability changes in lipid bilayers in response to ACh or carbachol, but the relationship to ACh receptor function is unclear.

We wish to report discrete changes of conductance after the incorporation of an ACh receptor preparation into artificial lipid bilayers. These events were related to a unit conductance that is probably associated with some fundamental function of the receptor.

Receptors were extracted by modification of published affinity chromatography techniques that make use of the curaric properties of neurotoxins from elapid snake venoms⁴⁻⁶. *Naja melanoleuca* d-toxin (10 mg) was attached to 15 ml of Agarose A-15 (Biorad) beads by the cyanogen bromide reaction⁹⁻¹¹ and a typical receptor extraction then proceeded as follows. Twenty-five whole mouse brains were homogenised in 10 volumes of 0.05 M sodium phosphate buffer, pH 7.4, containing 0.1% Triton X-100 detergent. The particulate matter was removed by centrifugation at 10,000g for 30 min and the supernatant was passed through the toxin-Agarose gel. The gel was then washed with the same buffer-detergent solution plus 1 M NaCl to disassociate material bound by nonspecific ionic interactions, followed by receptor elution with a 0.0 to 0.5 M carbachol gradient in buffer-detergent. Protein determinations¹² indicated the possible presence of two protein peaks following exhaustive dialysis against 0.05 M phosphate buffer.

The ability of the original fractions from peak 1 (containing about 0.2 M carbachol from the gradient) to affect ionic permeability were examined in lipid bilayers as described earlier¹³. This involved a dilution of the original fractions by 400:1 to at most 25 ng ml⁻¹ of total protein and 5×10^{-4} M of carbachol in 100 mM NaCl on one side (*cis*) of the bilayer. The other side (*trans*) contained 1 M NaCl and the gradient thus formed allowed both sodium and chloride conductances to be estimated separately, depending on the polarity of the voltage clamp across the membrane. The chamber was made of a fluorochlorocarbon material (Kel-F) and contained a 1.4-mm hole over which the bilayer was spread. A Keithley 427 current amplifier was used to amplify the current changes sufficiently for detection on a strip-chart recorder. Clamp was maintained at + or -80 mV.

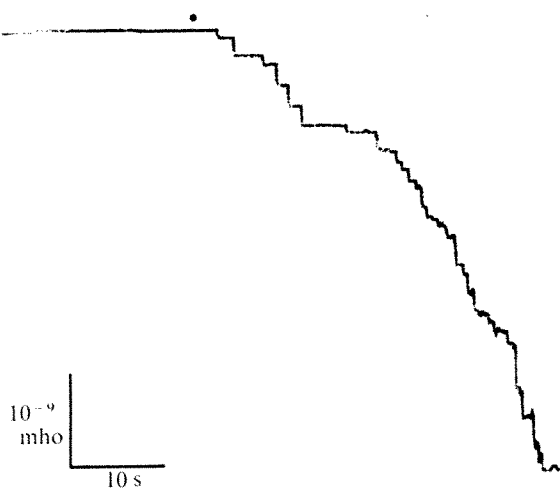


Fig. 1 Initial increase in chloride conductance after addition of receptor to *cis* side of bilayer. The final concentration of total protein was at most 25 ng ml⁻¹ with about 5×10^{-4} M carbachol.

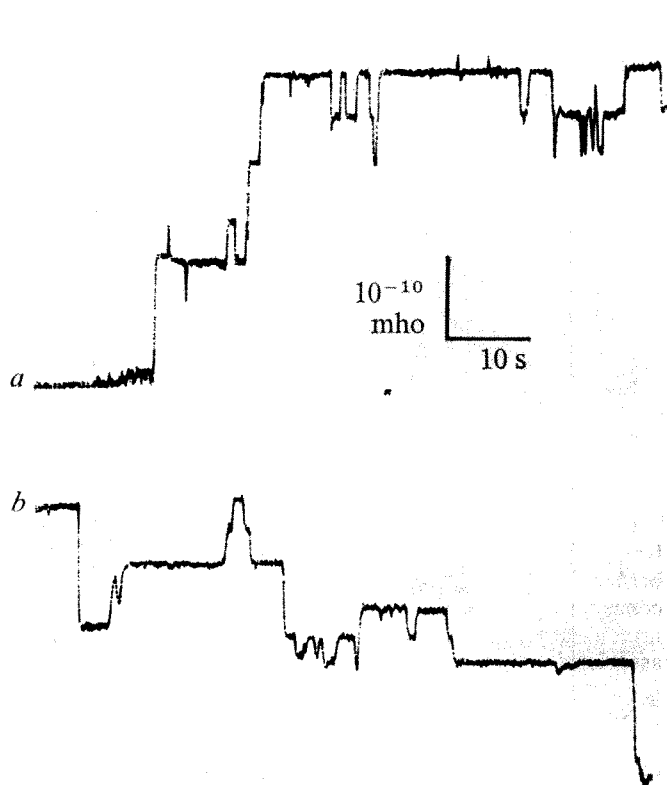


Fig. 2 The same receptor preparation as in Fig. 1 except that 2.5×10^{-3} M of tubocurarine was added to the *cis* side of the bilayer. The scale is at 10 times higher resolution with (a) the chloride conductance and (b) the sodium conductance.

Under these conditions, two sizes of conductance changes were electronically detected with step increases of 2.4×10^{-10} mho (Cl^-) and 1.5×10^{-10} mho (Na^+) for large quanta and 5.9×10^{-11} mho (Cl^-) and 3.7×10^{-11} mho (Na^+) for small quanta. The initial conductance increase was monotonic and the chloride conductance recording is shown in Fig. 1. Addition of tubocurarine at competitive concentrations (2.5×10^{-3} M) seemed to slow the rate of occurrence of large quanta by about 30:1 and the smaller quanta became prominent (Fig. 2a (Cl^-) and b (Na^+)). A similar effect seemed to occur with addition of similar concentrations of atropine, although the nature of the studies made quantitation difficult.

When the carbachol was removed by dialysis of the receptor preparation against phosphate-detergent solution, the rate of occurrence of large quanta appeared to be reduced relative to the occurrence of small quanta. When the receptor was dialysed against phosphate alone, however, activity ceased, indicating that detergent was necessary to preserve the electrical properties of the molecular entity involved. In addition, if the receptor was eluted by a carbachol gradient in phosphate but in the absence of detergent, no electrical activity was detected when the preparation was incorporated into the bilayer. This occurred in spite of the detection of similar elution peaks. We had previously found that Triton X-100 alone did not affect our bilayer system at the concentrations used. Gel electrophoresis demonstrated the presence of one primary protein peak, although the possibility that the electrical activity is caused by another protein component cannot be excluded. Peak 2 showed qualitatively similar electrical activity.

The ionic selectivities of the large and small quanta and their relative size suggests that the large quanta represent an aggregate of four of the small quanta. This might be

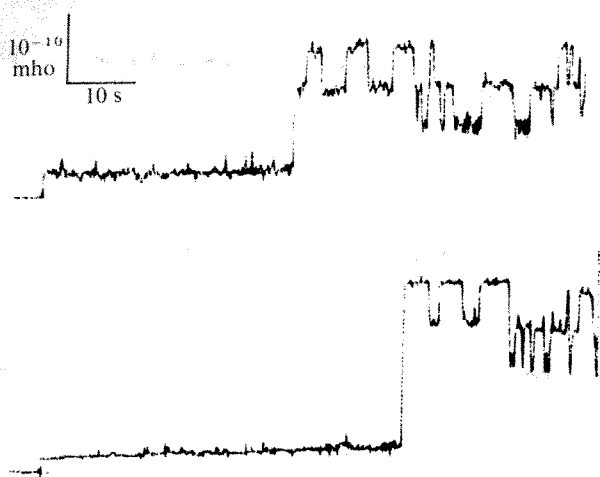


Fig. 3 Conductance changes (Cl^-) for two runs after the receptor had been dialysed against 0.05 M phosphate buffer, pH 7.4, containing 0.1% Triton X-100 for 72 h with six changes of dialysate.

related to theories that the receptor can exist as a tetramer^{14,15}. We note that the electrical activity observed is consistent with the theory that Ach-mediated membrane permeability changes might occur by ionic channel gating.

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- ¹ Changeux, J.-P., Kasai, M., and Lee, C. Y., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1241-1247 (1970).
- ² LaTorre, J. L., Lunt, G. S., and de Robertis, E., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 716-720 (1970).
- ³ Miledi, R., Molinoff, P., and Potter, L. T., *Nature*, **229**, 554-557 (1971).
- ⁴ Karlsson, E., Heilbronn, E., and Widlund, L., *FEBS Lett.*, **28**, 107-111 (1972).
- ⁵ Eldefrawi, M. E., and Eldefrawi, A. T., *Archs Biochem. Biophys.*, **159**, 362-373 (1973).
- ⁶ Klett, R. P., Fulpius, B. W., Cooper, D., Smith, M., Reich, E., and Possani, L. D., *J. biol. Chem.*, **248**, 6841-6853 (1973).
- ⁷ Bosmann, H. B., *J. biol. Chem.*, **247**, 130-145 (1972).
- ⁸ Jain, M. K., Mehl, L. E., and Cordes, E. H., *Biochem. biophys. Res. Comm.*, **51**, 192-197 (1973).
- ⁹ Axen, R., Porath, J., and Ernback, S., *Nature*, **214**, 1302-1309 (1967).
- ¹⁰ Porath, J., Axen, R., and Ernback, S., *Nature*, **215**, 1491-1492 (1967).
- ¹¹ Cuatrecasas, P., *J. biol. Chem.*, **245**, 3059-3065 (1970).
- ¹² Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- ¹³ Goodall, M. C., *Archs Biochem. Biophys.*, **147**, 129-135 (1971).
- ¹⁴ Khromov-Borisov, N. V., and Michelson, M. J., *Pharmac. Rev.*, **18**, 1051-1090 (1966).
- ¹⁵ de Robertis, E., *Science*, **171**, 963-971 (1971).

Opiate agonist-antagonist effects on Renshaw cells and spinal interneurons

ANALGESIC doses of morphine change the levels^{1,2} and release^{3,4} of acetylcholine from the central nervous system. At the single neurone level, however, little is known of the

effects of morphine on cholinergic transmission. On the only central neurones known to receive cholinergic endings, the Renshaw cells⁵, morphine administered electrophoretically from micropipettes has been shown to modify cell activity in several ways: to reduce the depressant action of glycine⁶, to increase the latency of action potentials evoked by a ventral root stimulus⁷ and to excite⁸.

To assess the relevance of these effects to the opiate syndrome⁹, we performed experiments with naloxone, a narcotic antagonist essentially devoid of agonist activity⁹. As a further test of specificity of effects observed with morphine, three morphinans were also studied: levorphanol, an active opiate; dextrorphan, an isomer devoid of narcotic activity; and levallorphan, a narcotic antagonist. The results indicate that excitation at nicotinic receptors for acetylcholine may be of relevance to the opiate syndrome.

Experiments were performed on 23 cats anaesthetised with pentobarbitone sodium. The spinal cord was prepared for stimulation and recording using conventional techniques¹⁰. Renshaw cells were located by the responses to stimulation of ventral roots. Extracellular recordings were obtained with the centre barrels of seven barrel micropipettes and the outer barrels contained the drugs to be ejected electrophoretically. These were: morphine SO_4 (70 mM), naloxone HCl (0.1 M), levorphanol tartrate (0.1 M and 50 mM in 100 mM NaCl), dextrorphan tartrate (0.1 M), levallorphan tartrate (0.1 M), acetylcholine Br (0.5 M), L-glutamate Na (0.5 M), L-aspartate Na (0.5 M), DL-homocysteate Na (0.2 M), glycine (0.5 M pH 3), γ -aminobutyric acid (0.5 M pH 3), atropine SO_4 (10 mM in 165 mM NaCl), dihydro- β -erythroidine HBr (10 mM in 165 mM NaCl), nicotine HCl (0.2 M), acetyl- β -methylcholine Cl (0.5 M).

Morphine (30-80 nA) excited 42 of 55 Renshaw cells tested. This effect was readily demonstrated on cells excited by low concentrations of acetylcholine (ejecting current less than 10 nA) and was not necessarily accompanied by bursts of firing. With cells relatively insensitive to acetylcholine, ejection of morphine with relatively high currents was more likely to produce bursts of action potentials with little rise in mean firing rate. Whereas the ejecting current of morphine required to produce cell firing was approximately ten times that of acetylcholine, much lower currents of morphine enhanced the excitant action of acetylcholine and/or an excitant amino acid. Excitation of a Renshaw cell by morphine acetylcholine and DL-homocysteate is shown in Fig. 1.

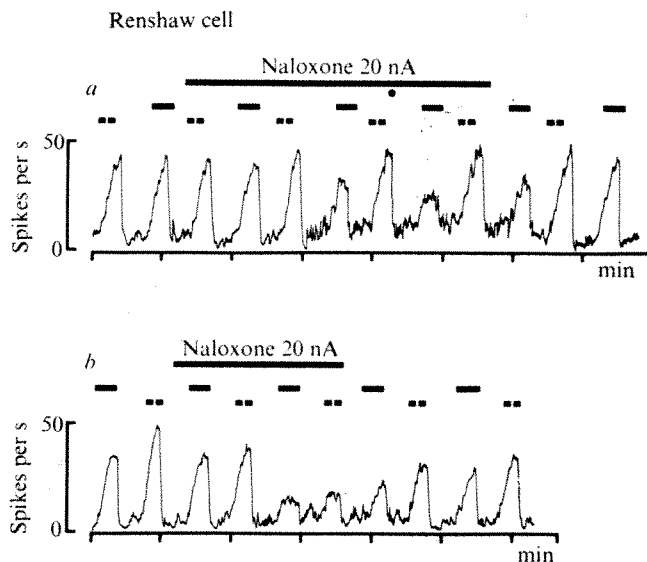


Fig. 1 The effect of naloxone on excitation of a Renshaw cell by, a, morphine (30 nA) (—) and DL-homocysteate (30 nA) (—) and, b, by morphine (30 nA) (—) and acetylcholine (7 nA) (—). The times of electrophoretic ejection of compounds are marked by horizontal bars above each record of cell firing.

Excitation by morphine was antagonised by naloxone. Ejecting currents of naloxone (10–30 nA) adequate to block the effects of morphine were usually without effect on cell firing but higher concentrations sometimes produced bursts of firing. Excitation by acetylcholine but not that by an amino acid was also reduced by naloxone. Figure 1 illustrates the differentiation between these two types of receptor by naloxone. Naloxone also reduced the excitant effect of nicotine but not that of acetyl- β -methylcholine on Renshaw cells, indicating a probable action at nicotinic receptors on these neurones. Dihydro- β -erythroidine (1–5 nA) readily reduced the action of acetylcholine on Renshaw cells and also reduced excitation by morphine. Atropine was a relatively poor antagonist of excitation of Renshaw cells by acetylcholine but again the action of morphine was also reduced in parallel with acetylcholine.

The other effects of morphine on Renshaw cells were not antagonised by naloxone. Naloxone acted like morphine in prolonging the latency of the initial action potentials of Renshaw cells in response to a ventral root stimulus but was without effect on the depressant action of glycine and GABA and did not modify the antagonism of glycine by morphine.

Morphine (5–100 nA) did not excite a sample of spinal interneurons located by firing in response to stimulation of peripheral nerves. Morphine (17 of 25 cells) and naloxone (10 of 12 cells) were depressants of these neurones and no antagonism of the action of morphine by naloxone was observed. This action of morphine has been previously reported¹¹ but the lack of antagonism by naloxone casts doubt on its relevance to the effects of systematically administered morphine.

All three morphinans tested reduced the sensitivity both to acetylcholine and an amino acid excitant of a proportion of Renshaw cells, an effect accompanied by a reduction in action potential amplitude. This was the only effect observed with dextrorphan (eight cells) and levallorphan (five cells) but with levorphanol, the active opiate, excitation was observed with five of twelve Renshaw cells. The reduction of effectiveness of all excitants is possibly a local anaesthetic effect¹² as these compounds are known to block conduction in squid axon¹³ and are more potent than morphine in this respect.

Concentration attained when drugs are administered micro-electrophoretically are unknown and therefore the relevance of effects observed with this method to those occurring after systemic administration can be difficult to assess. Nevertheless, as excitation by morphine at nicotinic receptors for acetylcholine was the only action of this alkaloid antagonised by naloxone the effect warrants further investigation. Studies of the distribution of opiate receptors in the brain of the rat based on the displacement of bound naloxone by active and inactive morphine-like compounds have found good correlation with the distribution of acetylcholine¹⁴. In this species electrophoretically administered morphine excited one half of the brain stem neurones sampled¹⁵ but no correlation with the effects of acetylcholine, noradrenaline or 5-hydroxytryptamine was observed.

Our experiments are being extended to cholinceptive cells in other regions of the central nervous system of the cat.

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¹ Howes, J. F., Harris, L. S., Dewey, W. L., and Voyda, C., *J. Pharmacol.*, **169**, 23–28 (1969).

² Crossland, J., and Slater, P., *Br. J. Pharmacol.*, **33**, 42–47 (1968).

- ³ Jhamandas, K., Pinsky, C., and Phillis, J. W., *Nature*, **228**, 176–177 (1970).
- ⁴ Mullin, M. J., Phillis, J. W., and Pinsky, C., *Eur. J. Pharmacol.*, **22**, 117–119 (1973).
- ⁵ Curtis, D. R., and Ryall, R. W., *Expl Brain Res.*, **2**, 81–96 (1966).
- ⁶ Curtis, D. R., and Duggan, A. W., *Agents Actions*, **1**, 14–19 (1969).
- ⁷ Duggan, A. W., and Curtis, D. R., *Neuropharmacol.*, **11**, 189–196 (1972).
- ⁸ Martin, W. R., *Pharmac. Rev.*, **19**, 463–521 (1967).
- ⁹ Blumberg, H., Dayton, H. B., George, M., and Rapaport, D. N., *Fedn Proc.*, **20**, 311 (1961).
- ¹⁰ Curtis, D. R., Phillis, J. W., and Watkins, J. C., *J. Physiol. Lond.*, **150**, 656–682 (1960).
- ¹¹ Dostrovsky, J., and Pomeranz, B., *Nature new Biol.*, **246**, 222–224 (1973).
- ¹² Curtis, D. R., and Phillis, J. W., *J. Physiol., Lond.*, **153**, 17–34 (1960).
- ¹³ Simon, E. J., and Rosenberg, P., *J. Neurochem.*, **17**, 881–887 (1970).
- ¹⁴ Pert, C. B., and Snyder, S. H., *Science*, **179**, 1011–1014 (1973).
- ¹⁵ Bradley, P. B., and Dray, A., *Br. J. Pharmacol.*, **47**, 642P (1973).

Lithium and the monoamine neurotransmitters in the rat hippocampus

ALTHOUGH the psychoactive properties and therapeutic value of lithium have long been recognised¹, relatively little is known about its mode of action in the central nervous system². This is partly due to the lack of a complete understanding of the biological basis of mental disorders and to the variety of biological functions that can be affected by lithium.

The putative monoamine neurotransmitters, noradrenaline, dopamine and serotonin have been implicated in such functions, and indeed lithium influences their metabolism in the brain^{3,4}. The precise distribution of monoamine-containing cell bodies, fibres and synaptic terminals has been well documented for several targets within the brain. Recently, the monosynaptic pathway between the catecholamine-containing pontine nucleus locus coeruleus (LC) and the hippocampus was studied physiologically and pharmacologically^{6,7}. In this pathway the iontophoretic application of noradrenaline suppresses spontaneous discharges of hippocampal pyramidal cells⁶ as does stimulation of the LC (ref. 7). These noradrenergic synapses in the hippocampus thus provide an ideal test system for the search for the mechanisms of psychoactive drugs in the nervous system. I describe here an attempt to elucidate a possible mode of action of lithium in the hippocampus. This was done by searching for possible interactions between the effects of iontophoretically applied lithium and several putative neurotransmitters as well as the electrical stimulation of the LC, on firing rates of hippocampal pyramidal cells.

I used male Zivic Miller rats (150–200 g) anaesthetised with halothane. Methods of recording and drug iontophoresis have been described elsewhere⁸. Briefly, spike discharges were recorded through the central barrel of a five-barrel micropipette. Three of the outer barrels contained lithium chloride (Li⁺, 0.1–0.2 M); acetylcholine chloride (ACh, Merck, 2.5 M); L-noradrenaline-HCl (Aldrich, 1 M); serotonin creatinine sulphate (Aldrich, 0.5 M) and γ -aminobutyric acid (GABA, Aldrich, 1 M). Each compound was ejected as cations, and retained with a negative holding current. The fourth outer barrel was filled with 3 M NaCl and ejected a continuous balancing current⁸. Stimulating electrodes were placed in the LC as described previously⁷.

The activity of thirty-five cells in the pyramidal cell layer of the dorsal hippocampus was monitored in eight rats. When applied iontophoretically (with a current range of

25–100 nA) Li^+ did not have a consistent direct effect on firing of hippocampal cells. Only three of the cells tested were slightly excited (120–140% of pre-drug rate) and two were slightly slowed (Fig. 1a), whereas the firing patterns or spike sizes of thirty other cells were not affected. Noradrenaline had a consistent and long lasting inhibitory action on spontaneous and Ach-enhanced hippocampal activity⁶. All fifteen cells tested were inhibited during application of noradrenaline (current range 50–100 nA applied in pulses of 15 s). In seven of the cells inhibition was reversibly antagonised during the concurrent application of Li^+ (Fig. 1b). In five more cases Li^+ (in 1.0 M concentration within the pipette) antagonised the cellular response to noradrenaline but no complete recovery was demonstrated even 10–30 min after application of Li^+ . Only three cells were unaffected by Li^+ .

The interaction between Li^+ and serotonin was tested in seven cells. Like noradrenaline, it was inhibitory with respect to spontaneous hippocampal cellular activity. Concurrent application of Li^+ for 2–3 min reversibly antagonised the inhibitory responses of three cells to serotonin. Four other cells were unaffected (Fig. 1a).

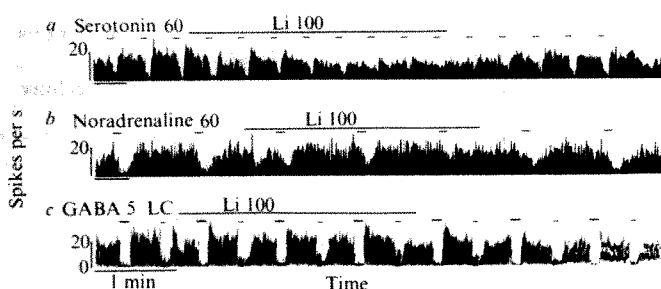


Fig. 1 Interactions between iontophoretic application of Li^+ , several putative neurotransmitters and LC stimulation. Hippocampal cellular activity in the pyramidal layer was integrated over 1-s intervals and recorded on an ink recorder. *a*, The effects of Li^+ on the responses to serotonin applied with an injection current of 60 nA at fixed regular intervals (bars). The concurrent application of Li^+ (with current of 100 nA), for 2–3 min caused a slowing of the spontaneous firing, but also blocked the response to serotonin. This response was reinstated 1–2 min after termination of Li^+ current. *b*, Same as in (*a*), with a different cell, Li^+ antagonised the response to noradrenaline with no noticeable effect on spontaneous activity. *c*, Li^+ effects tested against the iontophoretic application of GABA and stimulation of LC. Stimulation parameters: pulses of 0.1 ms, 0.4 nA, applied through a concentric stimulating electrode at a rate of 10 per s for 5 s. Li^+ had no effect on the response to GABA but there was an antagonism of the response to LC stimulation. Note the difference in time scale between (*a*), (*b*) and (*c*).

Among the other compounds tested, GABA exerted the most potent inhibitory action towards hippocampal activity and its effect was pronounced even with small ejection current (0–5 nA). But none of the responses of fifteen cells tested was affected by the iontophoretic application of Li^+ in the current ranges that proved to be effective for antagonism of the responses to noradrenaline and serotonin (Fig. 1c). Similarly, acetylcholine, a putative neurotransmitter in the hippocampus had an excitatory action which was not modified by the iontophoresis of Li^+ . The absence of interaction was noted in a number of cells, although no systematic tests were made.

Finally, electrical stimulation of the LC, the source of hippocampal noradrenergic fibres and terminals produce noradrenaline-like inhibition⁷ (Fig. 1c). In five out of six cells tested, LC stimulation was at least partially antagonised by concurrent iontophoretic application of Li^+ . This antagonism did not result, however, in a complete elimination of the response to LC stimulation. Since at least some of the

noradrenergic terminals on the recorded cell are probably remote from the pipette tip and may not be affected by the Li^+ ejected, a complete antagonism could be difficult to achieve.

Under the conditions of these iontophoretic experiments, there was a built-in control for defining the specificity of the effects of Li^+ . When Li^+ or the other compounds were not being ejected, they were retained in the pipettes with a continuous negative current. To neutralise the continuous retaining currents at the pipette tip between and during drug ejection, positive current, that is, Na^+ ions, was continuously ejected from the balance barrel. Therefore, in all cases, the effects of Li^+ were actually tested against the effects of Na^+ .

Lithium can exert its antagonistic action by changing the ionic balance in the recorded cell environment. Although this is considered to be a possible effect of Li^+ in living tissue^{8,10}, it is not likely to be the cause for the specific effect demonstrated here since spontaneous firing as well as GABA-induced inhibition were not modified in the recorded hippocampal cells. A more likely alternative mechanism for modification by Li^+ is the cyclic AMP system. It has been postulated that cyclic AMP mediates the electrophysiological response to noradrenaline in the rat cerebellum¹¹ and there is some evidence for such a case in rat hippocampus, where the phosphodiesterase inhibitor, papaverine, potentiates the response of hippocampal cells to noradrenaline applied iontophoretically, and prostaglandin E_1 antagonises this response⁶. Lithium has also been found to block noradrenaline-induced formation of cyclic AMP in rat cortical slices¹² with relatively low concentrations, and this, together with the data reported here supports the hypothesis that cyclic AMP mediates monoamine response in the rat hippocampus.

Although my data are compatible with the view that Li^+ could block the noradrenaline receptor through an action on cyclic AMP synthesis, earlier alternative assumptions of Li^+ action based on such findings as increased uptake of noradrenaline by brain synaptosomes¹³, decrease in the release of noradrenaline and serotonin from brain slices¹⁴, cannot be eliminated. The iontophoretic mode of application, in spite of its many advantages, tests actually the acute effects of Li^+ which might be different from the effects of a chronic Li^+ treatment. Further electrophysiological study of Li^+ actions in a chronically treated preparation are required to clarify these possibilities.

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- ¹ Cade, J. F. J., *Med. J. Aust.*, **36**, 349 (1949).
- ² Samuel, D., and Gottesfeld, Z., *Endeavour*, **32**, 122 (1973).
- ³ Greenspan, K., Aronoff, M. S., and Bogdanski, D. F., *Pharmacology*, **3**, 129 (1970).
- ⁴ Ho, A. K. S., Loh, H. H., Craves, F., Hitzemann, R. J., and Gershon, S., *Eur. J. Pharmacol.*, **10**, 72 (1970).
- ⁵ Ungerstedt, U., *Acta physiol. scand.*, **82** Suppl. 367, 1 (1971).
- ⁶ Segal, M., and Bloom, F. E., *Brain Res.*, **72**, 79 (1974).
- ⁷ Segal, M., and Bloom, F. E., *Brain Res.*, **72**, 99 (1974).
- ⁸ Salmoiraghi, G. C., and Weight, F. F., *Anesthesiology*, **28**, 54 (1968).
- ⁹ Bunney, W. E., Goodwin, F. K., Davis, J. M., and Fawcett, S. A., *Am. J. Psychiat.*, **125**, 444 (1968).
- ¹⁰ Baer, L., Kassir, S., and Fiere, R., *Psychopharmacologia*, **17**, 216 (1970).
- ¹¹ Siggins, G. R., Battenberg, E. F., Hoffer, B. J., and Bloom, F. F., *Science*, **179**, 585 (1973).
- ¹² Forn, J., and Valdecasas, F. G., *Biochem. Pharmacol.*, **20**, 2773 (1971).

¹³ Colburn, R. W., Goodwin, F. K., Bunney, E. W., and Davis, J. M., *Nature*, **215**, 1395 (1967).

¹⁴ Katz, R. I., Chase, T. N., and Kopin, I. J., *Science*, **162**, 466 (1968).

Suppression of phage nonsense and temperature-sensitive mutants by an *suA* mutant *E. coli*

BECKWITH and Scaife^{1,2} showed that the suppressor allele *suA* of *Escherichia coli* does not suppress nonsense codons in the lactose operon, but does relieve the polarity effect of UAG, UAA and possibly frameshift mutations. It is shown here that *suA E. coli* strongly suppresses phage T4 amber mutations and weakly suppresses phage T4 temperature-sensitive and amber

mutations. Suppression of the inhibitory effect of chloramphenicol on phage T4 growth by *suA* was also shown. In addition, amber mutants of phage T7 were observed to be suppressed by *suA*. These results suggest that *suA* in addition to suppressing polarity in *E. coli* also increases translational ambiguity in T4 and T7-infected cells, especially for the amber codon.

All phage T4 strains used in this study as well as *E. coli* strains CR63 (*su*-1⁺ UAG), *E. coli* B (*su*-) and *E. coli* BB (containing an amber suppressor, as described by Wilson and Kells³) were from the California Institute of Technology stock collection, under the case of W. Wood. *E. coli* CAJ68 (containing a UGA amber suppressor) was provided by D. Mount, University of Arizona. *E. coli suA* XA7007, F⁻, Δ(Lac-Pro), Mal⁻, B₁⁻, Str^r, Δλ and *E. coli su*- Sy106 were a gift from W. Summers, Yale University and were the strains used in all burst size determinations. Sy106 is an isolate of the original strain from which XA7007 was derived and has the same genetic markers. The presence of the identifying markers in *E. coli suA* XA7007 and *su*- Sy106 were confirmed here. A related *su*- strain, *E. coli su*- XA7014, was obtained from J. Beckwith. The phage T7 strains used were originally from the laboratory of W. Studier and were supplied by T. North at the University of Arizona.

The suppression of phage amber mutants (Table 1) was compared by burst size measurement in four *E. coli* strains (CR63, *suA*, *su*- and B). Unadsorbed phage, measured in a chloroform-treated aliquot taken before the end of the eclipse period, were subtracted when they constituted more than 5% of the final phage yield. Clearly *suA* is comparable with CR63 in the efficiency of its suppression of amber mutants whereas *su*- is about as inefficient as B. In general, amber mutants defective in catalytic functions showed greater burst sizes on suppression by *suA* than those defective in stoichiometric functions⁴. When further tested on plates, the efficiency of suppression by *suA* of most of the amber mutants was high enough to allow these mutants to form plaques. When either the *su*- strain obtained from Summers (Sy106) or the *su*- strain obtained from Beckwith (XA7014) was used as an indicator, no plaques were formed.

The suppression of phage temperature-sensitive (*ts*) mutants is compared in the *suA* and *su*- strains at various temperatures (Table 2). The *ts* mutants defective in catalytic functions seem to be significantly suppressed by *suA* at higher temperatures. Although there is some suppression of *ts* mutants defective in

Table 1 Suppression of T4 amber mutants by the *suA* allele, expressed in burst size

	CR63*	<i>suA</i> *	<i>su</i> -†	<i>E. coli</i> B†
Catalytic functions				
<i>amNG83</i> (30)	46	77	0.01	0.003
<i>amE1017</i> (30)	70	131	0.03	0.008
<i>amNG352</i> (42)	21	20	0.06	0.04
<i>amE93</i> (42)	32	22	0.0	0.01
<i>amB22</i> (43)	86	339	0.01	0.02
<i>am4301</i> (43)	83	189	0.06	0.01
<i>amB3</i> (46)	14	112	0.1	0.01
<i>amNG371</i> (46)	55	263	0.7	0.08
<i>amNG106</i> (47)	77	256	0.2	0.06
Stoichiometric functions				
<i>amN50</i> (20)	108	5	0.0	0.005
<i>amB17</i> (23)	62	102	0.06	0.01
<i>amB272</i> (23)	51	18	0.15	0.01
<i>amN65</i> (24)	66	2	0.0	0.01
<i>amB26</i> (24)	79	4	0.06	0.01
<i>amA453</i> (32)	56	25	0.03	0.06
<i>amE1</i> (36)	10	7	0.03	0.01
<i>amC15</i> (36)	63	5	0.06	0.03
<i>amN52</i> (37)	61	152	0.01	0.06
<i>amB280</i> (37)	37	1	0.5	0.08
T4D	143	265	47	226

Phage infections were carried out at 26.6° C. After each amber mutant designation the gene in which the mutant is defective is indicated in parentheses.

* Average of two experiments.

† Average of three experiments.

Table 2 Suppression of T4 *ts* mutants by the *suA* allele, expressed in burst size

	25.6°C	28.0°C	30.0°C	34.0°C	38.0°C*	39.6°C	41.5°C	42.0°C†
Catalytic functions								
<i>tsL88</i> (43) <i>suA</i>	200	336	290	407	93	55	14	2.1
<i>tsL88</i> (43) <i>su</i> -		226		370	36	29	0.02	0.087
<i>tsL91</i> (43) <i>suA</i>	43	129	107	65	25	61	27	2.1
<i>tsL91</i> (43) <i>su</i> -		126		179	26	8.4	0.5	0.506
<i>tsL109</i> (46) <i>suA</i>	227	335	436	305	119	46	6.0	1.1
<i>tsL109</i> (46) <i>su</i> -		199		378	21	8.1	0.5	0.622
<i>tsB10</i> (47) <i>suA</i>	192	419	488	326	101	4.2	4.0	5.3
<i>tsB10</i> (47) <i>su</i> -		79		16	2	0.8	0.1	0.531
<i>tsA52</i> (47) <i>suA</i>	258	390	470	385	90	26	5.0	6.6
<i>tsA52</i> (47) <i>su</i> -		190		181	8	4.0	0.3	0.436
Stoichiometric functions								
<i>tsL65</i> (23) <i>suA</i>	147	370	567	388	51	1.7	0.04	1.0
<i>tsL65</i> (23) <i>su</i> -		177		477	50	6.6	0.005	0.001
<i>tsN29</i> (24) <i>suA</i>	178	435	514	438	3.1	0.09	0.04	0.8
<i>tsN29</i> (24) <i>su</i> -		184		297	1.2	—	0.002	0.002
<i>tsA4</i> (37) <i>suA</i>	198	301	887	534	131	142	158	24
<i>tsA4</i> (37) <i>su</i> -		54		235	35	27	18	34
<i>tsA31</i> (37) <i>suA</i>	192	284	367	335	98	126	125	37
<i>tsA31</i> (37) <i>su</i> -		117		123	43	61	31	56
T4D <i>suA</i>	151	185	380	262	138	160	389	121
T4D <i>su</i> -		287		351	125	125	108	144

After each *ts* mutant designation the gene in which the mutant is defective is indicated, as well as the *E. coli* host infected (either *suA* or *su*-).

*Average of two experiments.

†Average of three experiments.

stoichiometric functions by *suA* at higher temperatures, it is less striking.

The suppression of the phage umber mutants by CAJ68, *suA* and *su⁻* is shown in Table 3. *SuA* seems to be a significantly more effective host in supporting growth of umber mutants than *su⁻*, but not nearly as effective as CAJ68, which contains a specific umber suppressor. This low efficiency of suppression by *suA* of umber mutants is similar to the low efficiency of suppression of *ts* mutants reported above and in contrast to the high efficiency found for amber mutants.

Table 3 Suppression of T4 umber (*um*) mutants by the *suA* allele, expressed in burst size

	CAJ68*	<i>suA</i> †	<i>su⁻</i> ‡
Catalytic functions			
<i>umC166</i> (56)	265	2.1	0.0
<i>umC123</i> (47)	391	6.5	0.1
<i>umC102</i> (42)	131	0.1	0.0
Stoichiometric functions			
<i>umC23</i> (37)	296	0.7	0.05
<i>umC105</i> (34)	361	3.0	0.0
T4D	245	337	308

Phage infections were carried out at 26.6° C. After each umber mutant designation the gene in which the mutant is defective is indicated in parentheses.

* Average of two experiments.

† Average of three experiments.

The burst sizes of phage T7 amber mutants in three *E. coli* strains (BB, *suA* and *su⁻*) are compared in Table 4. *E. coli suA* is comparable with *E. coli* BB (which contains an amber suppressor) in the efficiency of its suppression of T7 amber mutants. In contrast, growth of T7 on *E. coli su⁻* is at least an order of magnitude less efficient. Where *E. coli su⁻* was used as a plating indicator, no plaques were obtained. When *E. coli* BB or *suA* was used as a plating indicator, large distinct plaques were formed. *E. coli suA* thus seems to suppress T7 and T4 amber mutants with about the same efficiency.

In the experiments described in Table 5, chloramphenicol was added 1.5 min after infection. At 11.5 min after infection it was diluted out so that phage growth could proceed. Recovery from the chloramphenicol effect was then measured in terms of burst size after completion of the infectious cycle. As Table 5 shows, burst sizes after chloramphenicol treatment were only 22–27% of normal in *E. coli* CR63, S/6, and *su⁻*. However in *E. coli suA* the burst size after chloramphenicol treatment was 70% of normal.

Table 4 Suppression of T7 amber mutants by the *suA* allele, expressed in burst size

	<i>E. coli</i> BB*	<i>suA</i> †	<i>su⁻</i> ‡
<i>am28</i> (5)	141	178	15‡
<i>am29</i> (3)	137	124	22‡
T7 wild type	142	80	98

Phage infections were carried out at 30° C. After each amber mutant designation the gene in which the mutant is defective is indicated in parentheses.

* Average of three experiments.

† Average of two experiments.

‡ These apparent burst sizes are probably due to unadsorbed phage.

The results presented here indicate that the *E. coli suA* mutant suppresses amber mutations of phages T4 and T7, *ts* and umber mutations of phage T4, as well as the chloramphenicol-induced inhibition of phage T4 growth. The most plausible explanation

for the suppression of such varied blocks to growth is that the *suA* mutation of *E. coli*, when present in phage T4 and T7-infected cells, increases the level of translational ambiguity. In its ability to suppress various different mutations, the *suA* allele seems similar to ram, the ribosomal ambiguity mutation of *E. coli*⁵. It may be that *suA* is also altered in a translational element. The product of the *suA* gene has been partially purified by Wetekam and Ehling⁶ and has been shown to act at translation even when uncoupled from transcription.

Suppression of amber, *ts* and umber mutants defective in stoichiometric functions was usually inefficient compared with suppression of such mutants defective in catalytic functions (Tables 1, 2 and 3). Catalytically acting proteins may allow normal burst sizes even when present in reduced amounts whereas stoichiometrically acting proteins will not allow normal burst sizes unless present at near wild-type levels⁴. The burst sizes obtained after suppression of amber, umber and *ts* mutations in stoichiometric functions implies that suppression by *suA* usually restores a low level of functional gene product. The few stoichiometric mutants which were suppressed efficiently suggests that the level of suppression is context or gene specific.

Table 4 indicates that *suA* is an effective suppressor of phage T7 amber mutations, when burst size is the criterion of suppression. Summers⁷ using the *suA* strain used here (XA7007) observed that on infection by a phage T7 amber mutant defective in gene 1 (RNA polymerase) mRNA formation continued beyond the amber codon, whereas in *E. coli su⁻* it did not. Furthermore, he noted in the *suA* infection that short polypeptide fragments corresponding to a defective gene 1 product were formed. This indicates that at least some of the time translation is terminated at the amber codon in the *suA* host. However his result does not preclude the possibility that occasionally synthesis is continued beyond the amber codon. As discussed above, under conditions where essential enzymes are produced at a low level, phage yield may nevertheless be normal because of the catalytic nature of enzyme function. Thus Summers' results are compatible with our conclusion that *suA* is an effective suppressor of the amber phenotype of phage T7.

The effect of the *suA* allele on suppression of the amber codon as observed in phage T4 and T7-infected cells was not observed in uninfected *E. coli*^{1,2,8}, although only few bacterial mutants were tested. Hsu and Weiss⁹ and Schedl *et al.*¹⁰ have presented evidence that ribosomes extracted from uninfected cells are more active than ribosomes extracted from T4-infected cells *in vitro* when used in translating mRNA from *E. coli* or MS2. This suggests that T4 proteins alter ribosomal template specificity. Smith and Haselkorn¹¹ showed that proteins either induced or modified by phage T4 become associated with the *E. coli* ribosomes after T4 infection. Similar proteins may be produced by phage T7. It may be that one or more of these proteins alter the ribosomes' susceptibility to the effect of the host *suA* allele. If the *suA* allele acts at the level of the ribosome, which is then the initially determined phenotype of the mutant, suppression of polarity^{1,2} might be due to an increased tendency of *suA* cell ribosomes to continue along the mRNA after encountering a nonsense codon so as to be available at the next initiating codon. The continuation of the ribosomes would also protect the mRNA against the degradative action of RNases.

Morse¹² presented evidence that chloramphenicol induces an

Table 5 Suppression by the *suA* allele of the chloramphenicol-induced inhibition of phage T4D growth, expressed in burst size

	CR63	S/6	<i>su⁻</i>	<i>suA</i>
T4D, no chloramphenicol	272	170	116	130
T4D, with chloramphenicol	60	43	31	90
Burst size as % of normal burst	22%	26%	27%	70%

Phage infections were carried out at 30° C. To 0.5 ml of bacteria at about 2×10^8 cells ml⁻¹, 0.5 ml of phage were added to give a multiplicity of infection of 13 phage per bacterium. When the infection had proceeded for 1.5 min, 100 µg of chloramphenicol in 0.1 ml was added. At 11.5 min after infection, 0.1 ml of culture was diluted 4×10^{-4} -fold and growth was allowed to occur for 2 h. At this time phage progeny were determined by plating on *E. coli* CR63 and the burst sizes calculated. All figures are the average of three experiments.

artificial polarity effect on distal mRNA in the *E. coli* tryptophan operon, and that this polarity effect is relieved by the *suA* allele. Table 5 indicates that *suA* also suppresses the effects of chloramphenicol on phage growth. Thus there are somewhat parallel effects of *suA* with respect to chloramphenicol in the two systems. Chloramphenicol is thought to block the transfer of the amino acid from aminoacyl tRNA to the growing polypeptide chain¹³. Since chloramphenicol affects a process taking place on the ribosome it is reasonable to suppose that the *suA* reversal of chloramphenicol inhibition could be due to an effect of *suA* on ribosomal function.

Kuwano *et al.*¹⁴ found a greater release of mRNA fragments *in vitro* by a crude extract of *su*⁻ compared with a similar extract of *suA*. This release was thought to be brought about through the action of an endonuclease which they tentatively designated endonuclease A and which was presumed to be present in *su*⁻ cells, but defective in *suA* cells. Although there is no simple way to reconcile this result with the suppression effects I have observed, it could be supposed that endonuclease A binds to ribosomes and affects the accuracy of translation.

In conclusion, the observations reported here suggest that the *suA* allele produces an altered translational element which enhances mistranslation of the genetic code in phage T4 and T7-infected cells. The wild type form of the *suA* translational element may be necessary both for accurate translation and the dislodgement of the ribosome from the mRNA after reading a nonsense codon.

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- ¹ Beckwith, J., *Biochim. biophys. Acta*, **76**, 162 (1963).
- ² Scaife, J., and Beckwith, J. R., *Cold Spring Harbor Symp. quant. Biol.*, **31**, 403 (1966).
- ³ Wilson, J. H., and Kells, S., *J. molec. Biol.*, **69**, 39 (1972).
- ⁴ Snustad, D. P., *Virology*, **35**, 550 (1968).
- ⁵ Gorini, L., *Nature*, **234**, 261 (1971).
- ⁶ Wetekam, W., and Ehring, R., *Molec. gen. Genet.*, **124**, 345 (1973).
- ⁷ Summers, W. C., *Nature new Biol.*, **230**, 208 (1971).
- ⁸ Morse, D. E., and Primakoff, P., *Nature*, **226**, 28 (1970).
- ⁹ Hsu, W. T., and Weiss, S. B., *Proc. natn. acad. Sci. U.S.A.*, **64**, 345 (1969).
- ¹⁰ Schedl, P. D., Singer, R. E., and Conway, T. W., *Biochem. biophys. Res. Commun.*, **38**, 631 (1970).
- ¹¹ Smith, F. A., and Haselkorn, R., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 91 (1969).
- ¹² Morse, D. E., *J. molec. Biol.*, **55**, 113 (1971).
- ¹³ Monro, R. E., Staehlin, T., Celma, M. L., and Vazquez, D., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 357 (1969).
- ¹⁴ Kuwano, M., Schlessinger, D., and Morse, D. E., *Nature*, **231**, 214 (1971).

Correlations between plasma ACTH concentrations and breathing movements in foetal sheep

BREATHING movements *in utero* are a normal accompaniment of foetal life¹⁻³. Dawes *et al.*³ reported an instance of reduced foetal breathing in a sheep with spontaneously occurring foetal hypoxaemia. Subsequent observations have shown similar reductions associated with foetal hypoglycaemia, infection, and maternally administered hypoxaemia. In addition they are influenced by hypercapnia, temperature

and time of day⁴. In human pregnancy reduced foetal breathing is associated with maternal hypertension and foetal growth retardation⁵. Thus breathing movements have been used as an index of foetal health. The concentration of adrenocorticotrophic hormone (ACTH) in the plasma of foetal sheep increases during hypoxaemia, haemorrhage and catecholamine infusion (refs 6-8 and C. T. J., K. B., J. G. Ratcliffe and J. S. R., unpublished observations) and may therefore reflect foetal condition. At present blood gas values, pH and heart rate are used as indices of foetal health *in utero* in animals and man. But we have observed a wide variation in plasma ACTH concentration and foetal breathing movements in foetal sheep *in utero* in circumstances in which blood gas values, arterial pH and heart rate were in the normal range. The present report describes a close correlation between the amount of foetal breathing and the foetal plasma ACTH concentration.

In 18 sheep of mixed breeds 120-145 d pregnant with catheters previously implanted in the foetal trachea and carotid artery, carotid blood samples were taken from

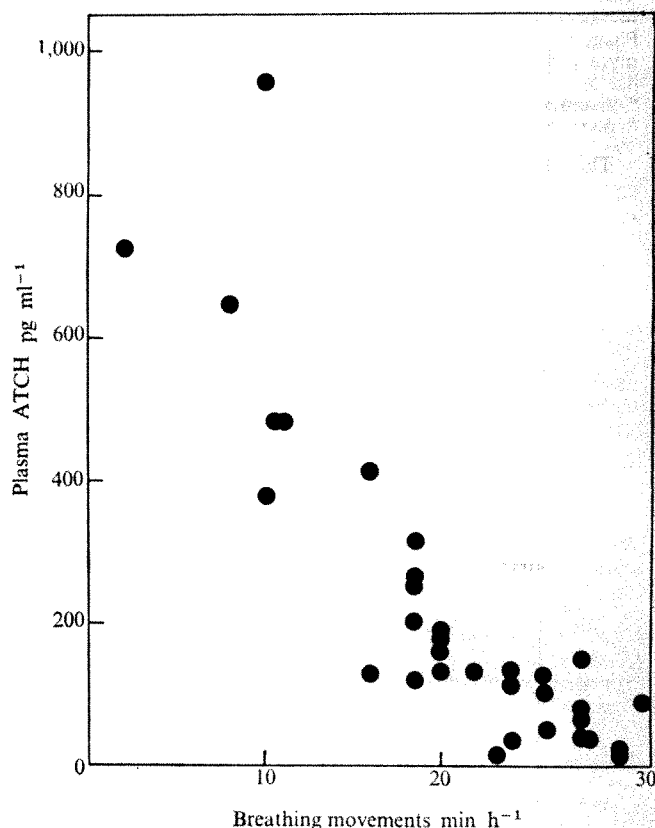


Fig. 1 Foetal carotid plasma ACTH concentration and incidence of foetal breathing movements. Each point, represents the foetal plasma ACTH concentration either at the beginning or the end of the hour that the incidence of foetal breathing was measured. Observations at both times were made on most animals.

mother and foetus and ACTH and corticosteroid concentrations were measured as previously described^{7,8}. Sampling took place at least 1 week after surgery, between 9000 a.m. and 1030 a.m. The mean foetal carotid arterial pH and P_{O_2} were respectively 7.37 ± 0.07 (s.e.m.) and 22 ± 0.5 mm Hg and the mean foetal heart rate was 157 ± 14 beats min^{-1} . There was a reciprocal correlation between the incidence of foetal breathing movements over an hour and the foetal plasma ACTH concentration at the beginning and end of that period. There was no correlation with foetal blood gas values, pH, plasma corticosteroid concentration or heart rate. The relationship observed between foetal breathing and plasma ACTH concentration was not directly related to gestational age.

In the foetal sheep dissociation of the electrocorticogram

into high and low voltage activity is first seen around 120 d gestation. Foetal breathing movements are only seen in association with the low voltage activity characteristic of rapid eye movement sleep³. Bernhard and coworkers⁹ have suggested in the foetal sheep that the development of the dissociation and hence rapid eye movement sleep probably originates in the corpus callosum and thalamus. It is possible that the activity in these structures during rapid eye movement sleep may have some connection with that which influences the hypothalamic control of ACTH secretion.

The inverse correlation between foetal breathing movements and ACTH concentrations is not surprising since both are influenced, in opposite directions, by hypoglycaemia, hypoxia, haemorrhage and infection (refs 4, 5, 8, 10–12; K. B., G. S. Dawes and J. S. R., unpublished observations). But none of these factors was directly responsible for the variation in breathing movements or plasma ACTH concentration in the present series of observations. The wide variation in ACTH concentration observed in the foetus and its sensitivity to various types of stimulation such as hypoxaemia or catecholamine infusion^{6,7,10} is surprising in view of the relative insensitivity to ACTH of the foetal adrenal output of glucocorticoids^{6,13}. This explains the absence of a correlation between breathing movements and plasma corticosteroid concentration in foetal sheep.

These observations indicate that the measurement of foetal blood gas values, arterial pH and heart rate alone do not fully reflect foetal condition. Other factors such as the incidence of foetal breathing movements and plasma hormone concentrations are required for the assessment of the physiological state of the foetus *in utero*.

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- ¹ Dawes, G. S., Fox, H. E., Leduc, B. M., Liggins, G. C., and Richards, R. T., *J. Physiol. Lond.*, **210**, 47 (1970).
- ² Merlet, C., Hoerter, J., Derielleneuve, C., and Tchobroutsky, C., *C. r. hebd. Séanc. Acad. Sci., Paris*, **270**, 2462 (1970).
- ³ Dawes, G. S., Fox, H. E., Leduc, B. M., Liggins, G. C., and Richards, R. T., *J. Physiol. Lond.*, **220**, 119 (1972).
- ⁴ Dawes, G. S., in *Foetal and Neonatal Physiology*, 49 (Cambridge University Press, Cambridge, 1973).
- ⁵ Boddy, K., and Mantell, C., *J. Physiol. Lond.*, **231**, 105 (1973).
- ⁶ Boddy, K., Jones, C. T., Mantell, C., Ratcliffe, J. G., and Robinson, J. S., *Endocrinology*, **94**, 588 (1974).
- ⁷ Jones, C. T., and Robinson, R. O., *J. Physiol. Lond.* (in the press).
- ⁸ Ganong, W. F., in *Advances in Neuroendocrinology* (edit. by Nalbandov, A. V.), 92 (University of Illinois Press, Urbana, 1963).
- ⁹ Bernhard, C. G., and Meyerson, B. A., in *Foetal and Neonatal Physiology*, 1 (Cambridge University Press, Cambridge, 1973).
- ¹⁰ Alexander, D. P., Britton, H. G., Forsling, M. L., Nixon, D. A., and Ratcliffe, J. G., in *The Endocrinology of Pregnancy and Parturition*, 112 (Alpha Omega Publishing, Cardiff, 1973).
- ¹¹ Takabe, K., Setaishi, C., Hirama, M., Yamamoto, M., and Horiuchi, Y., *J. clin. Endocr.*, **26**, 437 (1966).
- ¹² Nakai, Y., Imura, H., Yoshimi, T., and Matsukura, S., *Acta Endocr.*, **74**, 263 (1973).
- ¹³ Madill, D., and Bassett, J. M., *J. Endocr.*, **58**, 75 (1973).

Redistribution of endogenous gibberellins in geotropically stimulated roots

THE difference in growth rate of the two sides of geotropically reacting plant organs is generally explained by the well documented unequal auxin concentrations in the two halves. The auxin ratio in horizontally placed stems, coleoptiles, and roots usually attains a value of about 2:1 in favour of the lower half.

But, Phillips¹ and Railton and Phillips² have shown that the ratio of gibberellin activity in diffusates from opposite halves of geotropically reacting green *Helianthus* hypocotyls and etiolated *Zea* coleoptiles reaches values of about 9:1 and 4:1, respectively, in favour of the lower (convex) half. These findings raise the question of the possible role of gibberellins in the geotropic reactions of these organs.

In horizontally positioned main roots, auxin accumulating in the lower (concave) half, presumably in supraoptimal concentrations, has been postulated to retard the elongation of that half, thus bringing forth the positive geotropic curvature. The correctness of this view has been questioned for some time^{3,4}, and unspecified inhibitors have been suggested as mediators of the differential rates of elongation^{5–8}. In this situation, and in view of the reports on asymmetrical gibberellin distribution in geotropically stimulated shoots, it would be interesting to determine the distribution of gibberellins in horizontally positioned roots. For this purpose the following experiments were carried out.

Seeds of *Vicia faba*, cultivar Erfordia, were soaked for 4 h in tap water and sown in moist vermiculite in the dark at 25° C. All subsequent operations took place under a green safelight. After 4 d, 500 (or 250) seedlings having roots approximately 15 mm long were selected for root uniformity. Of these, 250 (or 125) were left upright and 250 (or 125) were kept horizontal for 30 min. Using a sharp blade, each root was then split lengthwise into two halves. Four batches of root halves were thus obtained: upper and lower halves of horizontally oriented roots, and left and right halves of upright roots. Each batch was extracted at 2° to 3° C with three changes of 80% methanol over 36 h. The acidic fractions soluble in ethyl acetate were passed through a column of polyvinyl pyrrolidone (PVP), eluted with phosphate buffer (pH = 8.0), and transferred in diethyl ether to small volumes of ethanol. These were loaded on silica gel thin layer chromatography plates, which had been prerun in a 98:2 (v/v) mixture of ethanol and acetic acid⁹ and activated at 105° C for 1 h. The plates were developed with isopropanol, 25% specific gravity 0.91 ammonia and water, 10:1:1 (v/v). The chromatograms were divided into 10 transverse strips each of which was scraped off, eluted with 2 ml distilled water, and bioassayed for gibberellin activity in the *Lactuca* hypocotyl assay¹⁰.

As a check on the accuracy of the splitting of the roots, the dry weights of matching, extracted halves (250 in each group) were compared. They were: upright roots, left halves: 0.54 g, right halves: 0.56 g; horizontal roots, upper halves: 0.42 g; lower halves: 0.43 g.

Our results confirm the few available reports on the occurrence of gibberellins or gibberellin activity in roots^{11–13}. As shown in Fig. 1, more gibberellin activity was found on chromatograms of extracts from the upper than from the lower halves of horizontally oriented roots, whereas the amounts of gibberellin activity detected in left and right halves of upright roots were nearly identical.

By comparison with gibberellic acid (GA₃) standards the gibberellin activities in the upper and lower halves of horizontal roots were found equivalent to 31.0 and 17.5 ng GA₃ per g dry weight of tissue, respectively, in experiment b (Fig. 1). In experiment c, representing a different set of roots, the figures were 35.0 and 9.5 ng, but the growth

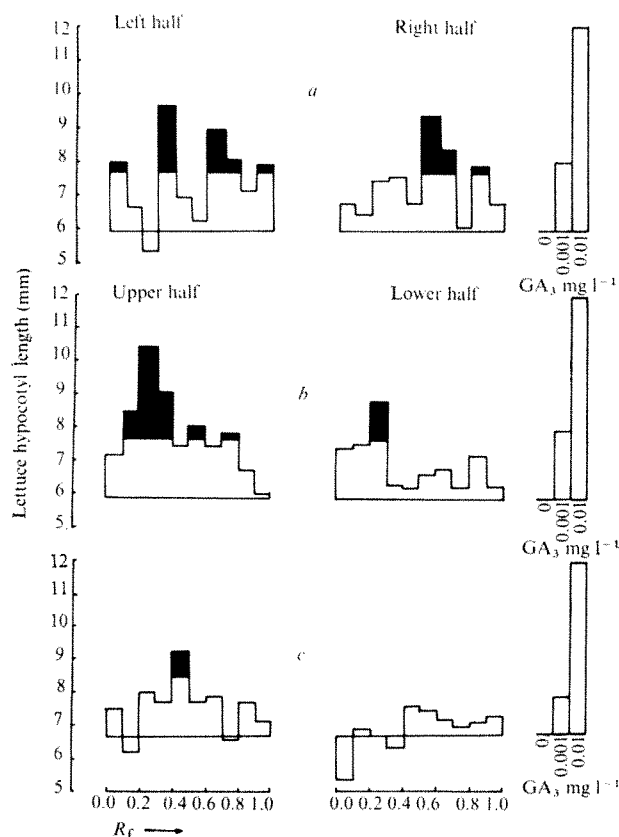


Fig. 1 *Lactuca* hypocotyl bioassays of acid, ethyl acetate-soluble fractions of chromatographed extracts of halved *a*, upright and *b* and *c*, horizontal *Vicia faba* roots. Extract equivalent to 0.2 g dry weight of roots in *a* and *b*, and to 0.1 g dry weight in *c*. A difference of 1.89 mm between any two readings is needed for statistical significance at the 5% level (base of the black parts). Gibberellic acid (GA_3) at 0.001 mg l^{-1} equals $2.6 \times 10^{-9} \text{ M}$.

promotions by the extract of the lower halves were too small to reach statistical significance. (The GA_3 ratio in favour of the upper half was 1.8:1 in experiment *b*; and in experiment *c* the calculated value was 3.7:1). In upright roots (*a*) the GA_3 activities in opposite halves were equivalent to 26 and 24 ng GA_3 per g dry weight.

In further experiments, to be reported in detail elsewhere, zones of the chromatograms running at the same R_f s as GA_3 were eluted with ethanol, methylated¹⁴ and subjected to gas liquid chromatography. The methyl ester of GA_3 was identified at the position (retention time) of methylated, authentic GA_3 . In terms of ng per g dry weight of root tissue, the following quantities of GA_3 were recorded: upper halves: 130, lower halves: 63 (ratio upper: lower = 2.06); right halves: 70, left halves: 74. Qualitatively the distributions were thus similar to those found by the bioassays.

The direction of the concentration gradient of gibberellins in the horizontally positioned *Vicia faba* roots is opposite to that found in shoot tips and coleoptiles^{1,2}. The direction of the gradient is also opposite to that of auxin. Theoretically, gibberellin might thus assist in the positive geotropic reaction by stimulating the elongation of the upper half, provided that gibberellin does stimulate root elongation.

Pilet¹⁵, working with intact seedlings of *Lens culinaris*, found no effect on root elongation by GA_3 at concentrations up to $1 \mu\text{M}$, whereas 10 and $100 \mu\text{M}$ GA_3 retarded growth. There are, however, a number of reports indicating a stimulation by gibberellins of root elongation in some plants: *Pseudotsuga*¹⁶, *Lycopersicon* (excised)^{17,18}, *Triticum*¹⁹, and *Carthamus*¹⁹.

In our own experiments (to be reported in full elsewhere) GA_3 was added to germinating *Vicia faba* seeds when their radicles had just broken the seed coat. The root lengths were measured after 24, 36, and 48 h. GA_3 was found to be stimulatory at concentrations from 0.001 to 10 mg l^{-1} (except at 1.0 mg l^{-1} at 24 h). At all times 100 mg l^{-1} was inhibitory. Seedlings of *Lepidium sativum* were transferred to GA_3 solutions on filter paper when their roots were about 20 mm long. The root lengths were measured after 1, 2, 3, 4 and 5 h. In the first hour, 10 and 100 mg l^{-1} were inhibitory, whereas lower concentrations had no significant effect. At 2 h there was a stimulatory trend at concentrations from 0.1 to 10 mg l^{-1} , whereas 100 mg l^{-1} was inhibitory. At 3, 4 and 5 h, all concentrations from 0.001 to 100 mg l^{-1} were stimulatory, the lowest concentration being the most effective.

Gibberellin may thus have a function in root geotropism, but since the geotropic response in roots involves a retardation of the elongation of the lower part, the action of an inhibitor of root elongation in that part cannot be dismissed. Although such a role has traditionally been assigned to auxin, several authors⁵⁻⁸ leave open the identity of the actual functioning inhibitor. The presence in roots of an inhibitor which is not auxin, but becomes distributed like auxin in horizontal roots, has been demonstrated in this laboratory and its characteristics are being studied.

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- Phillips, I. D. J., *Planta*, **105**, 234-244 (1972).
- Railton, I. D., and Phillips, I. D. J., *Planta*, **109**, 121-126 (1973).
- Wilkins, M. B., in *Gravity and the Organism* (edit. by Gordon, S. A., and Cohen, M. J.) 107-121 (University of Chicago Press, Chicago and London, 1971).
- Audus, L. J., in *Gravity and the Organism* (edit. by Gordon, S. A., and Cohen, M. J.), 137-149 (University of Chicago Press, Chicago and London, 1971).
- Gibbons, G. S. B., and Wilkins, M. B., *Nature*, **226**, 558-559 (1970).
- Pilet, P.-E., *Nature new Biol.*, **233**, 115-116 (1971).
- Pilet, P.-E., *Planta*, **111**, 275-278 (1973).
- Shaw, S., and Wilkins, M. B., *Planta*, **109**, 11-26 (1973).
- Lenton, J. R., Perry, V. M., and Saunders, P. F., *Planta* (Berl.), **96**, 271-280 (1971).
- Frankland, B., and Wareing, P. F., *Nature*, **185**, 255-256 (1960).
- Butcher, D. N., *J. exp. Bot.*, **14**, 272-280 (1963).
- Carr, D. J., and Reid, D. M., in *Biochemistry and Physiology of Plant Growth Substances* (edit. by Wightman, F., and Setterfield, G.) 1169-1185 (Runge Press, Ottawa, 1968).
- Jones, R. L., and Phillips, I. D. J., *Pl. Physiol.*, **41**, 1381-1386 (1966).
- Cavell, B. D., MacMillan, J., Pryce, R. J., and Sheppard, A. C., *Phytochemistry*, **6**, 867-874 (1967).
- Pilet, P.-E., *C. r. heb. Séanc. Acad. Sci. Paris*, **272**, 418-421 (1971).
- Richardson, S. D., *Nature*, **181**, 429 (1958).
- Butcher, D. N., and Street, H. E., *J. exp. Bot.*, **11**, 206-216 (1960).
- El Hinnawy, E., *Z. Pflanzenphysiologie*, **69**, 1-12 (1973).
- Abdalla, F. H., and El-Antably, H. M. M., *Res. Bull. Fac. Agric. Ain Shams Univ.*, **697**, 1-20 (Cairo, 1970).

Batesian mimicry without distastefulness?

MANY distasteful species are conspicuously coloured¹ and this is believed to aid their survival through the formation

of 'avoidance images'² by predators. It has been suggested that 'an efficient escape mechanism' either provided by a jumping response^{3,4}, or a fast escape flight⁵⁻⁷, could be as powerful as distastefulness in influencing a predator's strategy of prey selection, or could in some instances help to increase the relative frequency of an agile mimic of a distasteful but sluggish model⁴. Lindroth⁸ has made observations on several genera of flea-beetles (Alticinae, Chrysomelidae) and has found great external similarities between them and ground beetles of the genus *Lebia* (Carabidae), the larva of which is an ectoparasite of the flea-beetle's pupa. He suggests that the flea-beetle, which can escape suddenly from its exposed position on the food-plant by jumping, acts as a model and *Lebia* as a relatively sluggish mimic. As a parasite, *Lebia* is found in fewer numbers than the host, a situation also expected of some mimics in relation to their models. Neither was found to be distasteful to bird predators⁹. Thompson¹ makes use of Lindroth's⁸ hypothesis in explaining the occurrence of an apparently aposematic form in females of the non-mimetic common meadow spittlebug, *Philaenus spumarius*, which is acceptable to predators but for the possession of an efficient close-quarter escape mechanism. Thompson proposes that this mechanism accounts for aposematic morph *P. spumarius marginella* occurring at a frequency in the population above that maintainable through apostatic selection. In both cases it is proposed that a learning predator comes to associate a particular colour pattern with wasted effort and spurns all forms with a similar appearance.

This report describes a preliminary experiment to simu-

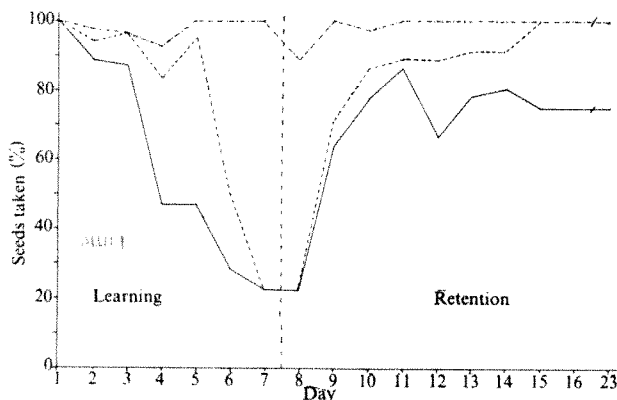


Fig. 1 Percentage of aposematic model/mimic, cryptic model/mimic and non-mimetic seed taken by the experimental birds during the learning period (model) and retention period (mimics). —, Green seed; ---, blue seed; ···, red seed.

late this efficient escape and assess its effectiveness on a predator's selection of prey. *Bathilda ruficauda*, the star finch, was used as the predator and the prey was white millet seed dyed red, blue or green with food colouring (1% acetic acid base). The escape mechanism was simulated by means of a manually-operated hinged feeding platform which could be lowered rapidly to remove the seed from the birds' sight. The platform base was coloured with dots of blue and green dye, the same size as the seeds, and on this the red seeds appeared 'aposematic' and the blue and green seeds 'cryptic' to the human eye and presumably to the birds⁹. In front of the platform was a feeding perch and a light was turned on above to signal the presence of seed.

On each testing day the birds were deprived of food for 1½ to 2 h, transferred to the test cage individually (to prevent observation learning¹⁰) and presented with nine seeds, three of each colour, individually in random order.

The time taken to come to the feeding perch and make a peck at the seed was noted, an upper limit of 2 min being imposed for each seed. After this time the platform was dropped to remove the seed if it had not already been eaten. This operation was repeated three times a day for each bird.

Two groups of four birds were used, one control and one experimental group. The controls were allowed to feed on all seed colours for 3 d. In the experimental group the green represented the 'non-mimetic' on which feeding

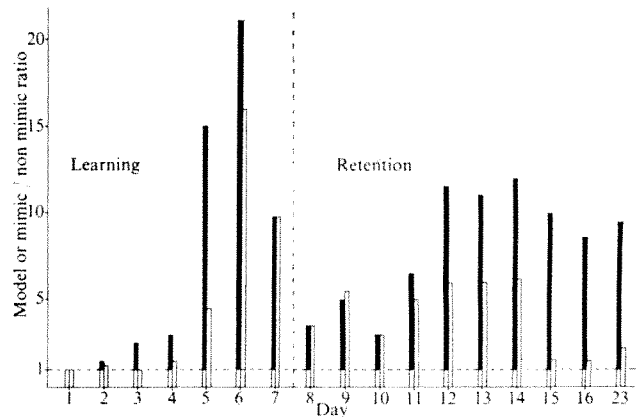


Fig. 2 The model-mimic/non-mimetic ratio (M/N Ratio) of mean feeding latency. A measure of the comparative advantage of the model-mimic complex over the non-mimetics. Feeding latency ratio; ■, red/green seed; □, blue/green seed.

was allowed throughout the experiment (days 1-16+23). The blue and red seeds acted as the 'models' during the first half of the experiment, the learning period (days 1-7), by being dropped out of sight as the bird attempted to feed. During the second half of the experiment, the retention period (days 8-16+23) the platform was not dropped for any seed until the end of the 2 min feeding time. The blue and red seeds were then being used as 'mimics'.

The results for the control group show that there was no colour preference in terms of mean feeding latency (Kruskal-Wallis one-way analysis of variance by ranks¹¹. $H=0.54$, $P=0.104$). The experimental group on day 1 also showed no colour preference (Fig. 1). During the learning period, discrimination took place between the non-mimetic (green) seed and the model (blue or red) seeds in terms of latency to feed. The aposematic (red seed) showed the greatest initial advantage over the non-mimetic (green seed), by being avoided before the cryptic model, and more often overall ($\chi^2=38.12$, d.f.=2, $P<0.001$), though the blue model also showed an advantage over the non-mimetic (green seed) ($\chi^2=26.4$, d.f.=2, $P<0.001$) (Fig. 1).

The use of daily mimetic advantage measurements (model or mimic/non-mimetic, feeding latency ratio) shows more clearly the relative advantage of the two model/mimics (Fig. 2); the aposematic model reaching the higher level on day 6. The figure also shows that the relative advantage of red over blue at first increases (up to day 5), then decreases to equality by day 7, the last day of the learning period; from day 11, the fourth day of the retention period, the aposematic mimic shows an advantage over the cryptic mimic. The existence of a mimetic advantage was also apparent when individuals were analysed separately (Table 1).

It was also noted that as the latency before taking a model/mimic seed increased so did the amount of activity unrelated to feeding, including flying around, moving along the perch and preening. A similar type of behaviour was

also exhibited by birds in an experiment on simulated distasteful Batesian mimicry¹² and is probably indicative of a conflict of behavioural tendencies in the animal. This slowing down of attack would give a real mimic more time to escape. Behaviour of this type was never shown towards the non-mimetic (green) seed by the experimental group, nor to any colour of seed by the control group.

The formation of an avoidance image² by the birds was due to the simulated escape mechanism and not to some feature of the apparatus, as the control group fed readily on all seeds from it and the experimental group went for the green seed without hesitation throughout the experiment. Avoidance image formation was therefore through 'frustration learning' which can be as effective as pain learning¹³, the mechanism operative in discriminating distasteful from palatable prey. It would seem then, that 'an efficient escape mechanism' as observed by Lindroth³ could be as powerful as distastefulness in qualifying its possessor as a model for Batesian mimicry. Further observations in the field may show the phenomenon to be less rare than is now supposed.

Table 1 Model-mimic/non-mimetic ratio for each bird during the learning period, retention period and both periods combined

Bird	Learning		Retention		Both periods combined (model/mimic complex)	
	B/G	R/G	B/G	R/G	B/G	R/G
B	2.7	4.1	4.0	7.8	3.5	6.0
M	6.8	10.7	4.0	4.5	5.5	7.8
WR	3.6	5.8	4.8	4.9	4.2	5.4
WL	2.5	3.5	2.1	2.8	2.4	3.3
All	3.1	4.7	3.8	5.7	3.3	5.0

B, Blue seed, (cryptic model/mimic); G, green seed, (non-mimetic); R, red seed, (aposematic model/mimic).

These findings also have implications for the phenomenon of balanced polymorphism in a prey population with aposematic morphs. Birds feed in a frequency dependent manner, the more common the prey the stronger the searching image¹⁴ formed. The selection of apostates (non-mimetic polymorphs) enables a prey species to occur in an area at a greater density than if it were monomorphic. Some of these morphs may appear aposematic and will create a searching image more readily than a cryptic morph. These aposematic forms, if in a species acceptable to a predator, will be found at a very low frequency in the population through apostatic selection mechanisms. *P. spumarius marginella* occurs in an area studied by Thompson⁴ at a level above that of other aposematic morphs, but below that of the cryptic morphs. As all four morphs have the escape mechanism the only difference is that *marginella* is strikingly coloured. In the experiment reported here, learning to avoid the aposematic model was more rapid and this learning was retained longer than for the cryptic model, though they had equally efficient escape mechanisms. In a natural situation the escape mechanisms would presumably be less than 100% effective but, as shown by the experiment, a predator would remember an encounter with the aposematic morph more readily than one with a cryptic morph³. If in a dimorphic species the aposematic form is, say, four times as easy to detect as the cryptic form then the strength of an avoidance or searching image formed would equal that formed by four of the cryptic form. In this simplified hypothetical situation with two morphs, a balanced polymorphic level would be reached when the cryptic morph was four times as common as the aposematic. If the aposematic morph becomes more frequent in the population 'the selective disadvantages of being conspicuous'¹⁴ and predation will increase until the aposematic morph is reduced to the balanced polymorphic level again.

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- ¹ Cott, H. B., *Adaptive Coloration in Animals*, 256-258 (Methuen, London, 1940).
- ² Clarke, B., *Systematics Association Publications No. 4 Taxonomy & Geography*, 47-70 (1962).
- ³ Lindroth, C. H., *Ent. Scand.*, **2**, 41-48 (1972).
- ⁴ Thompson, V., *Nature*, **242**, 126-128 (1973).
- ⁵ Jones, F. M., *Trans. R. ent. Soc. Lond.*, **79**, 345-385 (1932).
- ⁶ Sommeron, V. G. L., and Jackson, T. H. E., *J. Lepit. Soc.*, **13**, 121-150 (1960).
- ⁷ Hespenheide, H. A., *J. Ent. Ser. A.*, **43**, 49-55 (1973).
- ⁸ Rothschild, The Hon. M., in *Ecological Genetics & Evolution* (Edit. by Creed, R.) 202-223 (Blackwell, Oxford, 1971).
- ⁹ Pumphrey, R. J., *Ibis*, **90**, 171-190 (1948).
- ¹⁰ Klopfer, P. M., in *Behavioural Aspects of Ecology*, 10-34 (Prentice-Hall, New Jersey, 1962).
- ¹¹ Siegel, S., in *Non-parametric Statistics for the Biological Sciences*, 184-193 (McGraw-Hill, 1956).
- ¹² Reiskind, J., *Anim. Behav.*, **13**, 466-469 (1965).
- ¹³ Sutherland, N. S., and Mackintosh, N. J., in *Mechanisms of Animal Discrimination Learning*, 338-403 (Academic Press, 1971).
- ¹⁴ Tinbergen, L., *Archs neerl. Zool.*, **13**, 265-336 (1960).

Temperature effects on hearing in two species of *Amphisbaenia*

SEVERAL recent studies have documented diverse auditory responses in lizards and turtles as a function of body temperature. Previous work was with iguanid lizards, which are diurnal, and can regulate body temperature by basking; and with the aquatic turtle *Pseudemys*. We have worked with amphisbaenians, which are highly modified burrowing reptiles.

Werner (refs 1 and 2, and personal communication) showed that the ears of six species of iguanid lizards were most sensitive in terms of the electrical potentials of the cochlea at temperatures corresponding either to the 'eccritic' or to the similar 'preferred' temperature. At this optimum temperature the species-specific auditory sensitivity curve is regular and has a characteristic zone of maximum sensitivity². Temperature shifts produce fairly predictable species-specific variations. A temperature drop impairs the sensitivity, especially for the high tones, and makes the function highly irregular, with many sharp variations in frequency. A moderate rise improves the response to high tones while reducing it for low tones. A further elevation is likely to have little effect on high-tone sensitivity, but that to low tones is seriously impaired. In the region of best sensitivity the change often approaches 40 dB. Thus a torpid lizard is likely to have poor perception of sound, even though this may signal a predator or other sort of danger.

A lesser temperature effect was found in the turtle, *Pseudemys scripta elegans*³. For a head temperature range of 14° to 33° C the response to a constant tone of 330 or 630 Hz varied up to 4.3 dB, showing two peaks of sensitivity, respectively at 19° and 29.5° C. According to Patterson *et al.*, temperature has two effects: it acts on the energy source for the generation of potentials and involves either a conductive mechanism or blood pressure.

An earlier study⁴ reported limited observations on a single specimen of the African amphisbaenid *Chirindia langi*. Cochlear potentials differed up to 18 dB in the region around 3,000 Hz between 24.4° and 29.4° C.

Observations on two other species of amphisbaenians now demonstrate strikingly different behaviour. The animals were

four *Blanus cinereus cinereus* (Vandelli) from the vicinity of Jerez de la Frontera, Spain, and three *Diplometopon zarudnyi* Nikolski: one from the vicinity of Dammam, Saudi Arabia, and two from the vicinity of Kuwait. *Blanus cinereus* is probably the most primitive living amphisbaenian, and *Diplometopon zarudnyi* is a highly modified trogonophid.

Specimens were anaesthetised with ethyl carbamate (0.012 ml per g body weight, 20% in physiological saline), often supplemented. The cochlear potentials were recorded with a needle electrode in contact with the perilymph of the saccule while stimulating with various tones over the range of 100 to 10,000 Hz. Temperature was monitored by a thermal probe deep in the cloaca and controlled within 1° C by means of an electric blanket draped over the animal. For details see ref. 4.

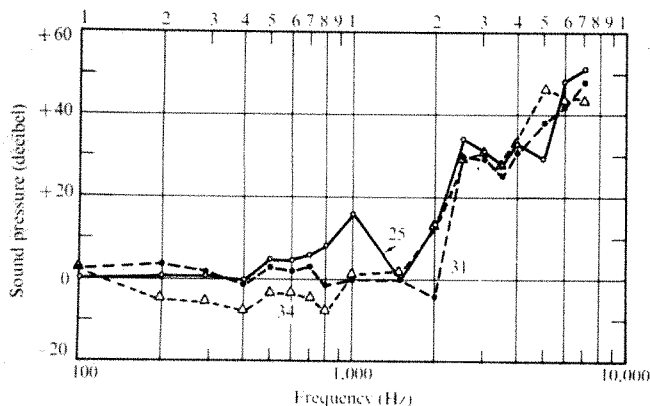


Fig. 1 Cochlear potentials of the left ear of *Blanus cinereus* (F868) taken at 25°, 31°, and 34° C in that sequence.

Figure 1 shows sensitivity curves (sound pressure in decibels relative to a pressure of 10^{-5} N m⁻², required at various frequencies to produce a standard potential of 0.1 μ V (that is, the lower the curve, the greater the sensitivity) for a specimen of *Blanus cinereus* at body temperatures of 25°, 31°, and 34° C. The curves differ, especially for the medium low and middle tones, but lack a clear trend as a function of temperature. Figure 2 shows similar observations on specimens of *Diplometopon zarudnyi* at four temperatures between 25° C and 40° C. All curves have the same general form.

A second specimen of *Diplometopon zarudnyi* tested at 37° C and then at 28° C yielded closely similar curves that cross one another several times. There was no obvious temperature effect, despite differences of 5–8 dB at two or three frequencies. The temperature was next set at 43° C, but the animal became hyperactive and a further test run had to be interrupted. After a period of time at a temperature of 35° C, a final series of measurements indicated reduced sensitivity. Possibly the high temperature of 43° C impaired the cochlear response.

These results reveal large differences among reptiles in the effects of temperature upon the response of the ear to sound. *Blanus* and *Diplometopon* maintain a remarkable constancy over a temperature range of as much as 15° C in sharp contrast to the lizards tested by Werner. *Pseudemys* and *Chirindia* evidently are intermediate.

It is as yet impossible to identify the process or processes involved in the observed temperature effects. Werner's observations seem definitely to exclude the sound conductive pathways, and point to the cochlear processes, and probably the hair cells themselves. The temperature effect may depend upon the magnitude of the polarisation potential set up at the surface membrane of the hair cell, and reflects the metabolic processes and membrane permeabilities by which this polarisation is established. Such possibilities should be tested and interspecies differences more broadly assayed. Do the responses reflect such environmental parameters as the range across

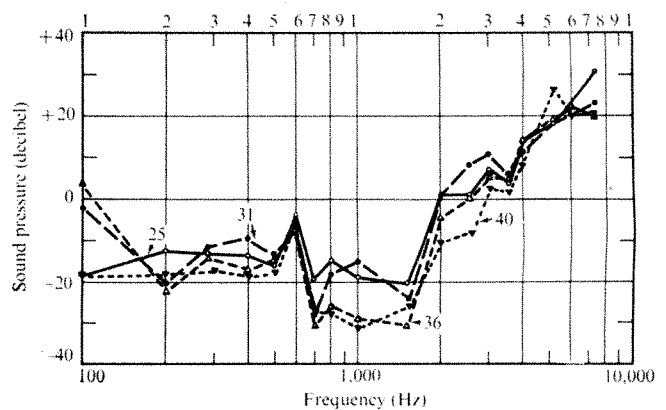


Fig. 2 Cochlear potentials of the left ear of *Diplometopon zarudnyi* (F869) taken at temperatures of 25°, 31°, 36°, and 40° C as indicated.

which a species normally thermoregulates? Can these amphisbaenians control the temperature of the otic capsule independent of general body temperature?

The sensitivity curves represented above show considerable variability tentatively attributed to the activity of middle ear muscles connecting to the terminal disk of the columella and probably affecting sound transmission to the inner ear⁵. Their activity may explain instability in the meter readings during presentation of a steady tone.

Efforts in one experiment to reduce this instability by the use of curare (*d*-tubocurarine) yielded uncertain results, perhaps because we were overcautious in the dosage used. Further experiments are needed on a more readily available amphisbaenian.

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¹ Werner, Y. L., *J. Herp.*, **2**, 178 (1968).

² Werner, Y. L., *J. Herp.*, **6**, 147 (1972).

³ Patterson, W. C., *J. aud. Res.*, **6**, 453 (1966).

⁴ Gans, C., and Wever, E. G., *J. exp. Zool.*, **179**, 17 (1972).

⁵ Wever, E. G., and Gans, C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2714 (1972).

Green cones of the piñon pine stimulate late summer breeding in the piñon jay

REPRODUCTION in birds is influenced by a variety of environmental factors, usually classified as ultimate or proximate¹. Ultimate factors determine efficiency of breeding, for example food supply for the young² both before and after they become independent of their parents. Proximate factors, on the other hand, are the timers or predictors which initiate breeding at the appropriate time. Photoperiod is the most reliable and

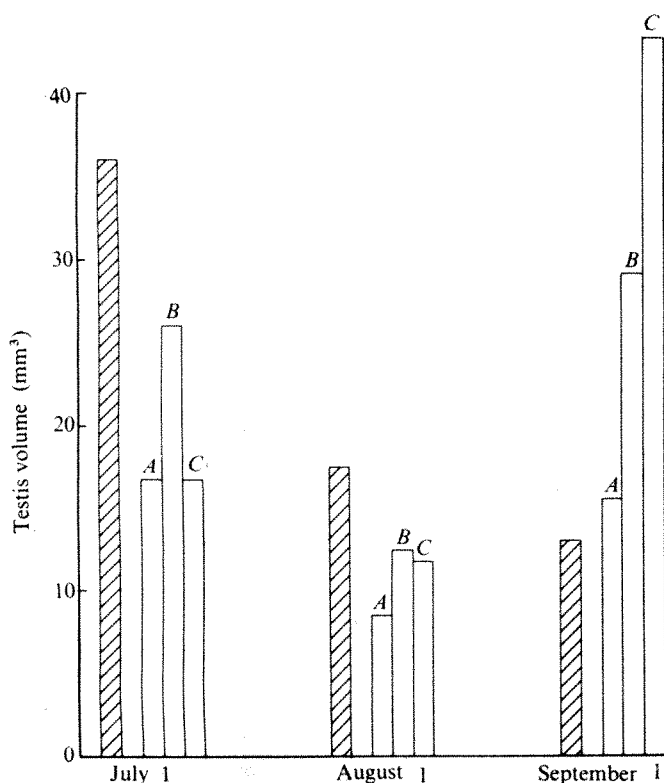


Fig. 1 Testicular response of three adult piñon jays provided freely with green cones of the piñon pine. Testes were regressing in ten experimental birds and ten controls through June and July. On August 1 the dried piñon seeds provided to the experimental jays were replaced with green unripened cones of the piñon pine. In three individuals shown here (A, B, C), testis regression was then reversed and the gonads enlarged. The unhatched bar represents maximum testis volume of the ten control piñon jays.

common proximate factor in those temperate zone species which breed exclusively in spring when daylength is increasing². Other proximate factors, such as rainfall, provide predictive information in many regions of the world, in that increased food availability follows the rains³.

Although most birds in temperate zones breed in spring and early summer, a few breed at other times of the year as well, in autumn or even winter⁴. In most such cases food abundance has been thought to be a primary stimulator of reproductive activity; however, with a single exception⁵, experimental support is lacking³. I report here field and experimental evidence that late summer–autumnal reproductive activity in piñon jays (*Gymnorhinus cyanocephalus*) of the south-western United States is triggered by the presence of large quantities of green cones of the piñon pine (*Pinus edulis*), which mature in late summer. Piñon pines in a given area produce cones and seeds largely in synchrony at irregular and infrequent intervals.

Piñon jays, like most other temperate zone bird species, are responsive to increasing photoperiod in spring (my unpublished work), and typically breed from late February or

early March to June⁶⁻⁸. In south-western New Mexico, however, near the south-eastern edge of this bird's range, climatic factors, and thus food availability, are unpredictable in spring and early summer and, as a result, breeding pattern and timing of the annual moult vary from year to year in a single population^{6,9}. It is here that late summer breeding sometimes occurs^{6,10}, and it seems to be related either to abundance of green cones of the piñon pine or to seeds contained within the cones (Table 1).

To test the roles of mature piñon seeds and the resinous green cones which contain maturing seeds, I spatially isolated two groups of ten adult male piñon jays in outdoor aviaries for 1 yr, beginning on September 1, 1971. One group received a nutritionally adequate diet, but with no piñon seeds or cones, whereas the other received the same diet, plus mature piñon seeds. The birds were laparotomised and their testes measured at monthly intervals.

No gonadal growth occurred in either group until the vernal increase in photoperiod, and through most of the year gonadal development was similar in both groups. By summer 1972 testes of all birds were regressing. On August 1, 1972, the ten jays receiving piñon seeds were instead given free access to intact, fresh, green piñon cones for a month. Regression of the testes was reversed in three birds (Fig. 1); that is, whereas dried piñon seeds did not prevent or delay regression of the testes, availability of green cones reversed gonadal regression and stimulated testis growth in some of the experimental birds. Gonads of the ten controls remained small (4.1–12.9 mm³, mean = 7.8 mm³), as did those of the seven experimental birds (1.4–12.5 mm³, mean = 7.8 mm³).

It is not surprising that most jays did not respond. The captive jays had neither mates nor suitable nesting sites, both of which are important in the attainment of reproductive competence in many birds¹¹. Further, in 1969, when autumnal breeding occurred, only about a third of the flock of about 300 jays actually bred⁶. As indirectly indicated in Table 1, natural selection favours no autumnal breeding in most years. Thus one could expect polymorphism in gonadal response to environmental stimuli in this population of piñon jays.

These data suggest that green piñon cones *per se* provide long term predictive information to the piñon jays; that is, the cones serve as proximate factors. In late summer, as a result of seasonal rains, other foods such as insects, are plentiful in every year, and it seems that piñon jays could easily provide for nestlings at this time. (It should be noted that piñon seeds form a very minor portion of the diet of nestlings^{6,12}.) Immature piñon jays become independent at about 8 weeks of age⁷. In birds hatched during autumn this occurs shortly before the onset of cold, temperate weather. Great quantities of cones in August provide the proximate cue that adequate food, in the form of piñon seeds, will be available to the callow young during the stressful winter season.

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Table 1 Breeding phenology and testis condition of piñon jays in south-western New Mexico, in relation to production of seeds of the piñon pine

Year	Breeding phenology	Piñon cone production	Dates taken	Testis volumes (mm ³)*		
				N	Mean	Range
1969	No spring breeding, breeding in autumn	Abundant	19–20 Aug.	4	301.0	59.6–413.4
1970	Breeding in spring, none in autumn	None	25 Aug.	3	13.9	8.8–18.4
1971	No breeding, spring or autumn	None	5–6 Sept.	14	7.7	2.5–23.9
1972†	Some spring breeding, none in autumn	Light	2–3 Aug.	8	102.2	11.9–115.8
1973‡	Some late spring breeding, none in autumn	Light				

* Testis volume in cubic millimeters was calculated using the formula for the volume of an ellipsoid: $V = 4/3\pi a^2b$, where $a = 1/2$ the shorter diameter and $b = 1/2$ the longer diameter.

† Some gonadal development and associated behaviour took place in early August; however, nesting did not occur.

‡ Specimens were not taken but nesting did not occur.

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- ¹ Thompson, A. L., *Ibis*, **92**, 173–184 (1950).
- ² Farner, D. S., and Lewis, R. A., *Photophysiology*, **6**, 325–370 (1971).
- ³ Immelmann, K., in *Avian biology* (edit. by Farner, D. S., and King, J. R.), **8**, 341–389 (Academic Press, New York, 1971).
- ⁴ Tordoff, H. B., and Dawson, W. R., *Condor*, **67**, 416–422 (1965).
- ⁵ Payne, R. B., *Univ. Calif. Publ. Zool.*, **90**, 1–115 (1969).
- ⁶ Ligon, J. D., *Condor*, **73**, 147–153 (1971).
- ⁷ Balda, R. P., and Bateman, G. C., *Condor*, **73**, 287–320 (1971).
- ⁸ Balda, R. P., and Bateman, G. C., *Living Bird*, **11**, 5–42 (1972).
- ⁹ Ligon, J. D., and White, J. L., *Condor* (in the press).
- ¹⁰ Bailey, F. M., *Birds of New Mexico* (New Mexico Department of Game and Fish, Santa Fe, 1928).
- ¹¹ Lehrman, D. S., Hinde, R. A., in *Sex and behavior* (edit. by Beach, F. A.), Ch. **15**, 355–380; Ch. **16**, 381–415 (Wiley, New York, 1965).
- ¹² Bateman, G. C., and Balda, R. P., *Auk*, **90**, 39–61 (1973).

Pentamerism and the ancestral echinoderm

ONE of the difficulties in an account of the origin of the echinoderms is their five-fold radial symmetry. Nichols¹ has proposed a hypothetical ancestral echinoderm derived from a lophophorate ancestor. Sipunculoids have a recurved gut and a hydraulically operated tentacular system using a special compartment of the coelom. These resemblances to echinoderms suggest that a sipunculoid-like animal might have given rise to an ancestor of the echinoderms by developing a calcite skeleton for protection. This ancestor is illustrated in cross section in Fig. 1, but Nichols was unable to say why it should be pentamerous. On analysis of the requirements of such an animal, however, it has been possible to produce a 'paradigm' (in Rudwick's sense²) which demands five rays.

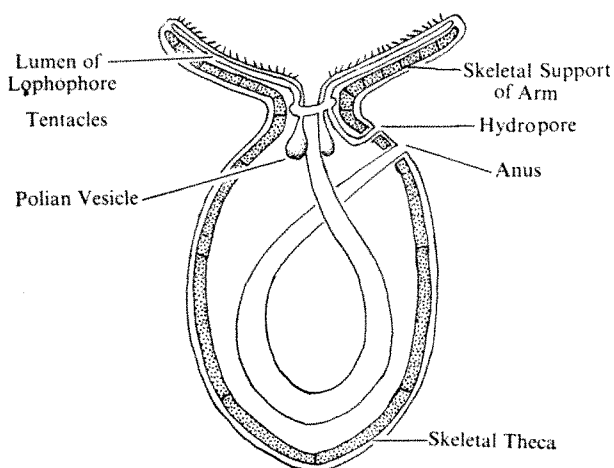


Fig. 1 Nichols' hypothetical ancestral echinoderm, in cross section.

Nichols' reconstruction, like many primitive echinoderms, has mouth, hydromore and anus in a straight line. Such an animal must live with the anus on the downstream side, to carry waste products away from mouth and hydromore (Fig. 2a). (In tidal waters, one current would probably be dominant, either because it would be stronger, or because it would carry food.) The hydromore fits best downstream of the mouth, where the current over it is filtered by the food-gathering apparatus. The lophophore of such an animal must serve three functions: first, to catch food settling vertically out of suspension; second, to catch food food carried along horizontally by the current; and third, to detect the approach of danger.

The third function needs a ray extended directly upstream (Fig. 2a), which would also serve the first two functions to some extent, but more rays are needed for food gathering. These must be bilaterally symmetrical about the axis established in Fig. 2a, or the animal would be twisted by the current. An additional ray extending downstream would only be possible if it did not lie on the surface of the theca, as in an edrioasteroid, but was extended over hydromore and anus as an arm. This would be undesirable as the arm would hinder the current in carrying away waste products, and might create eddies carrying these products into hydromore or food groove. There must, therefore, be an odd number of rays; one extended upstream and pairs on either side of the axis: one pair of rays at right angles to the axis (Fig. 2b) catches food carried horizontally by the current, but makes no provision for catching food settling vertically or for detection of danger on the downstream side of the animal. These functions would be better served if all the rays met at equal angles (Fig. 2c). If each lateral ray is of length r , it would then sample a section of the current width $w = r \sin 60^\circ = 0.866r$. Each ray would also extend 'early warning' downstream from the centre by a length

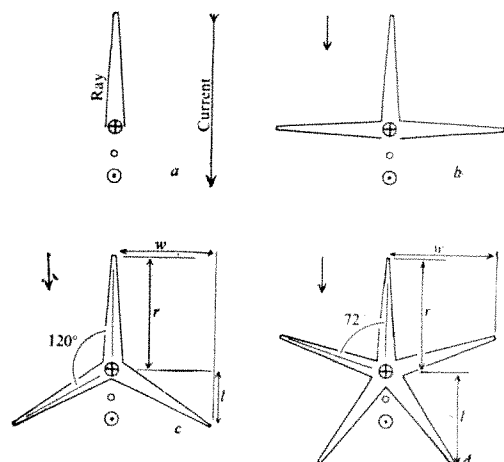


Fig. 2 Possible paradigms for an ancestral echinoderm. ⊕, Mouth; ○, hydromore; ⊙, anus; r , length of ray; w , current width searched; l , extension of 'early warning' facility downstream. Short downward arrows in b, c, d represent current direction.

$l = r \cos 60^\circ = 0.500r$. But this is a vulnerable side, predators may be attracted upstream to the animal by secretions carried by the current.

If another pair of arms is added, the requirements of the paradigm can be filled more satisfactorily. The animal of Fig. 2d has five arms of equal length and at equal angles to their neighbours. It is symmetrical to the current with one ray upstream and the anus downstream; each of the anterior lateral rays samples a width of current of width $w = r \sin 72^\circ = 0.951r$ (and it can be shown that this advantage of 5 rays over 3 persists even if the current veers considerably); each of the posterior lateral rays extends warning backwards by a distance $l = r \cos 36^\circ = 0.86r$, while the anterior lateral rays guard the flanks; and finally, five equally spaced rays provide a better net to catch food settling vertically. The addition of a third pair of rays does not produce corresponding advantages.

Five rays like this might be evolved by the branching of the lateral rays of a triad³, or by repeated branching of the

single ambulacrum of an animal like *Helicoplacus*. However, Hyman's summary⁴ of sipunculoids as having "less than 10 to numerous" tentacles suggests that it might fit Nichols' theory better to postulate a reduction of rays to the acceptable minimum of five so that the animal expends the minimum of metabolic energy in calcifying them. This reduction would mean further loss of food-gathering power to a lophophore already restricted in flexibility by calcification, which could be overcome by the provision of lateral branches to the rays as outgrowths of the coelomic canal. Nichols deduced such branches, but from analogies with recent crinoids, he considered the branches would be respiratory. Selection might have favoured their development to meet the needs of both feeding and respiration. Whatever the exact details, the hypothetical ancestral echinoderm has now been equipped with pentamerous symmetry and can give rise to a variety of primitive echinoderms.

I thank Professor D. Nichols for reading a draft of this paper.

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¹ Nichols, D., in *Symp. zool. Soc. Lond.* (edit. by Millott, N.), 20, 209-229 (Academic, London and New York, 1967).

² Rudwick, M. J. S., *Br. J. phil. Sci.*, 15, 27-40 (1964).

³ Bather, F. A., in *A Treatise on Zoology*, Part III (edit. by Lankester, E. R.), 11 (Black, London, 1900).

⁴ Hyman, L. H., *The Invertebrates, 5, Lesser Coelomate Phyla* (McGraw-Hill, 1959).

Fertilisation of sheep ova following their transfer to goats

AN interesting feature which has emerged from attempts to hybridise domestic sheep (*Ovis aries*) and goats (*Capra hircus*) is the marked difference in conception rate according to the direction in which the cross is made. In goats inseminated with sheep semen the conception rate is similar to that in goats mated naturally with male goats; whereas in sheep inseminated with goat semen fertilisation is an infrequent occurrence¹⁻³. The low fertilisation rate in sheep inseminated with goat semen has been confirmed by Hancock⁴ who found that only 5 of 93 (5.4%) sheep eggs were fertilised by goat spermatozoa.

Here I report an experiment undertaken with a view to isolating the factors which may be involved in the infertility of the sheep and goat cross, the approach being to circumvent any incompatibility between spermatozoa and environment by transferring sheep oocytes to the Fallopian tubes of goats which had been mated with fertile male goats. Results showed that most of the sheep eggs subsequently recovered had been fertilised and were undergoing cleavage, indicating that the sheep ovum presents no intrinsic barrier to the entry of the goat spermatozoon.

Eleven Border Leicester × Welsh Mountain ewes were treated with progestagen-impregnated intravaginal sponges (Veramix Plus; Upjohn Ltd) for 13 d, and were each given 1,500 i.u. pregnant mares' serum gonadotrophin (Folligon; Intervet Laboratories Ltd) by subcutaneous injection at the time of sponge removal. The ewes were then tested with a vasectomised ram twice daily for signs of oestrous activity, and were given either 6,000 µg luteinising hormone releasing factor (Roche Products Ltd) or 500 i.u. human chorionic gonadotrophin (Chorulon; Intervet Laboratories Ltd) by intramuscular injection,

18 h after the onset of oestrus. The animals were subjected to laparotomy approximately 24 h later. Six of the ewes were used as egg donors, the technique for recovery of eggs being that described by Hancock and Hovell⁵. The oocytes were transferred in a small volume of Hank's fluid into the ovarian end of the Fallopian tubes of four crossbred goats (G1, G2, G3 and G4) which had been mated with fertile male goats 30-60 min earlier. The remaining five ewes were inseminated with goat semen; the semen was collected with the use of an artificial vagina, and an equivalent of 2.15×10^9 (range 0.9-4.96) spermatozoa was deposited directly into each uterine horn. Goats G1, G3 and G4 and the inseminated sheep were subjected to laparotomy for egg recovery 3 d after the first operation; goat G2 was subjected to laparotomy 2 d after the first operation. A record was made of the number of corpora lutea in the ovaries of the goats at the time of the second operation.

Table 1 The numbers of sheep oocytes transferred to the left (L) and right (R) Fallopian tubes of mated goats, and the numbers of fertilised and unfertilised ova recovered

Goat	Sheep oocytes transferred		Corpora lutea		Ova recovered	
	L	R	L	R	L	R
G1	3	4	1	0	2 (6-8 cell) 1 (1 cell)	1 (5 cell)
G2	0	1	1	0	—	1 (2 cell)
G3	0	4	0	0	—	zona pellucida with spermatozoa
G4	3	2	0	1	2 (6-8 cell) 1 (1 cell)	1 (1 cell)

A total of six cleaved and three uncleaved ova were recovered from three of the goats (G1, G2 and G4). Assuming that, from the number of corpora lutea, one of the cleaved ova from goat G1 and one of the uncleaved ova from goat G4 were 'native' ova (see Table 1), the results indicate that seven of the transferred ova were recovered at the second operation. Five of the seven ova (71.4%) were cleaved. No ova were recovered from goat G4, but an empty zona pellucida showing the presence of embedded spermatozoa was found.

A total of ten ova were recovered from the five sheep in which goat semen had been placed directly into the uterine horns. None of the ova were fertilised, and there were no spermatozoa attached to, or embedded within, the zonae pellucidae.

Although the number of animals used in the experiment was very small, the fertilisation of transferred sheep oocytes in three goats and the presence of spermatozoa in the zona pellucida recovered from the fourth goat, indicate that there is no innate incompatibility between sheep ova and goat spermatozoa. These findings would seem to suggest that the low fertilisation rate in sheep inseminated with goat semen is due to factors affecting the survival, transport or capacitation of the goat spermatozoa in the ewe.

This work was supported by a grant to Professor J. L. Hancock from the Agricultural Research Council. I wish to thank Mr J. Dearden for technical assistance and for care of the experimental animals.

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¹ Warwick, B. L., and Berry, R. O., *J. Hered.*, 40, 297 (1949).

² Lopyrin, A. I., and Loginova, N. V., *Usp. sovrem. Biol.*, 36 (1953).

³ Lopyrin, A. I., and Loginova, N. V., *Anim. breed. Abstr.*, 22, No. 1019 (1954).

⁴ Hancock, J. L., *Proc. 5th int. Cong. anim. Reprod.*, A. I. Trento, 3, 445 (1964).

⁵ Hancock, J. L., and Hovell, G. J. R., *J. Reprod. Fert.*, 2, 295 (1961).

Swamp cancer

HYPHOMYCOSIS DESTRUENS EQUI, also called swamp cancer or bursattee, is a granulomatous disease commonly of the lower leg of the horse and is a well known condition in the tropics and subtropics¹. The associated fungus, 'Hyphomyces destruens', has neither been legitimately described nor satisfactorily placed in any group of fungi except broadly in the Phycomycetes, on the grounds of the morphology of the hyphae in tissue and culture.

Following recent work in Australia by Johnston and Hutchins², a request was made to these authors for isolates of the fungus from the New South Wales cases, but none had survived more than a few months in culture. This fate has also occurred to the isolates of Bridges and Emmons (H. Hasenclever, personal communication). In September 1973 isolates were obtained from four cases of swamp cancer in horses in the Central District of Papua New Guinea, which closely agreed with the limited description available of 'H. destruens'.

Because of suggestions by Bridges and Emmons³ and by Amemiya and Nishiyama⁴ and its general resemblance to

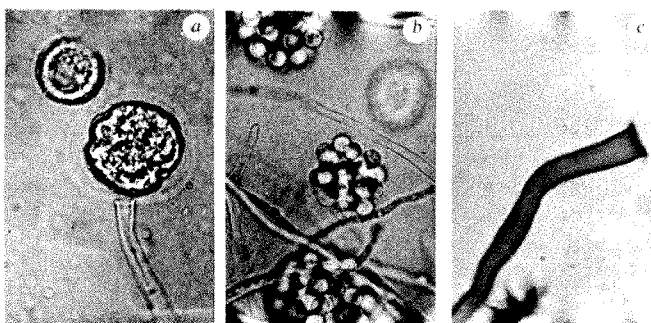


Fig. 1 *a*, Rotating vesicle of sporangial contents undergoing cleavage into zoospores immediately after emission ($\times 270$); *b*, zoospores encysted at sporangium apex ($\times 270$); *c*, sporangium aperture stained with Parker blue-black Quink ($\times 405$).

Mortierella spp., a number of methods developed to encourage these fungi to produce sporangia were tried out on the cultures, for example, hay and silage agars, but without success. A further attempt was made by placing portions of colonies from a Sabouraud glucose agar plate in sterile water in a Petri dish, to which a small piece of sterilised rotten maize silage had been added. After incubation for 2 d at 25° C biflagellate zoospores 9–10 μ m diameter were noticed arising from the cleavage of protoplasmic masses emitted from undifferentiated filamentous sporangia (Fig. 1).

This behaviour shows that 'H. destruens' is a Phycomycete belonging to the Pythiaceae in the Peronosporales and that it could be included in the genus *Pythium* Pringsheim⁵. Further work is in progress to establish whether it is a recognised or a new species, but the existence of a *Pythium* sp. pathogenic to mammals has not previously been suspected.

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- ¹ de Haan, J., and Hoogkamer, L. J., *Arch. wiss. prakt. Tierheilk.*, **29**, 395 (1903).
- ² Johnston, K. G., and Hutchins, D. R., *Aust. vet. J.*, **48**, 269 (1972).
- ³ Bridges, C. H., and Emmons, C. W., *J. Am. vet. med. Ass.*, **138**, 579 (1961).
- ⁴ Amemiya, J., and Nishiyama, S., *Bull. Fac. Agric. Kagoshima Univ.*, **17**, 215 (1966).
- ⁵ Waterhouse, G. M., *Mycol. Pap.*, **110**, 1 (1968).

Erratum

In the letter "Appearance of unusual mitochondria in rice coleoptiles at conditions of secondary anoxia" by B. B. Vartapetian, I. N. Andreeva and A. L. Kursanov (*Nature*, **248**, 258; 1974) the wrong illustration was published as Fig. 1*d*. The bottom half of Fig. 1 should be:

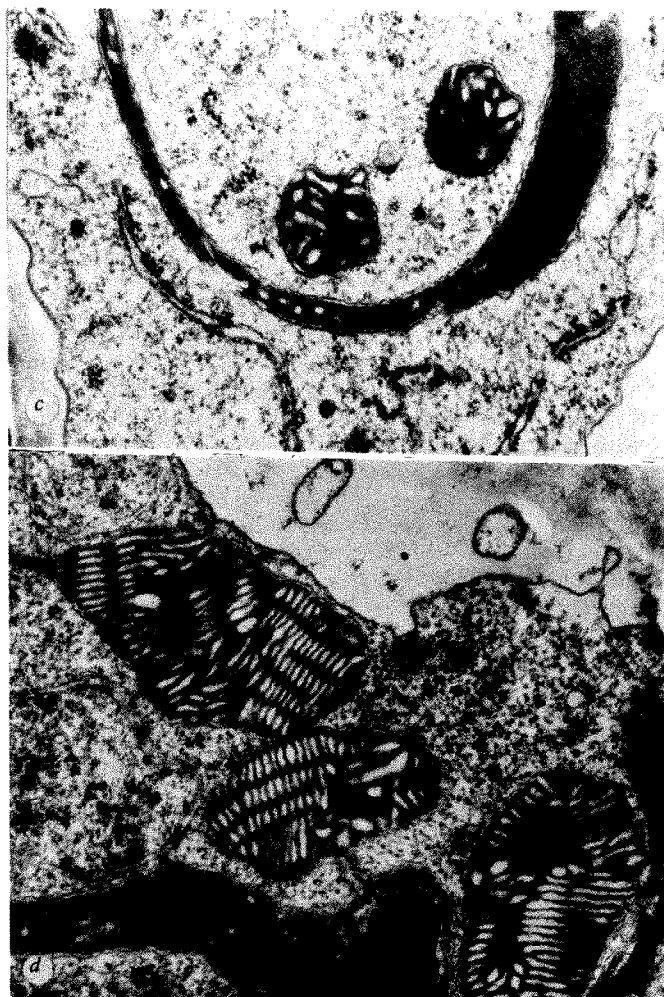


Fig. 1 Ultrastructure of mitochondria in cells of rice roots and coleoptiles in aerobic and anaerobic conditions. *a*, Root, 9 d after growth in aerobic conditions; *b*, root, 6 d of aerobic growth, then 3 d of growth in anaerobic conditions; *c*, coleoptile, 9 d of aerobic growth; *d*, coleoptile, 6 d of aerobic growth, then 5 d of growth in anaerobic conditions.

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book reviews

Pastures new

Introduction to Ecology. By Paul Colinvaux. Pp ix+621. (Wiley: New York and London, 1973.) £5.80.

Textbook of Theoretical Botany. Vol. 4. By R. C. McLean and W. R. Ivimey-Cook. Pp. viii+3317-3912. (Longman: London, October 1973.) £12.00

Introduction to Plant Ecology: A Guide for Beginners in the Study of Plant Communities. By A. J. Willis. Pp. 237. (Allen and Unwin: London, November 1973.) £5.25 boards; £2.95 paper.

THE publication of E. P. Odum's *Fundamentals of Ecology* (Saunders, Philadelphia, 1959) heralded a new era in the teaching of ecology at university undergraduate level. In particular it was Odum's emphasis upon the ecosystem as the basic unit of ecology which permitted the novel but long-awaited integration of plant and animal studies within a single cover. At last due emphasis was given to the principles governing the flow of energy, the cycling of elements and the dynamics of populations of organisms within the physical constraints imposed by the climatic and edaphic environment. As a result, this book did more than any previous text to influence the development of ecological teaching in universities throughout the world. Traditional plant and animal ecologists have been inspired not only to unite in their research efforts but also in their attempts to convey to their students an understanding of the functional mechanisms at work in natural communities of plants and animals.

The positive feedback system thus created has inevitably resulted in a logarithmic increase in the information accretion rate within the newly unified discipline of ecology. As a result, principles require modification, techniques require revision and new and better examples of many fundamental concepts emerge, all of which serve to make any text less and less useful as a teaching tool with the passage of time. Consumer demand must eventually result in the revision or replacement of traditional textbooks. Odum's text has undergone periodic revision, most recently in 1971, but for several years there has been a need for a fresh look at ecology in its totality by a new author uncommitted to the highly evolved, but often rigid, framework of a previously

published text. Two such texts have recently migrated across the Atlantic to its more conservative right-hand seaboard. One of these, Paul Colinvaux's *Introduction to Ecology*, is to be reviewed here; the other, Robert Ricklefs's *Ecology* (Nelson, London, 1973) was reviewed in these columns last year (245, 342; 1973).

Colinvaux's book is thoroughly refreshing in approach, content and style. The arrangement of material is systematic and sensible. Beginning with plant and animal geography, the author places an unusual, but highly desirable emphasis upon the time factor in the development of the formation (=biome) both on the successional time scale and over longer periods involving climatic change. Colinvaux's informal, even anecdotal, style makes this section particularly easy and exciting to read—his selection of material is so skilled that the founders of biogeography emerge as human beings rather than the stuffy Victorian protagonists pictured in so many texts.

The second part of the book deals with the ecosystem as a unit and, inevitably, it leans heavily upon Odum. But once again the author's highly selective and analytical approach leads to simplicity, consistency and flow in his style. For example, in the opening section of part 2 he reduces the major contributions of Elton, Lindeman and Hutchinson to the development of ecosystem concept to a mere paragraph. The paragraph nevertheless remains readable, full of infectious enthusiasm, yet pregnant with information and meaning. This paragraph forms the basis for his further development of the themes of production, energy transfer and nutrient cycling.

Part 3 of the book deals with competition and the relationship of the individual to the population. Inevitably, this section contains an abundance of animal data, mainly because so few botanists have regarded plants in these terms. Colinvaux's approach is made uniform by the way in which he relates all of the work he reviews to the processes of natural selection and evolution. In the final part of the book he continues the evolutionary theme by asking some of those enigmatic questions which make modern ecology so fascinating; why are some species of organism common whilst others are rare, what controls diversity in ecosystems and how is diversity

related to stability of ecosystems?

It is easy to criticise this book on many aspects of content, particularly the lack of detail in some sections. For example, the descriptive analysis of plant communities (a subject which has riveted the attention of British and Commonwealth plant ecologists for decades, often to the exclusion of all other considerations) is treated very briefly when compared with that of, say, Kershaw's *Quantitative and Dynamic Plant Ecology* (Arnold, London, 1973). Genetic aspects of natural selection and evolution rate receive a less rigorous treatment than in Ricklefs's *Ecology*. But in my opinion, any such defects are greatly outweighed by the sheer readability of Colinvaux's text as well as the effective way in which it stimulates the most fascinating ecological questions in the reader's mind. Any teacher who needs a general ecological textbook to introduce this vast subject to undergraduates need look no further than this book.

McLean and Ivimey-Cook's fourth volume in their *Textbook of Theoretical Botany* is, as its name implies, a massive collection of information relevant to certain aspects of plant ecology and plant geography. One may be forgiven for questioning the relevance of such an encyclopaedic textbook in these days. No longer can the plant sciences be adequately covered in one text, even if it runs to five expensive volumes as this one is intended to do. Indeed, I would even question the need for a textbook embracing plant ecology at a time when ecological synthesis is proving so much more valuable than analysis in the construction of a conceptually based and predictive science of ecology. Even the short preface to this volume conveys to me an air of incipient defeatism: "Many people who meet this unfamiliar word (ecology) for the first time are moved to ask, 'what is ecology?' and 'what does it do?' The present sketch may be useful in answering such questions so far as plant life is concerned."

How, one may ask, can such questions be answered by reference only to plant life? They cannot, and this fact becomes increasingly clear as one proceeds through this book. For example, many conceptual terms are treated inadequately, simply because they depend largely upon animal studies for their existence. The term 'niche' cannot be explained solely with

reference to plants; the semi-quote from Odum that in is the plants' profession is meaningless. Colinviaux asked a much more searching question when dealing with this topic; why are there so many species of grass in a meadow when they are all doing the same job? McLean and Ivimey-Cook not only fail to answer such a question, they do not ask it. Again, how sad it is that seven pages of this concept section are devoted to 'physiognomy' and only one to 'ecosystem'. That single page contains many misconceptions and errors, for example, the confusion of the terms 'population' and 'community', and the idea that an ecosystem must be stable or it will "disintegrate and disappear". Primary production is mentioned here, though not defined, but one has to hunt within the 'edaphic environment' section in order to find two more pages devoted to this topic in the 800-page book.

The coverage of the volume is patchy. Production ecology is almost non-existent, but soils and related environmental factors are dealt with very extensively. Even here, however, there is a tendency to depend upon older literature for much material with resultant inaccuracy; for example, their treatment of organic soils and peats is antiquated and misleading.

It is a little surprising to find three habitats selected for more detailed treatment in a book of this kind. These are freshwater habitats, marine habitats and sea beaches all of which are covered by specialised texts. The use of the term 'sea beaches' is also misleading. Instead of a discussion of the maritime beach habitat one finds a brief account of intertidal benthic algae and their zonation. Here again terms are used loosely; 'emergence' and 'exposure' are both used where 'emersion' would be preferable. The effects of exposure (to wave action) are not dealt with at all, despite their profound influence upon intertidal ecology.

The second (and smaller) part of the volume is concerned with plant geography. This subject is dealt with in a far more satisfactory way than is plant ecology. The approach is generally historical and due emphasis is placed upon tectonic and glacial changes in Tertiary and Quaternary times in influencing the present pattern of plant distributions. There is a very useful section which discusses Adams's criteria for the recognition of centres of origin and distribution of plant genera. This would have been even more useful if it could have been married with some ecological principles. The separation of plant ecology from plant geography, like that of plant and animal ecology, is an historical accident the effects of which

have benefited neither discipline. Our efforts should surely be directed towards healing these breaches rather than preserving them.

One final criticism of McLean and Ivimey-Cook's book is the absence of a bibliography with each volume. Presumably one is expected to buy the fifth volume to benefit from the citations of specific publications in the fourth volume.

The third book under review is the revision by Willis of A. G. Tansley's *Introduction to Plant Ecology*. Having stated my basic reservations about books devoted to general plant ecology and to the revision and up-dating of classic textbooks, I need not labour them further. This revision is a thorough one and has involved the introduction of a large proportion of new material since the final 1954 edition of Tansley's book. At the same time the basic Tansleyan influence remains dominant in concepts and approach.

There are certain areas, however, in which it is impossible to do justice to modern concepts and still to retain the Tansley tradition. For example, the chapter on vegetational succession remains true to Tansley only by omitting the contributions of Deevey, Livingstone, Hutchinson and Odum. A large proportion of the book is given over to the description of vegetation, yet Tansley's ideas on this subject, although a milestone in the history of plant ecology, are now to be regarded in the way a modern radioastronomer would look upon Galileo's telescope. Similarly, a modern ecologist would not look to Tansley if seeking a means of describing vegetation. Can we not allow these classic texts to rest in respected peace upon our shelves and, inspired by their truths but liberated from the straight-jacket of their historical context, set about their replacement with yet greater works? I am sure that Willis and many other revisors would have been more gainfully employed in such a task.

A comparison of the three books under review here sadly reflects the development of ecology on the two sides of the Atlantic. The New World has set about the task of integrating the science of ecology while the Old World remains hide-bound by its much treasured tradition.

PETER D. MOORE

Generations of insects

Insect Population Ecology: an Analytical Approach. By G. C. Varley, G. R. Gradwell and M. P. Hassell. Pp. x+212. (Blackwell Scientific: Oxford and London, 1973.) £2.75.

THIS is an excellent book about insect population dynamics, with emphases on

the construction and evaluation of life tables, on quantitative description of the effects of parasitoids, predators and competitors, and on application of these techniques in the field of biological control. It will be valuable to students and research workers, especially to those working on insects with discrete generations, since this is a basic assumption of most of the models developed here.

It is not a complete text of population ecology, partly because many important ideas have emerged only in the study of vertebrates, and partly because the scope of the book is less wide than the title implies. Other facets of population ecology such as dispersal, insect-plant relationships, effects of environmental heterogeneity and evolutionary aspects are not to be found here. But these omissions are not shortcomings, since this comprehensible treatment of population dynamics is particularly valuable now when the subject is no longer controversial. The disputes of fifteen to twenty years ago were founded partly on difficulties in definition of terms, partly on misunderstandings of cause and effect, and partly on the tendency of researchers to generalise from their own results. Subsequently, in the early sixties, most writers were particularly careful to avoid these pitfalls, but this salutary effect seems to be wearing off. This book should help to reverse the trend once more.

Not all the deductions from population models are strictly accurate. A case in point concerns the authors' own work on the winter moth and its parasites. A high and variable proportion of winter moth larvae dies each year when eggs laid on oak twigs hatch before the buds burst, causing starvation of the larvae. P. P. Feeny whose work is not referred to here, has given evidence (*Ecology*, **51**, 565-581; 1970) that the selection pressure responsible for this very early egg hatch stems from the accumulation of toxic chemicals in the oak leaves later in the season, such that larvae which hatched late would be unable to complete their development. If this is correct, then the interpretation given in the book—that the mortality is caused by "the wide scatter of bud opening times [which] prevents the time of hatch of winter moth eggs from being selected for very precisely"—is only half the story, and the assumption (page 133) that this mortality would be reduced if the buds opened synchronously on all the trees is incorrect. The same selection pressure for early hatching would still be present, and the resulting mortality would simply be more evenly distributed in space. The further assumption that this hypothetical situation would destabilise winter moth populations

seems equally unjustified, since, while the stabilising effect of parasites' responses to patchiness in host density would be removed, the major cause of instability—differences from year to year in the relative timing of egg hatch and bud burst—would, in the authors' interpretation, also be reduced.

M. C. SINGER

Obedient unto death

Obedience to Authority: An Experimental View. By Stanley Milgram. Pp. xvi+224. (Tavistock: London; May 1974.) £2.50.

"I WAS just obeying orders". From My Lai to Auschwitz and doubtless back to the time of Genghis Khan and before, the perpetrators of atrocities have excused their actions with some variation on that theme. Many people dismiss that excuse; they feel that the people involved in such horrors must be sadistic brutes not typical of the human race and comfort themselves with the secure knowledge that they would have the will and strength to refuse to obey such orders. Those comforting beliefs are shattered by Professor Milgram's account of his experiments at Yale during the 1960s. In the tests, subjects recruited from the general public to take part in what they believed to be a learning experiment were placed in a situation where they were required, under the orders of the "experimenter", to administer severe electric shocks to someone they thought to be another unsuspecting recruit for the learning experiment. Their willingness to do so is astonishing, and provides a new basis for understanding, if not excusing, the behaviour of people "just obeying orders".

Most of this book is taken up with descriptions of the various forms of the experiment and the responses of the subjects. Put baldly, it seems astonishing that anyone participated at all. Each volunteer was paired with an actor who was to be the victim in the experiment, and a rigged lottery ensured that the subject became the "teacher". The rationale of the test, as explained to him, was that the "learner" was to be encouraged to remember word pairs in a list by administration of electric shocks of increasing severity every time he made an error. The teacher had to administer these shocks while the genuine experimenter acted as the voice of authority. Many people were quite happy to administer what they genuinely believed to be shocks of up to 450 volts, while the learner screamed, protested, demanded to be released from the experiment and finally fainted.

Even when the teacher protested to the experimenter about the situation,

Fate, time, occasion, chance and change



Cartoon in *The Comic Muse*, July 18 1863, of the proposed removal of the Natural History collections to South Kensington (in fact not moved until the 1880s).

It hasn't always been 'all change' at the British Museum, but as readers of Edward Miller's scholarly and readable history (*That Noble Cabinet*, Deutsch, London, February 1974; £4.80) of the museum will soon discover, this famous institution has not been blessed with a particularly peaceful existence. For the first 150 years of the museum's life, since its foundation by Act of Parliament in 1753, if there weren't squabbles between the government, the trustees and the staff about money and buildings, there were worries about staffing, space and facilities; and in Victorian times especially there also seem to have been endless bickerings between certain of the senior staff.

The museum, however, weathered all the storms—including a heavy battering during the Second World War—and is all set to face the next

upheavals when, if present plans materialise, the library will be severed from the other departments and uprooted into new buildings. The museum was founded mainly as a department of natural history; ironically, it was this department—despised by the great librarian Panizzi (the architect of the present reading room)—which was the first major section to be severed physically if not completely emotionally from Bloomsbury when it was moved in the 1880s to Waterhouse's new buildings in South Kensington.

Miller—himself a museum man—has written a fascinating tale. There is no doubt that the BM has been favoured with more good luck, and loyalty from benefactors and staff, than its trustees and the British nation have at times deserved.

SARAH BUNNEY

he could usually be encouraged to carry on for some time, administering greater and greater shocks by such responses as "The experiment requires you to continue" or "It is absolutely essential that you continue". The voice of authority carried remarkable weight. So much so that the early simple versions of the experiment proved useless in defining exactly where people would draw the line. From having the 'victim' out of sight in the next room, successive modifications of the experiment eventually lead to physical contact, when the teacher had to hold the protesting learner in con-

tact with the plate supposedly giving shocks. And some people, acting under orders, carried out their duties up to the 450 volt level clearly labelled with "Danger Severe Shock". It is natural to ask how careful the experimenters were in their investigation—can their results be extrapolated to normal conditions? The answer seems to be yes. Over several years variations on the experiment were tried in which the "authority" was no longer respectable Yale University but a seedy (and fictitious) firm of "Research Associates", operating from a run down neighbourhood. Various permutations

on the roles were also investigated, with in some cases two experimenters giving conflicting orders, in others the experimenter himself offering to be the 'learner' to show how harmless everything was and then screaming to be let out, and so on. The results seem clear: given a defined chain of command and a guiding plan which must be obeyed most subjects were prepared to administer severe pain in a futile 'experiment' even when it caused them considerable emotional distress. In the most extreme cases, people were found who pressed on even when they thought the 'victim' had a weak heart and might be dying.

Thus far, the book is compelling reading and provides food not just for thought but for self analysis, but when Professor Milgram feels the need to produce theories to explain the behaviour he is on much weaker ground. This is as much because of the state of the art of psychology as through any fault of his, but it is a pity that able experimenters should so often feel the need to be theoreticians as well. The latter part of the book is, however, prevented from being in any sense an anticlimax by a double sting in the tail—an epilogue about My Lai, and an appendix discussing the ethics of carrying out the experiment at all.

MARY GRIBBIN

Eyes of the aged

The Human Lens in Relation to Cataract. Pp. x+324. (Ciba Foundation Symposium 19, New Series.) (Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers: Amsterdam, London and New York, 1973.) Dfl. 43.50; \$16.70.

ALTHOUGH the surgery of cataracts figures prominently in many ophthalmic practices, clinical research has been mainly concerned with the technological problems of avoiding the post-operative complications. The relative success of modern cataract surgery may have inhibited to some extent the need for research into the mechanisms responsible for, and the changes which occur as a result of, the development of lens opacities in the aged. There cannot be complete complacency on the clinical side, however, as the patient after a successful lens extraction is nevertheless challenged by a series of problems in achieving 'better sight'. The need for research at a more fundamental level is thus of great importance.

Publication of this volume was timed to coincide with the retirement of Dr A. Pirie from the post of Reader to the University of Oxford, which she has held with distinction from 1948 to 1973, and records the proceedings of a symposium on the human lens

in relation to cataract under her chairmanship. In a series of papers followed by wide ranging discussions investigators eminent in their respective fields present their current views arising from investigations into the physical, biochemical, immunological and epidemiological changes which occur in the ageing lens. In subsequent sections it is perhaps unfortunate that the problem of iatrogenic cataracts did not receive more attention, but this was more than offset by a communication indicating that at least in certain parts of the world the age at which cataracts develop in populations depends on the geographical habitat.

This volume will be of value to cataract surgeons who wish to expand their knowledge of the subject beyond the confines of operative technique, and provides an up-to-date review of concepts and investigation techniques for laboratory workers engaged in research on lenticular opacities in the aged.

J. C. DEAN HART

Ions enter crystals

Channeling: Theory, Observation and Applications. Edited by D. V. Morgan. Pp. xii+905. (Wiley: London and New York, November 1973.) £11.75.

THE discovery in 1965 that under certain conditions fast ions from nuclear particle accelerators could penetrate to anomalously great depths in single crystals came as something of a surprise to physicists. It was not that any new principle was involved but simply that the passage of fast ions through solids had been well studied for some 70 years and it was not to be expected that a phenomenon as striking as channelling could have avoided detection for so long. This greatly increased penetration occurs when the charged particles travel along the directions of the strings of atoms which make up the crystal lattice, for in these directions the particles can travel freely in the open channels between the atoms with little energy loss. In the past eight years there has been a considerable amount of research on the channelling effect and the principal features are now well understood. Since the effect is beginning to find a number of applications the appearance of a comprehensive treatment is both welcome and timely.

The first half of the book is devoted to discussing the fundamental aspects of channelling and of the corresponding blocking phenomenon which gives rise to the distinctive pattern of ions backscattered by single crystals. Classical models provide a good account of these processes and computer simulation has been successfully employed for predicting the trajectories of ions

in crystals. With increasing accuracy of measurement, however, the use of a wave description may become necessary as in the case of channelling of electrons.

The second half of the book contains accounts of a number of the applications in which the phenomenon has been used. Thus since the blocking process depends sensitively on the structure of the solid it can be used for crystallographic analysis, the pattern immediately leading to the identification of the principal planes and axes of the crystal. Again impurities embedded in otherwise perfect crystals obstruct the free passage of the channelled ions and the nature and location of these impurities can be determined by the change in the pattern of the scattered ions. This technique of atom location is already finding many uses and includes the fields of radiation damage, surface physics and semiconductor technology, each of which is discussed at some length.

One historically interesting application lies in the field of nuclear physics and is for measuring time intervals as short as 10^{-18} s; this was devised in 1960 by Dr P. B. Treacy, five years before the phenomenon of channelling had been discovered. The principle of the method was to observe the distance travelled by an excited nucleus in a crystal in the interval between excitation and disintegration. Dr Treacy's assumption was that the decay products would have a greater chance of escaping from a crystal if the nucleus disintegrated at a point in a channel rather than at the lattice point where it was excited. His experiment proved abortive owing presumably to the poor quality of his crystals and no effect was seen. Some years later, however, when the channelling phenomenon was known, this application was independently proposed by other authors and shown to work. It has now become an established technique and some beautiful experiments have been carried out using it.

The whole field of channelling is excellently described in this book. Its multiple authorship ensures that the chapters on the different aspects of the field bear the stamp of authority and yet they are sufficiently linked together to make a coherent whole without destroying the pleasant idiosyncrasies in style of the individual authors; the editor is to be congratulated on his successful blending together of the component parts. It is informative, stimulating and well presented. As a library book it is likely to remain of value for some time and should be available to all. It will be of special interest to those working in the fields of solid state physics and of the science of materials.

M. A. GRACE

Resource Recovery and Conservation

An International Journal

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Aims and Scope

The Journal is dedicated to detailed and comprehensive investigations of the interdisciplinary aspects of resource recovery and conservation. Resource recovery is recovery of materials and energy resources from wastes now destined to disposal. The wastes may be from household, commercial, or industrial sources and the recovery may be for reuse or recycling. Resource conservation is by appropriate management of natural resources, including implementation of public policies for conservation, and management of process and product design, fabrication, and production for more efficient utilization of materials. Resource recovery from wastes is one method of conservation. Implementation of resource recovery and conservation as a positive policy by the public or private sectors requires economical processes, scientifically based, soundly engineered, and properly planned for public acceptance.

The range of application to resource recovery is interdisciplinary and broad. Thus the aim of the journal is to act as an integrated forum, and a medium for the communication, information and guidance of experts from all over the world on the design, planning, and implementation of recovery and conservation.

Type of contribution

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Contributions may emphasize any of the aspects mentioned above as well as technological or institutional methodology. The latter includes politics, resource management and allocation, social and legal aspects. The writing style and level of presentation should be directed at a knowledgeable audience skilled in the specific subject or a related aspect of resource recovery.

Publication Schedule

RESOURCE RECOVERY AND CONSERVATION
will be published in quarterly issues, one volume per year,
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announcements

In the recently announced Honours List, **Sir John Wolfenden**, formerly chairman University Grants Committee and Director British Museum was made a Life Peer; **W. F. Anderson**, David Cargill Professor of Geriatric Medicine, University of Glasgow; **S. G. Clayton**, President, Royal College of Obstetricians and Gynaecologists; **A. F. Huxley**, Royal Society Research Professor, University College, London; **W. L. M. Perry**, Vice-Chancellor, The Open University; and **D. H. Wilkinson**, Professor of Experimental Physics, University of Oxford, were made Knights Bachelor.

Awards

The Society for Analytical Chemistry announces the **Robert Boyle Essay Awards**. (Entrants must be under 20.) For details write to: The Society for Analytical Chemistry, 9/10 Savile Row, London W1X 1AF.

Appointments

W. F. Vinen has been appointed to the Poynting Chair of Physics at the University of Birmingham.

D. R. Bates has been elected a Foreign Honorary Member of the American Academy of Arts and Sciences for his work on the study of the Earth's atmosphere.

William Lyall Ford has been appointed Professor of Experimental Pathology at the University of Manchester.

Ian Briercliffe Houston has been appointed to the additional Chair of Child Health at the University of Manchester.

Jean Kennedy McFarlane has been appointed Professor of Nursing at the University of Manchester.

J. Wiegold has been awarded a Personal Chair by the University of Wales.

Errata

In the article "Formation of methylmercury in a terrestrial environment" by W. F. Beckert *et al.* (*Nature*, **249**, 674; 1974) the units given in paragraph 3, lines 4 and 5 should be . . . 1 mCi $^{203}\text{Hg m}^{-2}$ and 2–20 Ci g^{-1} respectively.

In the article, "Palatability dynamics of cardenolides in the monarch butterfly" by L. P. Brower (*Nature*, **249**, 280; 1974) Table 2 contained several errors and the following corrections should be made: California butterflies, line one, number of birds should read 9†; line eight, absorbance range of subsample should read 0.320 to 0.399||; Massachusetts butterflies, line four, number of birds should read 10¶; Totals should read Males 186 and Females 169.

International meetings

September 19, **Thermodynamics and Fluid Mechanics/Environmental Science and Technology Joint Conference** (A. J. Tugwell, Groups Department, The Institution of Mechanical Engineers, 1, Birdcage Walk, Westminster, London SW1).

September 19–20, **Action of Drugs on Vertebrate Cells in vitro** (Dr M. Balls, School of Biological Sciences, University of East Anglia, Norwich NOR 88C, UK).

September 20–22, **Science, Technology and Society in Victorian England** (The Centre for Continuing Education, Education Development Building, University of Sussex, Brighton BN1 9RG, UK).

September 20–22, **1974 Annual Conference of the Education Group of the Institute of Physics** (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

September 22–24, **Rosenhain Memorial Conference** (The Conference Secretary, Dr D. E. Miles, Division of Materials Applications, National Physical Laboratory, Teddington, Middlesex, UK).

September 22–25, **International Symposium on the Electro-optical Properties of Macromolecular Solutions** (Dr C. Houssier, Laboratoire de Chimie Physique, Université de Liège au Sart-Tilman, B-4000 Liège, Belgium).

September 22–25, **48th Annual Conference of Aslib** (The Conference Organiser, Aslib, 3 Belgrave Square, London SW1X 8PL).

September 22–28, **Short Course on Environmental Health Engineering in Hot Climates and Developing Countries** (John Pickford, University of Technology, Loughborough, Leicestershire, LE11 3TU, UK).

September 23–26, **2nd Meeting of the European Geophysical Society** (Professor A. Marussi, Instituto di Geodesia e Geofisica, Università Degli Studi, Trieste, Italy).

September 23–26, **International Symposium on Contamination Control** (ICCCS, 6 Conduit Street, London W1R 9TG).

September 23–27, **9th World Energy Conference** (E. Ruttle, Secretary-General, World Energy Conference, 5 Bury Street, St. James's, London SW1Y 6AB).

September 23–27, **6th International Symposium on Problems of Listeriosis** (Dr Malcolm Woodbine, Microbiological Unit, Department of Applied Biochemistry and Nutrition, Faculty of Agricultural Science, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, UK).

September 23–27, **8th International Conference on the Properties of Water and Steam** (Conference I.A.P.S., Secrétariat, c/o S.F.T., 28 rue de la Source, 75016 Paris, France).

September 23–27, **6th International Congress of Infectious and Parasitic Diseases** (6th International Congress of Infectious and Parasitic Diseases, 01-201 Warszawa, Wolska 37, Poland).

September 23–27, **22nd Symposium of Vertebrate Palaeontology and Comparative Anatomy** (D. W. Yalden, Department of Zoology, The University, Manchester M13 9PL, UK).

September 23–October 3, **NATO Advanced Study Institutes Programme** (Miss C. H. Borrey, Institut de Recherche Interdisciplinaire, School of Medicine, Boulevard de Waterloo, 115, B-1000 Brussels, Belgium).

September 24, **Growth of Crystals from Biological Molecules** (Professor A. C. T. North, Astbury Department of Biophysics, University of Leeds, UK).

September 24–26, **5th International Symposium on Gallium Arsenides and Related Compounds** (Dr R. Veilex, Fifth International Symposium on Gallium Arsenide and Related Compounds, LEP, 3 avenue Descartes, F-94450 Limeil Brévannes, France).

September 24-26, **Radioimmunoassay and Related Topics in Clinical Biochemistry** (Mr J. Maxwell Jones, LKB Instruments Ltd., 232 Addington Road, Selsdon, South Croydon, Surrey CR2 8YD, UK).

September 24-27, **CAD 74 International Conference and Exhibition on Computers in Engineering and Building Design** (M. I. Dawes, IPC House, 32 High Street, Guildford, Surrey GU1 3EW, UK).

September 25, **Sounding Rockets and Experimental Results** (L. J. Carter ACIS, The British Interplanetary Society 12 Bessborough Gardens, London SW1V 2JJ).

September 25-29, **10th International Congress of Herpetology** (Dr K. Klemmer, Deutsche Gesellschaft für Herpetologie und Terrarienkunde, Senckenberganlage 25, Frankfurt (Main)-1, Germany).

September 26-27, **X-ray Astronomy by Rockets and Satellites** (L. J. Carter ACIS, The British Interplanetary Society, 12 Bessborough Gardens, London SW1V 2JJ).

September 27, **Annual General Meeting of the Bone and Tooth Society** (Royal Dental Hospital, 32 Leicester Square, London WC2).

September 28, **Nutrition Society Meeting** (Dr J. D. Sutton, Hon. Programmes Secretary, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, UK).

September 30-October 1, **Meeting of the Executive Committee of the International Union of Pure and Applied Physics** (Associate Secretary General of IUPAP, c/o Institute of Theoretical Physics, Fack, S-402 20 Göteborg 5, Sweden).

September 30-October 4, **2nd Symposium on Neutron Dosimetry in Biology and Medicine** (Symposium Secretariat, Gesellschaft für Strahlen- und Umweltforschung mbH, D-8042 Neuherberg/Munich, Ingolstädter Landstr. 1, Federal Republic of Germany).

September 30-October 5, **25th International Astronautical Congress** (International Astronautical Federation, 250 Rue Saint-Jaques, 75005 Paris, France).

Reports and Publications

Great Britain

- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 276, No. 1259: On the Chapman-Kolmogorov Equation. By J. F. C. Kingman. Pp. 341-369. (London: The Royal Society, 1974.) [95]
- Fossils in Caves. (Palaeontology Leaflet No. 4.) Pp. 7. (London: British Museum (Natural History), 1974.) 7p. [105]
- Tria Juncta in Uno: The Role of the Academic Medical Unit. By Professor Raymond Hoffenberg. (Inaugural Lecture in the University of Birmingham on 4 December 1973.) Pp. 15. (Birmingham: The Information Officer, The University, 1974.) 25p. [145]
- Science Research Council. Ion Implantation Panel Report 1973. Pp. 95. (London: Science Research Council, State House, High Holborn, WC2, 1974.) gratis. [145]
- Science Research Council. Annual Report of the Daresbury Laboratory for 1973. Pp. 159. (Daresbury, Near Warrington: Daresbury Laboratory, 1974.) [145]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 267, No. 888: Neuroanatomy of the Mesothoracic Ganglion of the Cockroach *Periplaneta americana* (L.). 1. The Roots of the Peripheral Nerves. By G. E. Gregory. Pp. 421-465 + plates 21-23. (London: The Royal Society, 1974.) [165]
- BP Statistical Review of the World Oil Industry—1973. Pp. 24. (London: The British Petroleum Co., Ltd., 1974.) [175]
- Public Health Laboratory Service. Monograph Series No. 6: The Prevention of Laboratory Acquired Infection. By C. H. Collins, E. G. Hartley and R. Pilsworth. Pp. vii + 59. (London: HMSO, 1974.) 50p. [175]
- Solar Energy Applications in Architecture. (An Interim Report as Part of a Project being carried out in the Department of Environmental Design, The Polytechnic of North London.) By Edward J. W. Curtis. Pp. 30. (London: The Polytechnic of North London, 1974.) [175]
- Social Science Research Council—Descriptive Brochure. Pp. 32. (London: SSRC, 1974.) [205]
- Medical Research Council—Laboratory Animals Centre. Manual Series, No. 1: The Accreditation and Recognition Schemes for Suppliers of Laboratory Animals. Second edition. Pp. 45. (Carshalton: MRC, Laboratory Animals Centre, 1974.) [205]
- Disaster Technology: an Annotated Bibliography. By Diana Manning. Pp. 331. (London: London Technical Group, Mining House, 55 Evelyn Gardens, SW7, 1973.) £4. [215]
- Bulletin of the British Museum (Natural History). Geology. Vol. 24, No. 7: The Dentitions and Relationships of the Southern African Triassic Mammals, *Erythrotherium parringtoni* and *Megazostrodon rudnerae*. By A. W. Crompton. Pp. 397-437 + 3 plates. £2.55. Instructions for Collectors No. 4a: Insects. Pp. vi + 169. Fifth edition, completely revised. £1.50. (London: British Museum (Natural History), 1974.) [225]

Other Countries

- Council for International Organizations of Medical Sciences. Diseases of the Urinary Tract and Male Genital Organs. Vol. 5: Provisional International Nomenclature. (A list of names of diseases recommended for international use as a complement to the WHO International Classification of Diseases.) Pp. 122. (Geneva: CIOMS, c/o WHO, Avenue Appia, 1974.) [254]
- New Zealand Meteorological Service. Summaries of Climatological Observations to 1970: Stations in New Zealand and Outlying Islands, including the Cook Group, Tokelau Islands, Pitcairn Island, Niue Island and Western Samoa. Pp. 77. (Wellington, NZ: New Zealand Meteorological Service, 1973.) \$1.50. [264]
- The Nucleus: Annual Review of the Science Foundation for Physics and the School of Physics within the University of Sydney. Pp. 96. (Sydney: The University, 1974.) [264]
- Journal of Urban Economics*. Vol. 1, No. 1, January 1974. Edited by Edwin S. Mills. Pp. 1-126. Subscriptions: Vol. 1 (4 issues), 1974, \$30. (New York and London: Academic Press, 1974.) [46]
- Therapeutic Effectiveness of Methadone Maintenance Programs in the USA. By Stephen S. Wilmarth and Avram Goldstein. (WHO Offset Publication No. 3) Pp. 53. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 17. [66]
- Smithsonian Contributions to Zoology. No. 149: Ecology and Behavior of the Giant Wood Spider *Nephila maculata* (Fabricius) in New Guinea. By Michael H. Robinson and Barbara Robinson. Pp. iv + 76. \$1.45. No. 164: Synopsis of the Families and Genera of Crayfishes (Crustacea: Decapoda). By Horton H. Hobbs, Jr. Pp. iii + 32. 80 cents. (Washington, DC: Smithsonian Institution Press, 1973 and 1974. For sale by US Government Printing Office.) [264]

National Research Council of Canada. Associate Committee on Scientific Criteria for Environmental Quality—Status Report, January 1974. Pp. 50. (Ottawa: NRC, 1974.) \$2. [264]

Lovo Geomagnetic Observatory Year Book 1972. Pp. 36. (Stockholm: Geological Survey of Sweden, Section of Regional Geophysics, 1974.) [294]

Companhia de Diamantes de Angola (DIAMANG). Servicos Culturais. Museu do Dundo. Publicacoes Culturais No. 87: Subsídios para o Estudo da Biologia na Lunda—Estudos Diversos (XXXVII). Pp. 186. (Lisboa: Companhia de Diamantes de Angola, 1973.) [304]

Deutscher Wetterdienst. Deutsches Meteorologisches Jahrbuch 1971. Bundesrepublik. Pp. xxxvi + 241. (Offenbach a.M.: Deutscher Wetterdienst, 1973.) [304]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 220: The Stratigraphy and Mineralogy of the Sokoman Formation in the Knob Lake Area, Quebec and Newfoundland. By I. S. Zajac. Pp. xi + 159. \$5. Bulletin 231: *Yohioia* Walcott and *Plenocaris* N. Gen., Arthropods from the Burgess Shale, Middle Cambrian, British Columbia. By H. B. Whittington. Pp. 27 + 18 plates. \$3. Paper 72-35: Geology of McBride Map-Area, British Columbia. By R. B. Campbell, E. W. Mountjoy and F. G. Young. Pp. v + 104 (14 plates). \$2.50. Paper 73-17: Pemberton (East Half) Map-Area, British Columbia. By J. A. Roddick and W. W. Hutchison. Pp. vi + 21. \$2. National Advisory Committee on Research in the Geological Sciences—Twentieth Annual Report, 1969-1972. (Annual Report and Reports of Subcommittees.) Pp. 123. \$2. (Ottawa: Information Canada, 1973 and 1974.) [25]

United States Department of the Interior: Geological Survey. Bulletin 1394-F: Four Newly Named Tongues of Eocene Green River Formation, Northern Piceance Creek Basin, Colorado. By D. C. Duncan, W. J. Hail, Jr., R. B. O'Sullivan and G. N. Pipiringos. Pp. iii + 13. 35 cents. Professional Paper 433-L: Distribution of Radionuclides in Bottom Sediments of the Columbia River Estuary. By D. W. Hubbell and J. L. Glenn. Pp. iv + 61 + 2 plates. \$1.75. Professional Paper 529-L: Atlantic Continental Shelf and Slope of the United States—Sediment Texture of the Northeastern Part. By John Schlee. Pp. iv + 64 + 6 plates. Professional Paper 814: Distribution of Selected Elements in Surficial Marine Sediments of the Northern Gulf of Mexico Continental Shelf and Slope. By Charles W. Holmes. Pp. iii + 7 + plate 1 \$3.35. (Washington, DC: Government Printing Office, 1973 and 1974.) [35]

Royal Ontario Museum. Life Sciences Occasional Paper, No. 24: Variation in the African Bat, *Tadarida lobata*, with Notes on Habitat and Habits (Chiroptera: Molossidae). By R. L. Peterson. Pp. 8. Life Sciences Contributions, No. 98: Electrophoretic Patterns of Serum Proteins of Neotropical Bats (Chiroptera). By D. Valdivieso and J. R. Tamsitt. Pp. 24. \$1.25. (Toronto: Royal Ontario Museum, 1974.) [75]

United States Department of the Interior: Geological Survey. Bulletin 1328: Estimation of Petroleum Exploration Success and the Effects of Resource Base Exhaustion Via a Simulation Model. By Lawrence J. Drew. Pp. iv + 25. 45 cents. Professional Paper 793: Geology of the South Pass Area, Fremont County, Wyoming. By Richard W. Bayley, Paul Dean Proctor and Kent C. Condie. Pp. iv + 39 + 1 plate. \$1.10. (Washington, DC: Government Printing Office, 1973 and 1974.) [75]

United States Department of the Interior: Geological Survey. Bulletin 1370: Bibliography of North American Geology, 1970. Pp. 1276. (Washington, DC: Government Printing Office, 1973.) \$8.70. [75]

United States Department of the Interior: Geological Survey. Professional Paper 774-B: Hetch Hetchy Reservoir Quadrangle, Yosemite National Park, California—Analytic Data. By Ronald W. Kistler. Pp. iii + 15. 65 cents. Professional Paper 799: Middle Devonian Rugose Corals of the Central Great Basin. By C. W. Merriam. Pp. iv + 53 + plates 1-14. \$1.85. (Washington, DC: Government Printing Office, 1973 and 1974.) [95]

National Research Council of Canada. NRC Associate Committee on Scientific Criteria for Environmental Quality. A Criteria Digest on Radioactivity in the Environment. By H. C. Rothschild. Pp. 53. (NRCC No. 13566.) (Ottawa: NRCC, 1973.) [155]

National Botanic Gardens of South Africa. Report 1972/1973. Pp. 33. (Kirstenbosch, Newlands, C.P.: National Botanic Gardens of South Africa, 1974.) [155]

World Health Organization. Technical Report Series. No. 538: The Selection of Teaching/Learning Materials in Health Sciences Education—Report of a WHO Study Group. Pp. 25. Sw. fr. 4. Technical Report Series, No. 540: Maturation of Fetal Body Systems—Report of a WHO Scientific Group. Pp. 33. Sw. fr. 5. (Geneva: WHO; London: HMSO, 1974.) [155]

Classified Advertisements

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Semi-displayed £3.60 per 10 mm. Minimum £7.20, each additional 2 mm 72p. Full page £230.00. Half page across £125.00. 30p is charged for the re-direction of replies to advertisements with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-242 6264. Telegrams: Textualist, London, W.C.2.

APPOINTMENTS VACANT

DIRECTOR

The Jackson Laboratory invites applications for the position of Director of the Laboratory, which will become open on October 1, 1975. Qualified applicants are invited to submit a curriculum vitae and summary of experience to one of the following:

Dr James D. Ebert, Chairman, Search Committee, Marine Biological Laboratory, Woods Hole, Massachusetts 02543;

Dr James F. Crow, Member, Search Committee, Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin 53706;

Dr Douglas L. Coleman, Secretary, Search Committee, The Jackson Laboratory, Bar Harbor, Maine 04609.

AN EQUAL OPPORTUNITY EMPLOYER.

(3)

PROFESSOR OF BIOMETRY OR BIOSTATISTICS

Applicants are invited to apply for a new senior faculty position in the Statistical Science Division, Department of Computer Science, SUNYAB, starting in September 1975. Applicants should have an outstanding record of research, training and collaboration in the Health and Statistical Sciences. Responsibilities include teaching, developing curriculum for undergraduate and graduate courses in Biometry and Statistical Science for specialists and non-specialists, and active participation in the collaborative activities within the Statistical Laboratory. Applications from women and minority candidates will be especially welcome. Please send curriculum vitae to:

Professor Marvin Zelen, Chairman
Search Committee
Statistical Laboratory
SUNY at Buffalo
4230 Ridge Lea Road
Amherst, New York 14226

(8)

RESEARCH POST AT THE OXFORD HAEMOPHILIA CENTRE

Applications are invited from medically qualified graduate scientists with some experience of research in some aspect of haemostasis for appointment as a member of a small team of Medical Research Council staff working at the Oxford Haemophilia Centre, under the direction of Dr Rosemary Biggs. The successful candidate will be expected to co-operate with other members of staff on projects already progressing at the Centre and to initiate a research project or projects in the general field of interest of the work at the Centre. The work of the M.R.C. staff is integrated with that of the N.H.S. Clinical Unit, many projects being undertaken jointly, and an Honorary Clinical contract with the United Oxford Hospitals may be sought at a level appropriate for the successful candidate. The M.R.C. and N.H.S. staff also work in close co-operation with the staff of the Lister Institute Plasma Fractionation Laboratory and joint research projects may be planned. The appointment will be to the M.R.C. staff on the appropriate scientific grade and will initially be for a period of five years with the possibility of renewal at the end of this period. Superannuation provision.

Applications should be addressed to Dr Rosemary Biggs, Director, Oxford Haemophilia Centre, Churchill Hospital, Headington, Oxford OX3 7LJ.

(2)

Senior Toxicologist

The Toxicology area of the Research Department is expanding and an experienced Toxicologist is required to take charge of a new section. This will entail the planning, conduct and assessment of acute and chronic tests in rodents and some general pharmacological investigations. The person appointed will be expected to maintain close liaison with other staff concerned in the safety evaluation of new Medical, Animal Health and Crop Protection products, and to contribute towards technical documents for registration of such products in the U.K. and overseas. Publication of results and attendance at scientific meetings are encouraged.

The modern, well-equipped research laboratories and associated facilities are located in Nottingham.

Applicants must have a good honours degree in pharmacology or relevant biological science and, preferably, a postgraduate qualification. Experience of working in Toxicology and a high standard of scientific report writing are essential.

Starting salary is negotiable and will reflect qualifications and experience. Conditions of employment are attractive and include good profit sharing and contributory pension schemes. Assistance with relocation expenses will be granted where appropriate.

Please write to:—J. F. Pattison, Employment Services,



The Boots Company Ltd.,
Station Street,
Nottingham NG2 3AA.

(9)

The University of Stellenbosch
Republic of South Africa

Senior Lectureship in Chemical Engineering

Applicants must have industrial and/or lecturing and/or research experience, and must indicate the direction(s) in which they specialise or their field(s) of research. The scope of work in the department is such that an engineer with almost any specialised interest in Chemical Engineering, will be able to lecture on subjects within his particular field of interest. Duties must be assumed on January 1, 1975, or as soon as possible thereafter.

Initial salary will be negotiated between £4,540 and £5,813 per annum, approximately, commensurate with experience and qualifications. Senior Lecturers in the Faculty of Engineering are permitted, on a limited scale, to carry out private

consultation work of a specialised nature.

In addition, the University offers a medical aid scheme, pension fund, group life insurance, housing subsidy, annual leave bonus, generous leave benefits and contributes towards moving costs of newly appointed staff. Applications, giving full details of research or teaching experience with certified copies of recent testimonials and the names and addresses of at least three referees, as well as present salary, age, marital status and general health condition, should, within one month of this advertisement, reach the Registrar (Academic), University of Stellenbosch, Republic of South Africa.

(53)

Senior Pharmacologist

Our client is a major British Research Organisation with extensive expertise in the field of biomedical sciences. Its function is to employ the latest skills, facilities and equipment to carry out research projects under contract for industry, government agencies and international bodies.

The laboratories are already well established, and are committed to a substantial growth and development programme over the next few years. In this connection we seek an experienced pharmacologist to spearhead the existing pharmacology projects, and also to identify and exploit new business opportunities in the UK and overseas.

Much importance is attached to publicising the specialised techniques developed in the unit, which currently include facilities for advanced psychopharmacological studies in primates, sensitive test systems for assessing the abuse-potential of new drugs and cardiovascular pharmacology. The successful candidate will be encouraged to develop interrelationships with other specialist research units, both within the organisation and outside it.

Applicants should be graduates in pharmacology or a closely related subject, ideally with a Ph.D. and several years' post-graduate experience in industrial pharmacology. A specific knowledge of behavioural pharmacology would

be of particular interest to our client. In addition, candidates should have an adaptable and outgoing personality, with the ability to sell their ideas and services.

Salary is negotiable within wide limits and will reflect the importance of this position. Benefits include a progressive contributory pension scheme with free life insurance, four weeks holiday and generous assistance with relocation expenses.

The laboratories are situated in pleasant countryside near a major University city.

Please write in complete confidence for an application form and further literature, or 'phone if you would like to discuss the position:

Mr. R. A. Forbes-Leith, Talentmark Limited,
King House, 5/11 Westbourne Grove,
London W2 4UA. Tel: 01-229 2266.



Talentmark
Biomedical and Scientific Consultants

(12)

THE UNIVERSITY OF NEWCASTLE UPON TYNE CHAIR OF PHYSICS

Applications are invited from experimental physicists for the Chair which was held until his death by Professor W. R. Hindmarsh. This is the third Chair in the School of Physics (the others being Geo- and Planetary Physics, and Theoretical Physics). Salary in accordance with the Professorial Scale (£5,625 by £96 to £5,721 by £195 to £6,501) with membership of F.S.S.U.

Further particulars may be obtained from the Registrar, the University of Newcastle upon Tyne, 6 Kensington Terrace, NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than August 31, 1974. (Applicants from outside the British Isles may submit one copy only.) (5)

SIR GEORGE WILLIAMS UNIVERSITY

MONTREAL, QUEBEC, CANADA DEPT. OF BIOLOGICAL SCIENCES THREE FACULTY POSITIONS

Three faculty positions available, July 1, 1974: 1 Associate Professor (\$15,400 to \$17,000), 2 Assistant Professors (\$12,000 to \$15,00) depending upon experience and qualifications. Research oriented persons qualified to teach in one or more of the following areas will be considered: Embryology, Plant Ecology, Biostatistics, Introductory Biology, Ph.D. required. Send resume and names of three references to: Dr. H. Enesco, Chairman, Dept. of Biological Sciences, Sir George Williams University, Montreal, Que. H3G 1M8. (10)

THE UNIVERSITY OF MANCHESTER LECTURER IN BOTANY

Applications invited for this post. Candidates should have special interests in taxonomy and biosystematics. Salary scale p.a.: £1,929 to £4,548 (under review), but initial salary will not exceed £2,388 p.a. F.S.S.U. Particulars and application forms (returnable by July 20th) from the Registrar, The University, Manchester M13 9PL. Quote ref: 136/74/N. (15)

FOOD HYGIENE LABORATORY

A.I.M.D.T. with experience in Bacteriology. The laboratory is concerned with the routine surveillance of foodstuffs and the investigation of problems associated with food hygiene and food poisoning. The successful applicant will be encouraged to take part in research projects which include work on the many types of organisms now associated with food poisoning.

The laboratory offers good facilities for study towards the special examination in bacteriology. Further information or arrangements to visit the laboratory can be made by telephoning the Head Technician, 205 7041, Extension 38. Application forms can be obtained from the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT. (6)

UNIVERSITY OF ABERDEEN SENIOR LECTURESHIP IN ANATOMY

Applications are invited for above post. The successful applicant will be responsible for teaching and supervising both medical and science students. Good facilities for research. Possession of a medical degree desirable but not essential. Post tenable from October, 1974, or as soon as possible thereafter. Salary on scale £4,707 to £5,844 per annum with appropriate placing.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (6 copies) should be lodged by August 3, 1974. (25)

University of London CHAIR OF ENVIRONMENTAL GEOLOGY AT CHELSEA COLLEGE

The Senate invite applications for this newly established Chair. The person appointed will be asked to collaborate closely with other Departments in the Environmental Sciences, and will play a leading part in developing both specialised and interdisciplinary studies at First and Higher Degree levels. Initial salary to be agreed but not less than £5,973 a year, plus £162 London Allowance. Applications (10 copies) should be received not later than July 23, 1974 by the Academic Registrar, (N) University of London, Senate House, WC1E 7HU, from whom further particulars may be obtained. (39)

AVON AREA HEALTH AUTHORITY (TEACHING)

BRISTOL HEALTH DISTRICT (TEACHING)

RADIOTHERAPY CENTRE WORKSHOP

Technician Grade IV or V

This interesting post includes the construction and development of apparatus for patient treatment and working on the mechanics of highly complex treatment machines. Would suit a Tool-maker or Instrumentmaker. Qualifications—Engineering Apprenticeship and either City and Guilds Final, O.N.C., H.N.C. or H.N.D. Salary Grade V £1,308 to £1,677 or Grade IV £1,530 to £1,953, depending upon qualifications.

Student Technician

Aged between 16 and 18 years old to train in the Bristol Health District (Teaching) Radiotherapy Workshop. A very interesting post, the work involves precision engineering and plastic techniques. Day release for further education. Someone with 4 'O' levels or equivalent preferred.

Applications, with the names and addresses of two referees and full details, should be sent to the Administrator, Radiotherapy Centre, Horfield Road, Bristol BS2 8ED. (45)

UNIVERSITY OF THE WEST INDIES BARBADOS

Applications are invited for (a) SENIOR LECTURER or (b) LECTURER IN BIOLOGY, tenable not later than 1st October, 1974. Appointee should have suitable qualifications in Biological Science including Biochemistry and/or Physiology.

Salary scales: (a) BD\$18,108-26,730 p.a. (b) BD\$13,200-20,904 p.a. (£1 sterling=BD\$4.8). F.S.S.U. Unfurnished accommodation at rent of 10% of salary for maximum of three years, thereafter 20% of salary paid in lieu of housing. Family passages. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees should be sent by air mail, as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Detailed particulars are available and should be obtained from the same source when an application is made. (22)

NATURE CONSERVANCY COUNCIL DIRECTOR — ENGLAND

£7,279-£8080 (currently under review)

Applications are invited for the new post of Director England of the Nature Conservancy Council (NCC). This post offers outstanding opportunities for a candidate with management ability and scientific understanding and a concern to conserve the wildlife of the country.

The Director will have authority delegated to him by the Director of the NCC for activities in England. He will work closely with the Chairman of the Council's Statutory Advisory Committee for England and will contribute towards the Council's policies as a member of the Great Britain Board of Management.

The Director England will have his own Headquarters and will direct six Regional Officers with responsibility for a staff of 160 and be responsible for 67 National Nature Reserves and 2,010 Sites of Special Scientific Interest. He must maintain high level contact with Central and Local Government Departments and Agencies, land managers and users, and a wide range of scientific, professional and voluntary bodies.

The post is graded Deputy Chief Scientific Officer. Candidates should have appropriate scientific qualifications and must have proven capacity for management and liaison.

Initially the post will be based in London with the possibility of relocation at a convenient site outside London. The successful candidate should take up office as soon as possible.

Interviews will be held in London on 8 August.

Application forms are available from Establishments (S), Nature Conservancy Council, 19 Belgrave Square, London SW1X 8PY.

Closing date for completed application forms: 22 July 1974. (20)

THE UNIVERSITY OF LEEDS PROCTER DEPARTMENT OF FOOD AND LEATHER SCIENCE

Applications are invited from chemists, biochemists, biophysicists, or other suitably qualified graduates for the post of LECTURER IN FOOD SCIENCE. Evidence of ability in research is required. The successful candidate will have teaching responsibilities in food chemistry. He will join the new Head of Department, Professor D. S. Robinson, in establishing research into the relation between chemical structure and function in foods, in particular in relation to proteins. An ability to acquire and apply many different specialised techniques to the study of protein function would be of advantage.

Salary on the scale £1,929 to £4,548 (under review).

Forms of application and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 40/8/D). Closing date August 30, 1974. (29)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the posts of SENIOR LECTURER and LECTURER IN THE DEPARTMENT OF PHYSIOLOGY. Appointees should possess a medical qualification or degree in Physiology. The duties will include lecturing and demonstrating in experimental and human physiology. Duties to be assumed soonest. Salary scales: (Medically qualified) Senior Lecturer J\$10,992-14,472 p.a. Lecturer J\$7,860-10,752 p.a. (Non Medically qualified) Senior Lecturer J\$10,992-14,472 p.a. Lecturer J\$7,860-10,752 p.a. (£1 sterling = J\$2.20). F.S.S.U. Unfurnished accommodation will be let by the university at a rental of 10% of salary. Family passages; triennial study leave. Detailed applications (6 copies) including a curriculum vitae and naming 3 referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Further particulars are available from the same source and should be obtained before an application is made. (21)

CENTRAL BIRMINGHAM HEALTH DISTRICT QUEEN ELIZABETH HOSPITAL, BIRMINGHAM B15 2TH BASIC GRADE BIOCHEMIST

required in Clinical Chemistry Department. This is a new post, for two years in the first instance, in which at least half the time will be devoted to a research project.

Job description and application form from Assistant Secretary (Personnel) at the above address. Closing date for applications July 31. (30)

Scientific Officers

Applications are invited for pensionable posts in the following Research Centres of the Department of Agriculture, Northern Ireland.

Agricultural and Food Chemistry Research Division

To join a team engaged in research projects in the various aspects of agricultural and food chemistry, plant and animal nutrition and plant and animal biochemistry.

Field Botany Research Division

To join a team engaged in research on growth and physiology of crops especially grasses, mainly in glass houses and controlled environmental growth rooms.

Agricultural and Food Bacteriology Research Division

To join a team engaged in research and investigational work into the bacteriological problems of the agriculture and food industry, including the microbiological examination of various foods and materials.

Fisheries Research Laboratory, Coleraine

Two officers are required; one to cover duties in the Department's angling development programme, which will include surveys of lakes and rivers. Laboratory work will include the examination of invertebrate fauna and fish samples and subjecting them to appropriate analyses.

The duties attached to the other post involve assessing salmonid production and populations in tributary rivers to Lough Neagh and especially in the Foyle system. In particular, studies of variations in annual recruitment and survival rates are required.

Candidates must be under 27 years of age on December 31, 1974 and have a degree, HNC or equivalent qualification in an appropriate Scientific subject. Applications will also be considered from final year students. Ability to drive and swim is essential for the fisheries post.

Salary scale (under review): £1,600 to £2,544. Starting salary will be related to qualifications and post-qualifying experience.

There are prospects of promotion to Higher Scientific Officer (£2,221 to £2,854), Senior Scientific Officer (£2,798 to £3,895) and Principal Scientific Officer (£3,715 to £4,895).

Please write or telephone for an application form and further information, quoting Ref. SB 172/74/N to the Secretary, Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms must be returned to arrive not later than July 25, 1974.

Applicants should state their preference for a particular Division. (24)



**NORTHERN IRELAND
CIVIL SERVICE**

Biochemist Team Leader

Analyst Team Leader

The Radiochemical Centre has created two posts for qualified scientists with experience in biochemistry and analysis.

The biochemical post calls for the capability of initiating and developing quality control techniques for the radioimmunoassay and biochemical assay of our radiopharmaceuticals.

The analytical post involves the development of chromatographic and other analytical techniques for organic chemicals by modern instrumental methods. A knowledge of statistical quality control techniques is desirable.

Both posts require at least an academic qualification of honours degree standard and relevant experience is essential.

The salary would be a minimum of £2500. Generous leave allowance, contributory superannuation scheme, staff canteen and social club.

The Radiochemical Centre is a world leader in the supply of radiochemicals and radiopharmaceuticals. If you want further information ring Martyn Bishop at Little Chalfont 4444 or write for an application form to



The Radiochemical Centre

Amersham

Bucks

(43)

THE GAMBIA Agronomist

Required by the Department of Agriculture for 2 tours of 12/24 months to assume responsibility for the experimental farm and the Agronomy section involved in field trials of upland crop production of pre-basic and foundation seeds and work on vegetables.

Candidates should have an honours degree in Agriculture or Agricultural Science with at least one year's field experience in agronomic research. Knowledge of Tropical Agriculture would be advantageous.

Salary in the range £2,270 to £4,350 p.a. which includes an allowance, normally tax free, of £1,266 to £2,400 p.a. Terminal gratuity 25% on basic salary only.

For a married man with two children paying tax at the standard rate the total emoluments described above, including gratuity, approximate to a gross (i.e. before tax) UK return of 3,800 to £6,200 and for a single man about £3,300 to £5,700 p.a.

Benefits include: generous leave, paid passages, education allowances, government quarters at reasonable rentals. A car loan of £600 and an Appointment Grant of up to £200 may also be payable.

The post described is partly financed by Britain's programme of aid to the developing countries administered by the Overseas Development Administration of the Foreign and Commonwealth Office.

For further particulars you should apply, giving brief details of experience to:

crown agents

M Division, 4 Millbank, London SW1P 3JD, quoting reference number MID/740611/NF (27)

Guy's Hospital Medical School

Graduate Research Assistant

required in Department of Obstetrics and Gynaecology for research on fat metabolism in pregnancy. Opportunity of higher degree. Experience of techniques of lipid analysis an advantage. Salary in range £1,569 to £1,833 (under review).

Apply in writing, with curriculum vitae, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT. (36)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the post of LECTURER/ASSISTANT LECTURER IN MATHEMATICS. Applicants should have teaching and research experience in Pure or Applied Mathematics, tenable 1 October 1974. Salary scale: Lecturer J\$6,168-9,768. Assistant Lecturer J\$5,006-5,486 p.a. (£1 sterling = J\$2.20). F.S.S.U. Unfurnished accommodation will be let by the University at a rental of 10% of salary. Family passages; triennial study leave. Detailed applications (6 copies) including a curriculum vitae and naming 3 referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Further particulars are available from the same source and should be obtained before an application is made. (23)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHYSICS

It is intended to appoint a University Demonstrator in the Department of Physics to hold office from October 1, 1974 or as soon after as may be convenient. Two of the research groups in the Department are concerned with the theory of condensed matter and with experimental particle physics using bubble chambers, and preference will be given to candidates with an interest in working in these fields. The teaching duties are not so specialised and importance will be attached to the evidence of teaching ability at an undergraduate level. The appointment will be subject to the Statutes and Ordinances of the University, and will be for three years in the first instance, with the possibility of reappointment for two years. The maximum tenure of a University Demonstratorship is five years.

The pensionable scale of stipends from October 1, 1974, is £2,247 p.a., rising by unequal annual increments to £2,931, or, if the person appointed is ordinarily resident in College, £2,148 p.a. rising by unequal annual increments to £2,832.

Applications (ten copies), together with the names and addresses of not more than three referees, should be sent to the Secretary of the Appointments Committee of the Faculty of Physics and Chemistry, Department of Physics, Cavendish Laboratory, Madingley Road, Cambridge, CB3 0HE, so as to reach him not later than July 29, 1974. (32)

UNIVERSITY OF SUSSEX

LECTURER IN ENVIRONMENTAL STUDIES

Applications are invited for the post of Lecturer in Environmental Studies in the School of Cultural & Community Studies from October 1, 1974. Applicants should be qualified in the biological or social sciences and have a particular interest in Population Analysis and Policy.

Initial salary will be within the range £2,118 to £2,757 per annum on the Lecturer scale (£2,118 to £4,896 per annum) plus Threshold payments and F.S.S.U.

Further particulars and application forms, returnable by July 19, 1974, are obtainable from the Establishment Section, Office of Arts & Social Studies, Arts Building, University of Sussex, BRIGHTON BN1 9QN (Brighton 66755, ext 712, Miss Holland) quoting reference 444/4. (31)

IMPERIAL COLLEGE
DEPARTMENT OF ZOOLOGY
AND APPLIED ENTOMOLOGY

LECTURERS/RESEARCH
FELLOWS IN ZOOLOGY

Applications are invited for the above positions: Preference will be given to candidates with interests in Parasitology (Animal Parasitic Nematodes and their Vectors) and applied Insect Ecology. The initial salary will be in the range of £2,118-£3,285. (Research Fellows) or £2,118 - £4,896 (Lecturers) plus F.S.S.U. and London Allowance (unless based at the field station). The positions are tenable from 1st October or later by arrangement. Further particulars may be obtained from Professor T. R. E. Southwood, Imperial College, Prince Consort Road, London, S.W.7. To whom applications, with the names of two referees, should be sent by 1st August. (59)

THE INTERNATIONAL CENTRE OF
INSECT PHYSIOLOGY AND
ECOLOGY

P.O. BOX 30772,

NAIROBI
Kenya

RESEARCH SCIENTIST
(ENDOCRINOLOGIST)

Ref: Res/74/12

Applications are invited from young scientists with Ph.D. degree or its equivalent.

Requirements: Candidates should have experience in the field of endocrinology and should have a strong background in experimental entomology. Tropical experience will be an advantage.

Responsibilities: The appointee will work on reproduction, development, seasonality, and diapause in the Sorghum Shootfly.

Salary: Will depend on qualifications and experience, but not less than K.Shs.44,000/= per year. Other benefits include ten per cent gratuity of basic salary earned, a housing allowance, and medical insurance.

Applications, in six copies, should give the following information: General education, Professional qualifications, experience, marital status, age, present salary and terms of service, reprints of any published articles, and names and addresses of four referees (including a personal reference). Photostat copies of relevant transcripts, thesis abstracts, and certificates should be enclosed.

Applications should be addressed to:

The Administrative Officer,
ICIPE Research Centre,
P.O. Box 30772,
NAIROBI
Kenya

to reach him before 31st July, 1974.

51

THE QUEEN'S UNIVERSITY OF
BELFAST

Lectureship in
Physical Chemistry

Applications are invited for the above post which is tenable from October 1, 1974 or such other date as may be arranged. Applicants should have some postdoctoral experience, including teaching. Research interests in the fields of polymer chemistry and/or nuclear magnetic resonance spectroscopy would be an advantage. Initial placing, which will depend on experience and qualifications, will be made at one of the first three points on the lecturers' scale, £2,118 to £2,247 to £2,412 rising to £4,896, with contributory pension rights under the F.S.S.U. The appointment will be subject to a period of probation of up to three years in duration. Applications should be received by August 15, 1974. Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland.

(Please quote Ref. 74/N.)

(52)

CAMBRIDGE UNIVERSITY PRESS

SENIOR EDITOR
PHYSICAL SCIENCES

The Press requires a senior editor with the ability to expand profitably an important physical science list, covering the entire range from undergraduate textbooks to research monographs. He will be expected to acquire manuscripts within the Press's overall editorial policy; to make and maintain contact with physical scientists in university departments and elsewhere; to establish good relationships with existing and potential authors; to brief production and marketing departments about new books; and to maintain the profitability of the backlist. Qualities required are initiative, judgment and flair, administrative skill, a professional interest in the sciences, a sense of their likely development, and the ability to define and exploit publishing opportunities. Travel, at home and abroad, is part of the job.

Preferred age 25 to 35; degree in one of the relevant sciences; experience desirable in editorial acquisition in the sciences, or in undergraduate teaching.

The starting salary will be fully competitive and is negotiable in the light of age and experience, with regular progression. Location central Cambridge; contributory pension scheme and other benefits; four weeks' holiday and resettlement expenses.

The Press is one of the world's leading scientific publishers. It is a department of the University, and is governed by a committee, the Press Syndicate, appointed by the University. The Publishing Division produces journals, Bibles and nearly 300 new books each year, and there is also a large Printing Division.

Candidates should apply, giving details of age, education, qualifications, career to date and present salary to:

Dr Alan Winter, Publishing Director,
Cambridge University Press,
The Pitt Building,
Trumpington Street,
Cambridge CB2 1RP

(42)

SUPERVISOR
VACCINE PRODUCTION

We need to recruit an additional Supervisor in our Vaccine Department to take charge of all aspects of the production of a new diagnostic reagent for the detection of hepatitis associated antigen.

There will also be opportunities to take part in advanced development work, mainly involving tissue culture techniques, within the department which is 85 strong.

We believe that the post would suit an F.I.M.L.T. or an A.I.M.L.T. (virology) who has microbiological/biochemical experience, and has an interest in production and process development. It would also be suitable for a graduate with a year or two of post-graduate laboratory experience who now wishes to move into a production environment.

We recognise that the work occupies a relatively narrow sphere of activity, but nevertheless is a rapidly evolving discipline.

Our plant and laboratories are situated in a pleasant location on the Kent Coast and we offer the range of fringe benefits to be expected of an expanding, international organisation.

Pfizer

If you feel that you combine the qualities required for production and the inspiration necessary for development work, please write, giving brief relevant details to: J. E. T. Haile, Personnel Department, Pfizer Limited, Sandwich, Kent.

(73)

NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the posts of:

**BUILDING RESEARCH ENGINEER
BUILDING RESEARCH ARCHITECT
BUILDING MATERIALS SCIENTIST**

Applicants for the post of Building Research Engineer must be engineers or technologists or architects with experience in construction research and development. Residential experience in tropical or sub-tropical areas of building would be desirable. The successful candidates will be required to develop a building research and development programme geared to the requirements of the Zambian construction authorities and industry.

Applicants to the post of Building Materials Scientist must be industrial chemists or engineers with wide experience in the production, usage and testing of building materials. The successful candidate will be required to establish a building materials laboratory service which will undertake research and development and give advice on the production and usage of building materials.

Salary according to qualifications and experience on the following salary scales:

Professional Officers	K3240 × 240 — K4440
Senior Professional Officers	K4680 × 240 — K5640
Principal Professional Officers	K5840 × 240 — K6800
Senior Principal Professional Officers	K7000 × 200 — K7600

For Zambians there is a superannuation scheme. Non-Zambians will be paid a gratuity of 25% of aggregate basic salary earned during service of not less than thirty months.

Accommodation will be provided on an economic rental basis but not exceeding 10% of basic salary. Hard furniture will be provided. For Non-Zambians travel and educational allowances are available for minor dependent children attending school outside Zambia.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to:

The Secretary-General,
National Council for Scientific Research,
P.O. Box CH. 158,
Chelston, LUSAKA, Zambia.

(50)

UNIVERSITY COLLEGE LONDON Chemistry Department TEMPORARY LECTURER

Applications invited for the post of Temporary Lecturer in Inorganic Chemistry. The successful applicant will be expected to carry a normal teaching load and to devote the remainder of his time to research in inorganic chemistry; he will probably have a PhD degree and some teaching experience.

The appointment will start on October 1, 1974 and be tenable on a yearly basis up to a maximum of three years. Salary will be on an appropriate point on the lecturer scale.

Applications giving details of career, qualifications and the names of two referees, as soon as possible to Mr. F. Widdas, Department of Chemistry (N), 20 Gordon Street, London WC1H 0AJ, from whom further particulars may be obtained. (41)

NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified candidates for the posts of

CERAMIC SCIENTIST OR TECHNOLOGIST

Applicants for the post of ceramic technologist must have a University degree in ceramic technology or equivalent qualifications. The successful candidate for the post of ceramic technologist will be required to participate in the research programme of the Ceramic and Refractories Development Research Laboratory. A practically minded person is required to meet the challenge of developing ceramic products from Zambian raw materials.

Salary according to qualifications and experience on the scales

Professional Officer	K3,240x240-K4,440
Senior Professional Officer	K4,680x240-K5,640
Principal Professional Officer	K5,840x240-K6,800

There is a superannuation scheme for Zambians. Non-Zambians will be paid a graduated gratuity of 20% to 30% depending on the length of service.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to:

The Secretary-General,
National Council for Scientific Research,
P.O. Box CH. 158,
Chelston,
LUSAKA.

(48)

Histology Technician

required for Pharmacological and Toxicological Laboratory. Experience essential in the preparation and processing of animal tissues. Good working conditions. Pension and Assurance Scheme. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (71)

NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited for an immediate vacancy for a **SENIOR PRINCIPAL PROFESSIONAL OFFICER IN ANIMAL PRODUCTION, ANIMAL PRODUCTIVITY UNIT.**

Applicants must hold postgraduate University degrees in agriculture, animal science, veterinary science, or animal nutrition, animal physiology or animal management. Candidates should have extensive practical research experience. The successful candidate will be responsible for developing, in relation to the Animal Productivity Research Programme, policy on animal production research and supervising the work of the Research Unit. He would be required, under the direction of the Council to collaborate with Government Departments and parastatal organisations.

The salary scale is in the range of K7,000 x 200-K7600. A gratuity of 20 to 30 per cent, depending on length of service is paid to non-Zambians. Travel and educational allowances are available for minor dependent children. Comfortable accommodation with basic furniture will be provided at low rental.

Applications giving full personal details, qualifications and experience and naming three referees should be sent to:

The Secretary-General,
National Council for Scientific Research,
P.O. Box CH. 158,
Chelston,
LUSAKA,
Zambia.

(49)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF AERONAUTICS AND ASTRONAUTICS RESEARCH ASSISTANT/STUDENT

Applications are invited for the post of Research Assistant/Student for work on the dynamic stability of axisymmetric bodies at high Mach numbers. The work involves the development of a new type of aerodynamic facility and the exploitation of existing equipment for the measurement of dynamic stability derivatives.

Applicants should possess a good honours degree, or the equivalent, in a subject relevant to this research. The salary will depend on qualifications and experience. In the case of a Research Assistant, the salary will be in the range up to £1,750 per annum. The successful applicant will be expected to register for a higher degree.

Applications, including the names and addresses of two referees, should be addressed to the Deputy Secretary's Section, The University, Southampton SO9 5NH, quoting reference Na/182/R. (55)

AGRICULTURAL RESEARCH COUNCIL UNIT OF DEVELOPMENT BOTANY

Applications are invited from **PLANT PHYSIOLOGISTS** and **BIOCHEMISTS** for a two year temporary appointment as a **SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER** at the Agricultural Research Council's Unit of Developmental Botany to investigate an aspect of the hormonal control of plant growth development. Candidates should hold post graduate qualifications and have some further experience in original research.

Appointment in grade of Scientific Officer £1,707-£2,329 p.a. or Higher Scientific Officer £2,221-£2,854 p.a. Starting salary in accordance with qualifications and experience. At least two years post graduate experience is required for appointment to H.S.O. Superannuation under F.S.S.U. with an allowance of 41% of basic salary to off-set contributions.

The unit is attached to the Botany School of Cambridge University and is under the Direction of Professor P. W. Brian. Applications should be addressed to the Deputy Director, Dr D. J. Osborne, Unit of Developmental Botany, 181A Huntingdon Road, Cambridge, CB3 0DY. (64)

THE
UNIVERSITY OF MANCHESTER
INSTITUTE OF SCIENCE
AND TECHNOLOGY
DEPARTMENT OF CHEMICAL ENGINEERING
Thermodynamics of Liquid Mixtures

Applications are invited from prospective Graduates in Physical Chemistry, Chemical Engineering or related disciplines for research leading to the degree of Ph.D. in the field of thermodynamic and physical properties of fluids. The work is associated with the extensive Distillation and Absorption research carried out in the Department and is supported by the S.R.C. For suitably qualified candidates financial support can be arranged at a level equivalent to S.R.C. studentships. Further details and application forms may be obtained from Professor G. L. Standart, Department of Chemical Engineering, U.M.I.S.T., Manchester, M60 1QD. (60)

BRUNEL UNIVERSITY

DEPARTMENT OF POLYMER
SCIENCE AND TECHNOLOGY

LECTURER

Applications are invited for the post of Lecturer in Polymer Science and Technology to teach polymer processing and polymer physical properties to students studying for degrees in Polymer Technology and Materials Science.

The person appointed will have a good degree in an appropriate discipline e.g. Polymer Technology, and relevant teaching and research or industrial experience. A demonstrated ability to relate polymer studies to the broader study of materials would be a commendation.

Salary scale (from 1 October 1974) £2,118-£4,896 plus £162 London Allowance, with F.S.S.U. Appointment will be made at one of the first three points on the Lecturer scale.

Postcard for application form and further details to Senior Establishment Assistant, Brunel University, Uxbridge, Middlesex UB8 3PH or telephone UXBRIDGE 37188 extension 49. Closing date: 17th July, 1974. (58)

CHELSEA COLLEGE
University of London

The Biophysics Laboratory has a vacancy for a **LABORATORY TECHNICIAN GRADE 2B**. The appointment of a trainee technician would be considered. The work involves research into electrical properties of biological materials and general teaching laboratory duties. Qualifications required are O.N.C. or equivalent, but two A-levels in appropriate Science subjects would be preferable; encouragement will be given for further study. Salary Scale: £1,699 - £1,969 per annum (including London Allowance) of for a trainee £973-£1,426 per annum (including London Allowance). Please write, giving personal details, to Dr. D. Rosen, Biophysics Laboratory, Chelsea College, Manresa Road, London, SW3 6LX. (61)

**AGRICULTURAL RESEARCH
COUNCIL
UNIT OF DEVELOPMENTAL BOTANY**

Applications are invited for a two year temporary appointment as a **SCIENTIFIC OFFICER** at the Agricultural Research Council's Unit of Developmental Botany. The successful candidate would be engaged in a supporting role in a small team engaged in a study of plant nucleic acids.

Applicant should have an honours degree in a biological subject, preferably Biochemistry, and be prepared to work closely with the team leaders. Starting salary, in the range £1,707 - £2,329 p.a. will be dependant upon qualifications and experience. Superannuation with an allowance to offset personal contributions.

Apply giving full details of qualifications and experience to the Secretary, Unit of Developmental Botany, 181A Huntingdon Road, Cambridge CB3 0DY. (63)

Opportunity Overseas

British Solomon Islands Protectorate

FISHERIES OFFICERS

With the Department of Agriculture to assist in the development of improved fishing and storage techniques mainly for commercial purposes; advise, train and supervise supporting staff.

Applicants should have BSc (Biological Science/Zoology/Marine Biology) with experience in fisheries management. Practical experience preferably in a tropical country. Appointment 2 years.

Salary in scale £2,691-£5,834 pa which includes a non-gratuitable allowance payable in the United Kingdom and normally tax-free, in scale £1,560-£3,186 pa. Terminal gratuity 25% of basic salary.

Other benefits include paid leave, free family passages, children's education allowances and subsidised accommodation. A tax free appointment grant of up to £200 and an interest free car purchase loan of up to £600 may be payable in certain circumstances. Applicants should normally be citizens of, and permanently resident in the United Kingdom.

For full details, application form and booklet about the British Solomon Islands, please apply giving age and brief details of qualifications and experience to:—

Appointments Officer:

Overseas Development Administration

Room E301, Eland House,
Stag Place, London SW1E 5DH

(84)



Senior Biochemist

A leading British pharmaceutical Research Organisation has a vacancy for a **Senior Biochemist** to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines. Persons with appropriate experience are invited to apply in writing for further details to: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB, marking the envelope **CONFIDENTIAL**. (72)

An opportunity in PHOTOSYNTHESIS RESEARCH

at post-doctoral level is available immediately at the Department of Biology within a programme sponsored by the Deutsche Forschungsgemeinschaft at the **University of Konstanz Germany**.

The applicant should have the Ph.D. or equivalent qualification and should undertake research in basic biochemical problems of photosynthetic electron transport in green algae.

Scientists who have some relevant research experience are preferred, but also those are encouraged to apply who have a thorough training in plant physiology, biochemistry or possibly organic chemistry and like to work in the field indicated above.

Please send your application, curriculum vitae including details of previous experience, names of two referees, by July 15, 1974, to Professor P. Böger, Unit of Plant Physiology and Biochemistry, University of Konstanz, 7750 Konstanz, POB 733, Germany. (78)



Brixham
Laboratory

Experimental Biologist or Animal Physiologist

We are looking for a person with a good Honours degree and preferably a Ph.D. to work on the investigation of the long term effects of conservative substances, including bio-accumulated materials, found in industrial wastes, on the vital processes of marine organisms.

The work will initially involve the development of suitable laboratory techniques for measuring the non-lethal effects of pollutants. A good knowledge of chemistry is essential.

Please write, giving brief details of education and experience to:

Dr. V. T. J. Schenk, Head Office Services Dept.
ICI Ltd, Fulshaw Hall, Wilmslow, Cheshire.

(102)

THE UNIVERSITY OF SHEFFIELD ACADEMIC DIVISION OF PATHOLOGY

Applications are invited from medically or non medically qualified persons for the following posts:

LECTURER in CHEMICAL PATHOLOGY, tenable from a date to be arranged. Interest in hormonal control of calcium metabolism or the endocrinology of cancer desirable but not essential. Initial salary in the range (clinical) £2,535 to £3,165 on the scale £2,535 to £4,917 or (non-clinical) £2,118-£2,580 on the scale £2,118 to £4,896.

LECTURER in TUMOUR IMMUNOLOGY, a new post tenable from October 1, 1974. Candidates should have experience in immunochemistry or biochemistry. Work will involve characterization and isolation of antigens and marker substances from human tumours. Salary scale (clinical) £2,535 to £4,917 or (nonclinical) £2,118 to £4,896.

Further particulars (state for which post) from the Registrar and Secretary, the University, Sheffield S10 2TN to whom applications (8 copies) should be sent by July 31, 1974. Please note reference R105/G. (47)

UNIVERSITY OF SINGAPORE CHEMISTRY

Applications are invited for teaching appointments in the Department of Chemistry.

Special consideration will be given to those with teaching/research interests in the field of Chemical Engineering and who have appropriate industrial experience.

Salary in the range S\$1,100 to S\$3,500 per month depending on qualifications and experience, and level of appointment offered. A 13th month annual allowance of one month's salary is payable according to University's regulations.

Leave, provident fund, medical, housing and other benefits are available.

Candidates should write to:

The Registrar,
University of Singapore,
Singapore 10.

giving curriculum vitae (bio-data), with full personal particulars, and also the names and addresses of three referees. (91)

BENDIGO INSTITUTE OF TECHNOLOGY PRINCIPAL LECTURER IN ELECTRICAL ENGINEERING

Applications are sought from persons suitably qualified to lead the Department of Electrical Engineering and to complement the experience and interests of the Head of the Mechanical Engineering Department in the development of a degree course which has contributions from both fields of engineering.

The applicant should possess a first degree in electrical engineering, and be able to provide evidence of post-graduate study in the general field of control systems. Practical experience of industrial controls, or their simulation, would be an advantage.

The applicant should have a sound knowledge of the state-function and the classical approaches to control design. Initially, most control course-work would be in the latter topic. The applicant should be willing to lecture in electrical engineering subjects outside the control field.

Salary: £15,260 per annum.

The successful applicant would be expected to take up duty prior to the commencement of the academic year, early March 1975.

The Bendigo Institute of Technology is a College of Advanced Education offering tertiary level degree and diploma courses across a wide spectrum of disciplines. The Institute is pleasantly situated on a new 85 acre campus six kilometres from the city centre. Bendigo, located some 150 kilometres northwest of Melbourne offers a wide range of schooling at primary and secondary levels.

Further particulars are available on request from the Athe Academic Officer, Bendigo Institute of Technology Flora Hill, Bendigo 3550, Victoria, Australia. Closing date for applications is 20 September, 1974. (65)

UNIVERSITY OF SUSSEX SCHOOL OF BIOLOGICAL SCIENCES TEMPORARY LECTURER IN BIOCHEMISTRY

Applications are invited for a temporary lectureship in Biochemistry, tenable for one year from October 1, 1974. Preference will be given to candidates with an interest in protein or RNA metabolism.

Salary will be on the University Lecturer scale with initial placement in the range £2,118 to £3,285 per annum.

Applications including a curriculum vitae and the names of three referees should be sent to the Secretary of Science, Science Office (E), University of Sussex, BRIGHTON BN1 9RH from whom further details can be obtained. (82)

UNIVERSITY OF BIRMINGHAM FACULTY OF MEDICINE AND DENTISTRY DEPARTMENT OF PHYSIOLOGY

Applications are invited for a POST-DOCTORAL RESEARCH FELLOWSHIP to work on problems of nerve-muscle interaction. Candidates should be interested in using morphological techniques while working in close collaboration with physiologists.

Starting salary up to £2,553 p.a. plus F.S.S.U., depending on age and experience.

Applications, with curriculum vitae, naming two referees, should be sent to Professor S. M. Hilton, Head of the Department of Physiology, The Medical School, Birmingham B15 2TJ. (69)

UNIVERSITY OF LIVERPOOL Department of Dental Sciences

Applications are invited from Honours graduates in Biology, Biochemistry or a related subject for a Research Studentship available in the Department of Dental Sciences from 1st October, 1974. The research programme will involve a study on the metabolism of hard tissues with particular reference to the influence of fluoride on the processes involved.

Applications, giving details of age, academic qualifications and experience, together with the names of two referees, should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/276112/N. (87)

PHARMACEUTICAL RESEARCH AND DEVELOPMENT

Our extensive research and development laboratories at Loughborough offer an excellent career opportunity for young graduates interested in contributing to our current research programme into respiratory and cardiovascular diseases.

These posts would particularly suit a recent graduate with a good honours degree or equivalent in pharmacology or pharmacy and the enthusiasm and ability to carve out a successful career in a large, progressive research environment.

An attractive competitive starting salary will be offered and excellent conditions of employment include a full range of fringe benefits and opportunities for movement and advancement throughout our R and D function.

Please write with brief details of qualifications held or expected, and information concerning research activities to date:

A.B. Johnston, Fisons Limited, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire.



(70)

UNIVERSITY OF MELBOURNE DEPARTMENT OF GEOGRAPHY

The University wishes to fill the CHAIR OF GEOGRAPHY which will become vacant on 1st January 1975, following the retirement of Professor J. Andrews.

SALARY: \$A19,614 per annum.
Further information, including details of application procedure, superannuation, travel and removal expenses, housing assistance, and conditions of appointment is available on request. All correspondence (marked "Confidential") should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia. Further information is also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.
Applications close on 30 September 1974.

(74)

THE HANNAH RESEARCH INSTITUTE DEPARTMENT OF APPLIED STUDIES MILK UTILIZATION

Applications are requested for the post of section-head of the milk utilization group within the Department of Applied Studies. This group is presently working on various aspects of heat treatment and stability of milk proteins, the physical properties of milk fat and the deterioration in the manufacturing properties of milk consequent upon storage at refrigeration temperatures. Applicants should be qualified in chemistry, and some knowledge of the food industry is desirable. They should have considerable research experience and the adaptability to work on various projects of an applied nature.

The appointment will be made at the SSO level (£2,798 - £3,895 plus 4½% compensatory allowance for F.S.S.U. payments), which requires candidates to possess a first or upper second class honours degree and at least four years appropriate post-graduate research experience. Applications, with a full curriculum vitae and giving the names of two referees, should be sent not later than 9th August 1974 to The Secretary, The Hannah Research Institute, AYR KA6 5HL, Scotland, from whom further information may be obtained.

(76)

POST-DOCTORAL Fellow required for Scientific post (MRC Grade II) in the Division of Pathology at the Institute of Cancer Research, to work on aspects of cell differentiation with particular respect to the adrenal gland and its tumours. This work will give experience in methods of tissue culture, steroid analysis and radioimmunoassay. Salary in scale £2,385 - £3,540 plus Stage III and threshold awards. Full applications should be sent as soon as possible, in duplicate, to the Secretary, 34 Sumner Place, London SW7 3NU, quoting ref. 300/G/77.

(80)



Wellcome

Ph.D. for Diabetes Research

The Wellcome Foundation, the £multi-million pharmaceutical company, has interests throughout the world. It is growing rapidly and the majority of the production at Dartford, which is our largest UK site, is for export.

We require a Senior Scientist to augment the current research programme being carried out by the diabetes research unit. This programme involves basic research into diabetes and general metabolic problems.

Candidates, male or female, should hold a Ph.D. in one of the following fields: physical, protein or membrane chemistry. At least three years postdoctoral experience in a biological field and proven ability for originality, are essential.

Salary will be in the range £3,549 to £4,869. Conditions of employment are attractive and include 4 weeks' holiday, excellent pension and life assurance schemes and generous assistance with relocation expenses, where appropriate.

Please write giving brief career details and quoting reference U/397 to the

Site Personnel Officer (CS),
The Wellcome Foundation Limited,
Temple Hill,
Dartford, Kent.

(95)



ENTOMOLOGIST—

Agrochemical Research

Fisons Agrochemical Division, one of the major manufacturers of chemicals for crop protection, are expanding their programme of Research and Development. To satisfy part of our needs, we now wish to appoint a Senior Research Assistant in the Biological Screening Department of our Chesterford Park Research Station, near Saffron Walden, Essex.

Ideally, we are seeking either a young graduate in the Biological Sciences, or someone who expects to graduate in 1974, as experience is less important than the ability to fit into an existing team.

As part of that team, the person appointed would be required to carry out insecticide and nematocide screening tests to evaluate the effectiveness of novel compounds, and to assist in the culture of the insect colonies, including the

development of methods for new species.

We can offer a competitive starting salary and our other Company conditions of employment are amongst the market leaders.

For further details and an application form, please write, quoting reference 633/ to:

Personnel Department,
Fisons Limited—Agrochemical Division,
Chesterford Park Research Station,
Near Saffron Walden,
Essex, CB10 1XL.



(79)

Microbiologist

A vacancy has arisen within our expanding organisation at Havant, Hants, for a Microbiologist to join a small team involved in the microbiological quality control of pharmaceutical nutritional products.

The candidate should have a degree or equivalent qualifications in microbiology, age 25/45 and have at least two years experience.

A competitive salary will be paid depending upon age and experience.

The Company offers a non-contributory Pension and Life Assurance Scheme. Assistance with re-location expenses will be considered.

Please apply in writing to:—

Cameron Wolstenholme,
Director of Personnel & Industrial Relations,
John Wyeth & Brother Limited,
Huntercombe Lane South,
Taplow,
Maidenhead,
Berks, SL6 0PH.

Wyeth

(68)

A RESEARCH ASSISTANT is required by the Department of Virology for a project on respiratory viral infection. Salary scale £1,440 - £1,883 depending on experience. The post would be suitable for graduates in Microbiology or Biology wishing to gain experience in Virology and offers opportunities of obtaining higher degrees. For further information please contact Dr R. B. Heath, Virology Department, St. Bartholomew's Hospital, London EC1A 7BE (Tel 01-606 7777). Applications in writing to the Personnel Office quoting Ref: R/4577/N. (81)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS. AL5 2JQ

PHYSICAL or INORGANIC CHEMIST to assist in research on ion transformations in soils in relation to their classification and development, and to crop growth. Minimum qualifications: Degree, HNC or equivalent in Chemistry or Soil Science.

Interest in and knowledge of computer programming an advantage. Work includes the use of radiotracers.

Appointment in grade of Scientific Officer £1,435-£2,329 (scale under review). Superannuation with 51% non-pensionable allowance to offset contributions.

Applications giving full personal details, names of two referees, and quoting reference 2273 to the Secretary by 26th July, 1974. (86)

UNIVERSITY OF WESTERN AUSTRALIA Perth

ASSOCIATE PROFESSOR IN CLINICAL BIOCHEMISTRY (Royal Perth Hospital)

Applications are invited from experienced clinical biochemists with appropriate medical or science qualifications for the position of Associate Professor in Clinical Biochemistry on the staff of the University Department of Pathology. The appointee will be located at the Royal Perth Hospital and will be Head of the Hospital's Department of Biochemistry. He will participate in undergraduate and postgraduate teaching in Clinical Biochemistry and will undertake research and the supervision of M.Sc. and Ph.D. students. He will direct the Hospital Department of Biochemistry which has a well equipped service laboratory including multi-channel analysers and a computer.

The salary for an Associate Professor is \$A16,389 p.a. and a medically qualified appointee would also be entitled to a differential of \$A3,000 p.a. for clinical responsibilities. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme. Applications in duplicate stating full personal particulars, qualifications and experience should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia, 6009, by 3 August 1974. Candidates should request three referees to write immediately to the Staffing Officer.

Conditions of Appointment, application procedure and further details are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (75)



JUNIOR TOXICOLOGIST

We shall shortly require an additional graduate to assist the Senior Toxicologist. The work will involve carrying out a wide range of toxicity tests in various subjects. The person appointed will have a degree in Pharmacology, Veterinary Medicine, or other biological science. Experience in toxicology, though desirable, is not essential. Reference No. 0316.

TERATOLOGIST

A vacancy occurs for a teratologist to carry out evaluations of drugs and environmental chemicals in various subjects. The person appointed will be aged 22-30 years and will be either an experienced technician in the field, or a graduate seeking to specialise in teratology. Suitable training will be given where necessary. Reference No. 0924.

CYTOGENETICIST

We have a vacancy for a cytogeneticist to carry out evaluation of drugs and environmental chemicals in mammalian cells for mutagenic activity. The person appointed is likely to have several years experience in this field. Reference No. 0923.

Please write for application form to:—

Mrs. M. Moul,
Inveresk Research International,
Inveresk Gate,
Musselburgh EH21 70B,

quoting Reference No.

(83)

AGRICULTURAL RESEARCH COUNCIL

UNIT OF DEVELOPMENTAL BOTANY

Applications are invited for a three year temporary appointment as a **SCIENTIFIC OFFICER** at the Agricultural Research Council's Unit of Developmental Botany. The successful candidate would be engaged in a supporting role in a small team engaged in a study of plant nucleic acids.

Applicants should have an honours degree in a biological subject, preferably Biochemistry, and be prepared to work closely with the team leaders. Starting salary, in the range £1,707 to £2,329 p.a., will be dependant upon qualifications and experience. Superannuation with an allowance to offset personal contributions.

Apply giving full details of qualifications and experience to the Secretary, Unit of Developmental Botany, 181A Huntingdon Road, Cambridge CB8 0DY. (63)

UNIVERSITY OF READING DEPARTMENT OF MICROBIOLOGY RESEARCH DEMONSTRATOR

An unexpected vacancy has occurred for a Research Demonstrator in the Department of Microbiology, commencing October 1, 1974. Applicants should have qualifications in microbiology, bacteriology, biochemistry or related subjects and have a special interest in the physiology and biochemistry of micro-organisms. Applicants should expect to demonstrate in practical classes up to 10 hours per week during term and to engage in research for a higher degree (M.Phil., Ph.D.). Salary scale £1,047 by £51 to £1,149 p.a. (under review). Further particulars from Dr L. J. Zatman, Department of Microbiology, University of Reading, London Road, Reading RG1 2AQ. (Ref. T.N. 44). (96)

THE CAMPDEN FOOD PRESERVATION RESEARCH ASSOCIATION

invites applications for the following post:

GRADUATE ANALYTICAL CHEMIST

with special responsibility for development of automated techniques. Ability to undertake committee work, prepare and present reports will be required. Knowledge of statistical techniques regarded as essential qualification for post.

Posts are graded on Research Officer salary scale. For further details write to: The Director, Campden Food Preservation R.A., Chipping Campden, Gloucestershire GL55 6LD. (93)

POSTDOCTORATE FELLOW IN IMMUNOGENETICS

Biochemist/Immunochemist with Ph.D. degree needed to work on the genetics of man's immune response toward limiting doses of naturally inhaled protein antigens (highly purified components of pollens, etc.). Opportunity to join a group which has had 3 years experience in this new research area. Excellent facilities and stimulating environment. Candidate must have a sound background in protein purification and characterisation. Experience in radioimmunoassay and leukocyte culture techniques advantageous. Demonstrated creativity in previous research essential. Send detailed curriculum vitae, list of publications and names of two referees to: Dr David G. Marsh, Johns Hopkins University School of Medicine at Good Samaritan Hospital, 5601 Loch Raven Boulevard, Baltimore, Maryland 21239.

Equal Employment Opportunity Employer M/F. (92)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY

Applications are invited for the following posts to commence work on September 2, 1974:—

1. **TECHNICIAN** to work in the microbiological laboratories and to help in the preparation of materials for culture of bacteria and fungi for both teaching and research. Some experience of handling microbes is essential. Initial salary within a range up to £1,794 per annum (plus threshold payments).
2. **TECHNICIAN** to help with teaching and research in ecology. An interest in both field work and chemical analytical techniques would be an advantage. Initial salary within a range up to £1,920 per annum (plus threshold payments).

Candidates for both these posts should possess at least Part I C. & G. or O.N.C. and preferably H.N.C. Application forms which should be returned as soon as possible may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref RV/N/276117. (103)

NATURE CONSERVANCY COUNCIL SCIENTIFIC OFFICERS/ HIGHER SCIENTIFIC OFFICERS

Salary Range £1435-£2854

Applications are invited for the following three Assistant Regional Officer posts:

South Wales: The ARO will in the first instance be based at Cardiff. He will be responsible to the Regional Officer for work in that part of South Wales covered by the counties of Gwent and South and Mid Glamorgan. The area includes countryside of considerable interest to nature conservation but special emphasis will be required on the Council's activities in the urban/industrial districts.

Hebrides: Based initially in or near Portree in Skye, the ARO will be responsible to the Regional Officer for a wide range of conservation duties in Lewis, Harris, the Uists and in the Smaller Isles of the Outer Hebrides. He will be closely concerned with the management of the National Nature Reserves at North Rona and Sula Sgeir, St Kilda, the Monach Isles and Loch Druidibeg and the preparation of plans for these and other areas.

South Strathclyde: Based on proposed new Regional Headquarters near Glasgow, the ARO will be responsible to the Regional Officer for a wide range of conservation duties in the south Strathclyde area—excluding Argyll. He will be closely concerned with the management of the Loch Lomond National Nature Reserve and the preparation of plans for this and other areas.

Assistant Regional Officer duties include the survey and monitoring of wildlife resources, conservation of important nature conservation sites, and providing advice to a wide range of organisations. They will be expected to collaborate with Local Authorities and other official bodies, research and teaching establishments and Voluntary Conservation Organisations.

Candidates should normally be under 30 years of age with a degree (or equivalent) in an appropriate scientific subject. A current driving licence is essential.

Starting salary according to qualifications and experience.

Application forms and further particulars are available from Establishments (S) Nature Conservancy Council, 19 Belgrave Square, London SW1X 8PY. Please quote reference number E2/01/16.

Closing date for completed application forms: August 5, 1974. (85)

Overseas Development Administration Tropical Products Institute, London

Vegetable Oil Technologist

■ Research and advisory work on the processing of fats, oilseeds and edible nuts in order to assist developing countries to improve and utilise these resources ■ Considerable amount of field work overseas.

- ☐ 1st/2nd hons degree or equivalent in chemistry or biochemistry
- ☐ Experience of oilseed technology and of working in tropics an advantage
- ☐ Age under 32
- ☐ Appointment as Senior Scientific Officer (£3026-£4123 under review, plus threshold payments)
- ☐ Ref: SA/26/JD.
- ☐ Application forms (to be returned by 26 July 1974), from Miss C A Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

**Science
group
CIVIL SERVICE**

North Western Regional Health Authority

Regional Administrative Scientific Officer

Owing to the impending retirement of the present incumbent, applications are invited for the post of Regional Administrative Scientific Officer at a salary of £5,409 rising to £6,648 per annum (subject to revision).

The successful applicant, who will be accountable to the Regional Medical Officer, should have appropriate professional qualifications in medicine or the sciences and should possess a broadly based scientific background.

This is a large region with three teaching hospitals and the Regional Administrative Scientific Officer must have the ability to plan, co-ordinate and advise on the development of National Health Service scientific services over the whole field of research, training, manpower, equipment, building design and service planning at regional level.

Further particulars and application forms to be returned by July 20, 1974, are available from the Regional Administrator, North Western Regional Health Authority, Gateway House, Piccadilly South, Manchester M60 7LP. Please quote reference No. 862.

(110)

CHIEF TECHNICIAN

At Ewell County Technical College, in the Biological Sciences Department.

To be responsible for organising the technical services, supervising technical staff and controlling the maintenance of modern equipment.

Applicants should have relevant experience and possess qualifications suitable for this important post.

36 hour week; 18 days holiday; staff restaurant and car park.

Salary: £2,031 to £2,340 according to age, qualifications and experience (currently under review).

Application form from Acting Chief Administrative Officer, Ewell County Technical College, Reigate Road, Ewell, Epsom, Surrey. Tel: 01-394 1731 (or 24 hour answering service). Please quote ref: CAO/E18/CTBS.

(145)



SURREY
COUNTY COUNCIL

RESEARCH SCIENTISTS CAN YOUR CAREER MAKE THE GRADE?

At Metal Box it will, because much of our success in providing the most profitable packaging for our customers' goods can be attributed to our Research Department. We are one of the largest packaging companies in the world supplying a comprehensive international service through subsidiary and associate companies.

We now require graduates to embark on a satisfying and challenging career in our Corporate Research and Developing Department. The work covers two different aspects of research—making innovative contributions to the Company's product range and solving problems arising in the manufacture and use of current packaging systems.

We are looking for men or women, who have first or higher degrees and are interested in Materials Science with particular reference to Polymers, Physics, Physical Chemistry and Metallurgy. The posts would ideally be suitable for either the recently qualified or for people with some post-graduate experience.

If you're looking for a really interesting career in a well-established organisation, please apply to: The Personnel Manager, **THE METAL BOX COMPANY LIMITED**, Corporate Research Department, Twyford Abbey Road, London, N.W.10.

(97)

UNIVERSITY OF IBADAN VACANCIES

Applications are invited from suitably qualified persons for the following posts:

DEPARTMENT OF BOTANY (JOS CAMPUS)

- (a) Senior Lecturer.
- (b) Lecturers.
- (c) Assistant Lecturer.

Applicants for Senior Lecturer should possess a doctorate degree and should have had extensive University teaching experience. The applicant will be responsible for the organising of Botany teaching at the Jos Campus.

Applicants for Lecturers should possess research experience and a good honours degree in Botany. University teaching experience will be an advantage.

Applicants for Assistant Lectureship should possess good honours degree in Botany.

DEPARTMENT OF ZOOLOGY (JOS CAMPUS)

- (a) Lecturers with interest in Invertebrate Zoology, Ecology, Genetics or Physiology.
- (b) Lecturer in Vertebrate Zoology.

An applicant should have a good Honours degree and a research degree in Zoology. University teaching experience will be an advantage.

DEPARTMENT OF PHYSICS (JOS CAMPUS)

Senior Lecturer/Lecturer/Assistant Lecturer.

Interest in Geomagnetism, Ionospheric Physics, Solid Earth Physics, Atmospheric Physics and Applied Geophysics as well as Solid State Physics (Theoretical or Experimental) will be of advantage.

Conditions of Service

Appointments are to commence as soon as possible, and successful candidates will be on probation for the first three years, but their appointments will be confirmed to retiring age thereafter if their services were considered satisfactory. Passages are paid for family on appointment, approved overseas leave and terminations where applicable. F.S.S.U./N.U.J.S.S. Children's and car allowances. Part-furnished accommodation or housing allowance is provided.

Method of Application

Detailed application (4 copies) stating age, full qualifications, experience, and naming three referees by July 31, 1974, to the Registrar, University of Ibadan, Ibadan, Nigeria, from whom further particulars may be obtained.

Salary Scales:

Senior Lecturer—N£5,030 by N£150 to N£5,480 by N£270 to N£5,750.

Lecturer—N£2,760 by N£150 to N£3,660/ N£3,810 by N£150 to N£4,260 by N£270 to N£4,530 by N£150 to N£4,830.

Assistant Lecturer—N£2,140 by N£100 to N£2,240 by N£220 to N£2,460 by N£100 to N£2,660 (if holding a higher degree N£2,460 by N£100 to N£2,660).

(77)

**UNIVERSITY COLLEGE LONDON
DEPARTMENT OF GEOLOGY
HYDROGEOLOGIST**

An appointment in the grade of DEMONSTRATOR will be made from October 1, 1974, to assist with the postgraduate training course in Hydrogeology and to work with Mr. G. P. Jones in one of the fields of research with which the Department is currently engaged.

Applicants should preferably have had some experience or qualification in Hydrogeology or Hydrology, and an interest in teaching and research. The Demonstrator may register for a higher degree. Salary range £1,494 to £1,743. Appointment will be for two years in the first instance.

Applications, together with names and addresses of two referees, as soon as possible to Assistant Secretary (Personnel) (N), University College, London, Gower Street, LONDON WC1E 6BT. (101)

**THE POLYTECHNIC
OF CENTRAL LONDON
SCHOOL OF ENGINEERING AND SCIENCE
LIFE SCIENCES**

RESEARCH ASSISTANT
£1,427 to £1,537

Applications are invited from good Honours graduates in Biology for a Research Assistantship tenable from September 1, 1974. The successful candidate will join the Pollution Research Group and will investigate the effects of pollutants from motorways on aquatic ecosystems. It may be possible for the successful candidate to register for a higher degree.

Details and application form from The Establishment Officer, PCL, 309 Regent Street, London W1R 8AL. 01-580 2020 Ext 212. (113)

**ST. GEORGE'S HOSPITAL
MEDICAL SCHOOL
HYDE PARK CORNER, LONDON SW1**

The Department of Medical Microbiology has a vacancy for an ELECTRON MICROSCOPE TECHNICIAN. The work involves the development of diagnostic methods in virus infections and research topics related to virology and to molecular genetics. Arrangements can be made for training in electron microscopy and related techniques. Starting salary in the range of £1,825 to £2,095 (including London Allowance). Application giving curriculum vitae to Professor H. Stern, Department of Virology, St. George's Hospital Medical School, Hyde Park Corner, London SW1. (144)

**AGRICULTURAL RESEARCH
COUNCIL**

**INSTITUTE OF ANIMAL PHYSIOLOGY
BARRAHAM, CAMBRIDGE CB2 4AT**

TWO RESEARCH POSTS are available for one year in the first instance at postgraduate or post-doctoral level. The work concerns the hormone activity of early embryonic tissue in relation to the establishment of pregnancy. Applications are invited from a candidate qualified to study the ontogeny of hormone activity in tissue culture and from a biochemist to investigate protein synthesis. The salary (scale currently under review) will be £1,435 to £2,329 p.a. according to qualifications and experience. Superannuation under FSSU with a non-pensionable allowance to offset contributions. Applications with full details and names of referees to the Secretary of the Institute, quoting reference P.H.4. (105)

**UNIVERSITY OF MALAYA
FACULTY OF SCIENCE
CHAIR OF ZOOLOGY**

Applications are invited for appointment to the Chair of Zoology in the Faculty of Science. Candidates should have high academic qualifications and wide experience in teaching and research at University level. Administrative experience would be an advantage. Subject to academic suitability and experience, preference will be given to candidates who are proficient in Bahasa Malaysia (Malay) but this requirement is not applicable to overseas staff. If selected, overseas staff may be offered a short-term contract subject to the possibility of renewal by mutual agreement.

Salary scale (approx. stg. equiv.): There is a range of basic salaries, to a point on which a Professor is appointed, depending on his qualifications and experience. These are £2,919, £3,032, £3,146, £3,259, £3,373, £3,486, £3,600, £3,713. In addition the following allowances are payable: Variable Allowance £243 min. £649 max. p.a. calculated at 35% of basic salary. Supplementary Housing Allowance £503 p.a., and medical benefits.

Further particulars and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The closing date for the receipt of applications is August 6, 1974. (106)

HEAD OF TISSUE CULTURE

£3,000 p.a. plus Pension

Applicant should have a sound theoretical and practical appreciation of the problems of tissue and organ culture based on several years postgraduate experience and an interest in toxicology and carcinogenicity studies.

This is a rapidly expanding contract research company located in modern, well equipped laboratories in a rural setting within 40 minutes of London. Please telephone Stock 840101 for further information or send full career details in strictest confidence to the director:

LIFE SCIENCE RESEARCH

Stock, Essex CM4 9PE, England

(111)

INFORMATION SCIENTIST

Borax Consolidated Limited, an international company within the RTZ Group, requires an Information Scientist for the Information Department at its Research Centre in Chessington, Surrey.

The information team is responsible for providing the Borax Companies throughout the world with technical, patents, and commercial information.

Candidates should have a degree (or equivalent qualification) in chemistry or a related field. Previous experience in information work is desirable and fluent reading ability in one or more modern languages will be a distinct advantage.

Every opportunity will be given to the successful candidate for fullest utilisation of ability and initiative.

The post offers an attractive starting salary and excellent fringe benefits including non-contributory pension and life insurance schemes.

Please write giving a brief outline of your career to:— The Personnel Manager, Borax Consolidated Limited, Borax House, Carlisle Place, London SW1P 1HT. (98)

**UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY
RESEARCH FELLOW (CELL BIOLOGIST)**

Applications are invited for the post of Research Fellow (Postdoctoral preferred) for two years in the first instance to work on drug effects on cultured human cells.

Salary within the range £2,118 to £2,412 plus FSSU.

Applications (quoting R24/74) with curriculum vitae and names of two referees should be addressed, as soon as possible, to Dr Mary Dawson, Department of Pharmaceutical Technology, University of Strathclyde, Royal College, 204 George Street, Glasgow G1 1XW, from whom further particulars may be obtained (Tel. 041 552 4400 ext 2113). (112)

UNIVERSITY OF RIYADH MEDICAL SCHOOL—SAUDI ARABIA

(In association with the University of London)

Applications are invited from FEMALE honours graduates preferably holding a doctorate and with teaching experience for the following posts:—

SENIOR LECTURER/LECTURER IN PHYSICS SENIOR LECTURER/LECTURER IN CHEMISTRY SENIOR LECTURER/LECTURER IN BIOLOGY

Since the opening of the Medical School in 1969 only male staff and students have been accepted. In the 1974-75 academic year, a start is to be made in the medical training of women. Ten premedical female students are to be accepted at the School in the first instance. Appointees will be responsible for a teaching programme for these students according to an agreed syllabus which has already been prepared. The teaching load will be shared by all lecturers who will also be jointly responsible for examining the students' progressive attainment and for organising the practical work of the course.

The basic premedical sciences course will last for approximately 1½ academic years. In the premedical course the female students will be taught in segregated classes, but in the preclinical and clinical years, classes will be coeducational.

Salary Scales:—Senior Lecturer Saudi Ryals 4,500 x 200—5,700 per month. Lecturer Saudi Ryals 3,500 x 200—4,700 per month. Housing allowance of SR10,000 p.a. for Senior Lecturers and SR 8,000 p.a. for Lecturers. (Present rate of exchange is £1 sterling = 8.5 Saudi Ryals). In Saudi Arabia currencies are freely convertible and transferable. Appointments are for one year or longer; renewable.

Detailed applications (three copies) with the names of three referees should be addressed to the Inter-University Council for Higher Education Overseas, 90-91 Tottenham Court Road, London W1P 0DT, not later than July 26, 1974. (99)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the post of LECTURER/ASSISTANT LECTURER IN CHEMISTRY

Appointee should have an interest in Organic Chemistry. Salary scale: Lecturer J\$6,168 to \$9,768 p.a., Assistant Lecturer J\$5,006 to \$5,486 p.a. (£1 sterling = J\$2.20). FSSU. Unfurnished accommodation will be let by the University at a rental of 10% of salary. Family passages; triennial study leave. Detailed applications (6 copies) including a curriculum vitae and naming 3 referees should be sent as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Further particulars are available from the same source and should be obtained before an application is made. (100)

UNIVERSITY OF AUCKLAND NEW ZEALAND

SENIOR LECTURESHIP/ LECTURESHIP IN GEOGRAPHY

Applications are invited for the above-mentioned post. Preference will be given to candidates with qualifications in the Geography of East Asia and in either Climatology or the Geography of Transport. Applications will also be considered from persons with systematic specialist interests in other branches of physical or cultural geography.

Salary scales: Commencing salary within the appropriate scale will be determined in accordance with qualifications and experience. Senior Lecturer: NZ\$8,713 to \$10,232 p.a.; in exceptional cases the NZ\$8 may extend this scale up to \$11,139. Lecturer NZ\$6,753 to \$8,568 p.a. An allowance is made towards travel and removal expenses and superannuation is available on an FSSU basis.

Further particulars, conditions of appointment and application procedure obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on July 31, 1974. (108)

UNIVERSITY OF SYDNEY DIRECTOR OF THE SAMPLE SURVEY CENTRE

Applications are invited from persons qualified and experienced in the theory and practice of sample surveys for the position of Director of the Sample Survey Centre. The appointee to this position will have professorial status.

Salary rate: \$A19,614 p.a.

A statement of Conditions of Appointment and Information for Candidates may be obtained either from Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, University of Sydney, N.S.W. 2006, Australia. Applications close on August 15, 1974. (109)

UNIVERSITY OF SYDNEY MARINE STUDIES CENTRE

FIXED-TERM LECTURESHIP IN PHYSICAL OR CHEMICAL OCEANOGRAPHY

Applications from suitably qualified scientists are invited for a Fixed-term Lectureship in Physical or Chemical Oceanography in the Marine Studies Centre. The appointee will be expected to develop courses in the principles of physical and chemical oceanography to third year students in the Faculty of Science with a background in the biological or earth sciences.

The appointment will commence as early as possible in 1975 and will terminate on December 31, 1975, in the first instance.

Salary range: \$A9,002 to \$12,352 p.a.

Applications including curriculum vitae, list of publications and names of three referees, by July 26, 1974, to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information is available. (107)

TECHNICIAN

required to assist in research on cancer and immunology in Dept. of Experimental Pathology. Previous laboratory experience required. Salary depending on qualifications and experience. Apply: The Secretary, St. Mary's Hospital Medical School, Paddington, London W2 1PG, quoting Ref. EP 2. (148)

UNIVERSITY OF READING DEPARTMENT OF CHEMISTRY RESEARCH DEMONSTRATORS

required in inorganic and physical chemistry from October 1, 1974, for three year appointment. Candidates should have a good Honours Degree in Chemistry, or equivalent qualifications. Demonstrators help supervise practical classes and undertake research leading to a higher degree (M.Phil or Ph.D.). Salary in the scale £1,046 x 51 to £1,149 p.a. (Under review). Apply to Professor G. W. A. Fowles, Department of Chemistry, University of Reading, Whiteknights, Reading, from whom further information about research topics can be obtained. (Ref: T 52.) (147)

LIVERPOOL POLYTECHNIC SCHOOL OF PHARMACY SCIENTIFIC ASSISTANT IN MEDICAL CHEMISTRY

Applications are invited for a scientist experienced in organic methodology to work on a project in the field of narcotic analgesics approved by MRC. Salary £2,100 for a minimum of 3 years which may be supplemented by teaching duties.

Enquiries and applications to: Dr. A. F. Casy, School of Pharmacy, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF. (146)

RESEARCH STUDENTSHIPS

Applications are invited for two three-year Research Studentships, tenable from October 1, 1974, at the M.R.C. Dunn Nutritional Laboratory, Cambridge, in the following areas:

(1) The relationship between protein metabolism and hormonal status in animals subjected to protein-energy malnutrition.

(2) The biochemical effects of riboflavin deficiency in an experimental animal, to relate the known parameters of subclinical riboflavin deficiency with biochemical lesions which may have pathological consequences. These studies will form part of a new programme, the aim of which is to examine various aspects of human nutritional status in relation to the water-soluble vitamins.

Applicants should have, or be expecting this summer, a first or upper second class honours degree, preferably in Biochemistry or Nutritional Science, and should send their curriculum vitae and names of two referees for (1) to Dr P. G. Lunn and (2) to Dr C. J. Bates, at the Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ, not later than June 30, 1974. (4)

KING'S COLLEGE, CAMBRIDGE FELLOWSHIP IN APPLIED PROBABILITY THEORY IN RELATION TO POPULATION BIOLOGY

Applications are invited for Fellowships in the above field, including population and human genetics, ecology, evolutionary theory, demography and stochastic diffusion processes to be held (from October 1975 or by arrangement) in the King's College Research Centre where computing facilities are available. Senior (age limit of 32) and Junior (age limit of 28 years and not more than about 3 years research experience) fellowships are available, together with an unrestricted fellowship which could be held by a senior worker on sabbatical leave. Maximum tenure, 4 years. Closing dates: **October 1, 1974**, for seniors and open; **November 15, 1974**, for juniors.

Salary: This is based on age and linked to the University lecturer scale. For an unmarried Fellow who is provided with free-accommodation in the College it ranges at present from about £1,500 at age 24 or below to £3,450 at age 36. Married Fellows receive £500 more, £625 if they have a dependent child or children.

Details of accommodation, research facilities, etc., from: The Convener, King's College Research Centre, King's College, Cambridge CB2 1ST, England. (11)

UNIVERSITY OF GLASGOW DEPARTMENT OF CELL BIOLOGY RESEARCH STUDENTSHIP

Applications are invited for a research studentship to work on the role of proteins in the structure and organisation of cell membranes. Candidates should expect to have a 1st or upper second class honours degree.

Applications, giving age, experience, and the names of two referees, to Dr A. J. Lawrence, Department of Cell Biology, University of Glasgow, Glasgow G11 6NU.

In reply please quote Ref. No. 3498M. (17)

UNIVERSITY OF GLASGOW

Applications are invited for a research studentship to work on aspects of bacterial electron transport or nitrogen fixation. Candidates should have, or expect to obtain this year, a first or upper second class Honours degree in an appropriate subject, and the successful applicant will be expected to register for a Ph.D.

Applications stating age, qualifications, experience, and the names of two academic referees, should be sent as soon as possible to Dr R. M. Daniel, Department of Cell Biology, University of Glasgow, Glasgow G11 6NU.

In reply please quote Ref. No. 3491M (18)

UNIVERSITY OF KENT AT CANTERBURY Studentships in Chemistry

Applications are invited for the following three SRC (C.A.S.E.) Studentships.

(1) To work with Drs A. V. Chadwick and J. D. Wright, in collaboration with the Safety in Mines Research Establishment (Dept. of Energy), on the effect of gases on electrical properties of semi-conducting molecular crystals.

(2) and (3) To work with Prof E. F. Caldin and Dr B. H. Robinson, in collaboration with two major oil company research establishments, on the properties and applications of micelles formed in non-aqueous solvents, using fast-reaction techniques.

Applicants should have good Honours degrees in Chemistry, Chemical Physics, Physics (1) or Biophysics (2) and (3).

Applications should be made to the Assistant Registrar, Chemical Laboratory, The University, Canterbury, Kent, CT2 7NH., from whom further details may be obtained. Closing date July 26, 1974. Please quote ref. PG 7/74. (35)

FELLOWSHIPS AND STUDENTSHIPS

LEUKAEMIA RESEARCH FUND RESEARCH FELLOWSHIPS

The Leukaemia Research Fund invites applications by August 2, 1974 from suitably qualified graduates, medical or non-medical, for a Fellowship in leukaemia research in a field of special interest to the Fund, tenable in France for one year in association with I.N.S.E.R.M. A second year's support in Britain is also offered. The salary in France will be up to a maximum of 61,500 French francs per annum. Applications, with the names of two referees, to the Secretary, Medical Advisory Panel, Leukaemia Research Fund, 61 Great Ormond Street, London WC1N 3JJ. (88)

WATER POLLUTION STUDIES

Imperial College
POSTGRADUATE
STUDENTSHIP

Applications are invited from suitably qualified graduates in Biological Sciences, for an S.R.C. Postgraduate Studentship tenable in the Public Health Engineering Laboratory.

The Research project which the successful candidate will undertake comprises part of a programme of bacteriological studies intended to obtain fundamental information about the ecology of the bacterial populations involved in sewage treatment.

Applications, enclosing a curriculum vitae, should be sent to:

**Dr R. Perry,
Public Health Engineering,
Imperial College,
London, SW7.**

Telephone: 01-589 5111 Ext. 1362.

(44)

DEPARTMENT OF PHYSICS University of Leicester RESEARCH STUDENTSHIPS IN X-RAY ASTRONOMY

Two S.R.C. Studentships, for research leading to the Ph.D. degree, are available from October 1974 in the X-ray Astronomy Group. Research opportunities are expected to exist in the following areas:

- analysis and interpretation of data from the Group's sky survey and spectrometer-polarimeter experiments on the UK-5 X-ray satellite, due for launch in September;
- experimental work on cosmic X-ray rocket payloads concerned with the mapping and spectroscopy of galactic supernovae and close binary sources;
- experimental and theoretical study of the X-ray spectrum and structure of solar coronal active regions and flares;
- laboratory development of imaging devices and readout systems appropriate to future X-ray astronomy missions.

Applications are invited from students in Physics, Electrical Engineering or Mathematics who expect to obtain a good Honours Degree this summer and should be addressed to Professor K. A. Pounds at the above address. (16)

RESEARCH STUDENTSHIP

available for a graduate (Class I or 2i) in Biochemistry to participate in a programme of research on haemoglobin and other blood proteins of lower Primates. Apply to Professor N. A. Barnicot, Dept. of Anthropology, University College London, Gower Street, London WC1E 6BT. (40)

UNIVERSITY OF BATH SCHOOL OF BIOLOGICAL SCIENCES Studentship

Applications are invited from biochemists and microbiologists with a first or upper second degree for appointment to an SRC studentship for work on yeast membranes and vesicles. Further information can be obtained from Professor A. H. Rose, School of Biological Sciences, University of Bath, Claverton Down, Bath, BA2 7AY. (34)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY Department of Biochemistry

A Science Research Council postgraduate research studentship, is available either for work in an existing group on the synthesis and regulation of pyruvate carboxylase in thermophilic bacteria, or on some related topic in microbial biochemistry. Applications with the names of two referees should be sent to Professor A. A. Eddy, Department of Biochemistry, U.M.I.S.T., Manchester, M60 1QD. (67)



The Jack Charrington Memorial Fellowship 1974/75

Applications are invited for the award of The Jack Charrington Memorial Fellowship from British Subjects, normally resident in the U.K., who are associated with the Coal Industry or Coal Trades.

The object of the fellowship is to encourage the study of new developments associated with the coal industry or coal trades in other countries with special reference to the application of such developments in the United Kingdom. Applicants must nominate the new development and country in which they propose to carry out the study.

The holder of the fellowship will be granted a stipend of up to £5,000 for a year's tenure or proportionately less for a shorter study. In addition travelling expenses will be met. Part of the tenure will be spent in writing a report.

Applications with full curriculum vitae, a synopsis of the new development and country in which they propose to undertake the study should be submitted by July 31, 1974, to the Secretary, The British Coal Utilisation Research Association Limited, Dept. N.I., C/O National Coal Board, Coal Research Establishment, Stoke Orchard, Nr. Cheltenham, Glos. GL52 4RZ. (33)

Research Studentship

at the Institute of Marine Biochemistry,
Aberdeen

Applications are invited from graduates with a first of upper-second class Honours degree in biochemistry for a research studentship (3 years) provided by the Natural Environment Research Council. The work will be on the biochemistry of cellular and humoral immune mechanisms in marine invertebrates and will probably centre around the nature and function of constitutive glycoproteins. Some collaboration with bacteriologists and virologists with marine interests is envisaged. The successful applicant will be expected to register as a PhD student with the University of Aberdeen and will receive a basic grant, currently £695 per annum, plus approved fees.

Applications to:

**Dr P. T. Grant,
Institute of Marine Biochemistry,
St Fittick's Road,
Aberdeen AB1 3RA.**

(46)

UNIVERSITY OF MANCHESTER DEPARTMENT OF CHEMISTRY POSTGRADUATE STUDENTSHIP

Applications are invited for an S.R.C.—C.A.S.E. studentship to work on the mechanism of chelation and demetallisation of metal complexes with Dr. J. A. Connor and in collaboration with Dr. R. Price of I.C.I. Organics Division. The project will involve both preparative organic and inorganic chemistry together with the application of physical methods of measurement in the elucidation of a problem which is of basic importance to transition metal chemistry and of significance to the dyestuffs and metallurgical industries. Applicants should write as soon as possible to Dr. J. A. Connor, Department of Chemistry, University of Manchester, Manchester M13 9PL. (54)

THE UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for a research studentship to work on chemical modifications of B-lactam antibiotics. The work is supported by a grant from Beecham Research Laboratories and the studentship (£695 p.a. plus fees) is for three years. The successful candidate, who must have a good Honours Degree in Chemistry or an equivalent qualification, will be expected to study for the Ph.D. Degree.

Applications, naming two referees, should be sent to Dr. R. P.J. Stoodley, Department of Organic Chemistry, The University, Newcastle upon Tyne NR1 7RU, as soon as possible. (57)

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Science Research Council Studentships are available in the Polytechnic for research in topics selected from the following:

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Computer Animation of Non-Rigid Objects
Ironmaking in the Blast Furnace
Control and Instability of Fluid Distribution Systems
Algebraic Semigroups and mappings of S-sets

Candidates for these studentships should hold, or expect to be awarded, an upper second class honours degree in an appropriate branch of science or engineering, or an equivalent qualification.

Further details and application forms are available from the Staffing Section, Department N, Teesside Polytechnic, Borough Road, Middlesbrough, Teesside TS1 3BA and should be returned within 14 days. (38)

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Candidates should possess a good degree in Microbiology or Biochemistry. Applications, together with the names and addresses of two referees, should be forwarded to Professor D. Hughes, Department of Microbiology, University College, P.O. Box 97, Cardiff CF1 1XP. Please quote ref. 0599. (28)

FELLOWSHIP IN SALMON
DISEASES

Applications are invited by the Atlantic Salmon Research Trust for a Research Fellow to be based at the Farran Laboratory of the Salmon Research Trust of Ireland, Newport, Co. Mayo, Ireland, in association with the Unit of Aquatic Pathobiology of the University of Stirling, with whom the successful candidate will be expected to register for a Ph.D. Application forms and conditions of service are available from the Director, Atlantic Salmon Research Trust Ltd., 29 South Street, Farnham, Surrey. Completed application forms to be received by July 31. (90)

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Applications are invited from graduates in pharmacology or related subjects for a three-year research post at the postdoctoral level, supported by The Nuffield Foundation. The project concerns the rôle of genetic variation in the ability to metabolise foreign substances in the mouse. Starting date October or November 1974. Technical help will be available.

Salary £2,091 to £2,220 to £2,385 p.a. Plus F.S.S.U. Applications, with curriculum vitae and two referees, to Dr. I. E. Lush, Department of Biology, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. (94)

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A research studentship of NERC which involves working for a Cambridge Ph.D. is offered for radio echo studies of polar ice sheets. Field work involves extensive sounding flights over Antarctica in Hercules aircraft from the USA. General and computer analyses of results and development of equipment is carried out in Cambridge. Requirements: II.1 degree in physics, geophysics, electronics or engineering. These could be concerned with electromagnetic propagation in or properties, flow, etc., of ice sheets or ice shelves, sub ice geology and/or associated geophysical problems.

Please contact Dr Robin, Director, SPRI, Cambridge CB2 1ER. Telephone 0223-66499 ext 400. (104)

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Two Co-operative Awards in Science and Engineering, will be available from October 1974 for 3 years. Applications are invited from graduates with a good honours degree.

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Applicants should hold an honours degree or be about to complete an honours course in Biochemistry or Chemistry, to carry out research on protein chemistry of collagen with particular reference to the chemical nature of stability of the crosslinks stabilising the collagen fibres of various tissues used in gelatin manufacture. The project, which will be undertaken in collaboration with the Gelatine Division of the British Food Manufacturing Industries Research Association, will be supervised by Dr A. J. Bailey.

2. Meat Research Institute—Research Studentship

A good honours degree and background in physiology/biochemistry/pharmacology, would be advantageous to join a multidisciplinary research team concerned with the physiology of growth and development, examining lipid metabolism and the effects of lipolytic and autolipolytic agents in farm animals. The work is expected to form a basis of a PhD thesis registered at University of Bristol. It will be carried out in collaboration with May & Baker Ltd at their Veterinary Research Division at Ongar, Essex and supervised by Dr D. Lister.

Further particulars of award and allowances, together with a form of application from:

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The value of the Studentship, which is for two years and may be extended to a third year, will be not less than £700 per annum, exclusive of fees. Candidates must register for a higher degree. Closing date for applications 30th September 1974.

Further details and application form may be obtained from:- The Academic Registrar, The University, Southampton SO9 5NH. (56)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL SCIENCES
Applications are invited from graduates with a final or upper second class honours degree in Microbiology or in Botany or Biochemistry with subsidiary Microbiology for a

NERC RESEARCH STUDENTSHIP

to be held for up to three years in the above Department for work under Dr. C. M. Brown on bacterial interactions in an estuarine environment.

Applications, naming two referees, should be sent to Dr. Brown at the Department of Biological Science, The University, DUNDEE DD1 4HN as soon as possible. (66)

UNIVERSITY OF BRISTOL
Department of Anatomy
POSTDOCTORAL RESEARCH
FELLOWSHIP

Applications are invited for a Postdoctorate Fellowship, sponsored by the Medical Research Council, to work in collaboration with Dr D. W. Lincoln and Dr Carol A. Mason on the electrophysiology of the neurosecretory nuclei of the rat hypothalamus, with a view to understanding the mechanisms that determine the pulsatile release of oxytocin in milk ejection and labour. Previous experience of intra- and extracellular recording from single neurones and the computerised analysis of this data would be useful, though not essential.

The appointment is for one year from September 1, 1974, renewable for two further years. Initial salary up to £2,247 p.a.

Applications giving details of age, qualifications and experience, together with the names of two referees, should be sent by July 20 to Dr D. W. Lincoln, Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD, from whom further particulars are available. (37)

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September 9-14, 1974

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The Secretary,
School of Environmental Sciences,
Plymouth Polytechnic,
PLYMOUTH PL4 8AA.

(36)

MEETING

NUDE MICE—THEIR HUSBANDRY
AND USE

An informal one-day meeting on the husbandry and use of the Nude mouse will be held at the Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey, SM5 4EF (Tel: 01-643 8000) on Tuesday, July 16, 1974. Applications to attend this meeting (for which there will be no charge) are invited from interested research workers. Numbers will be restricted to a total of 40 people. Copies of the programme are available from Dr. M. Festing at the above address. (13)

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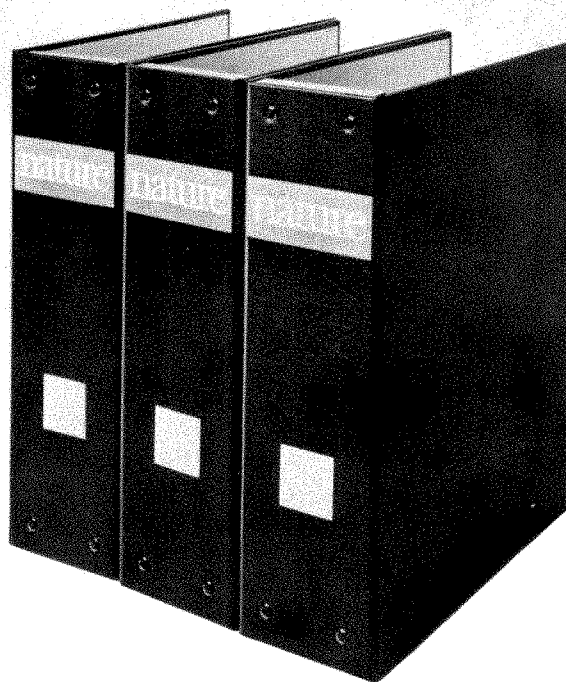
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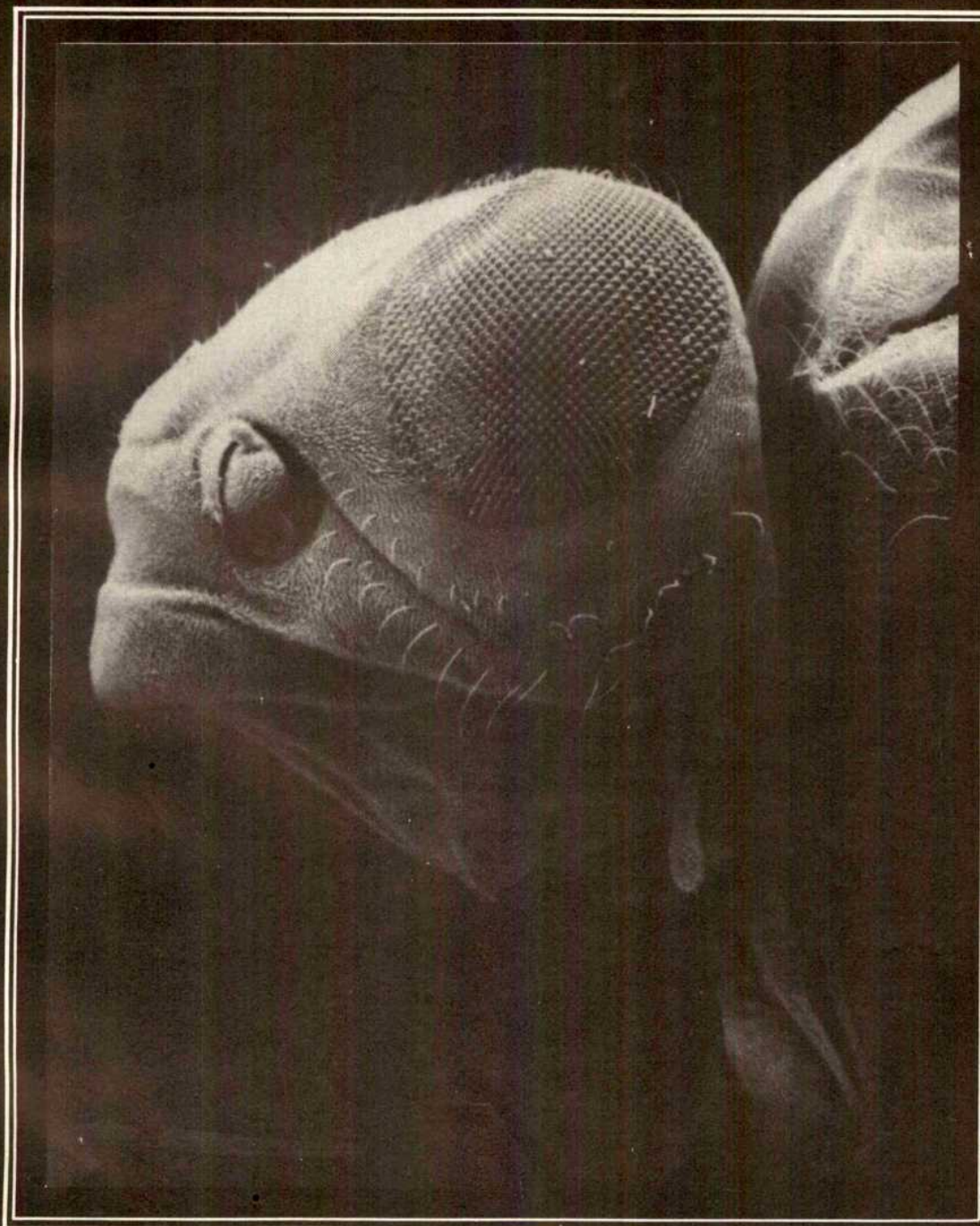
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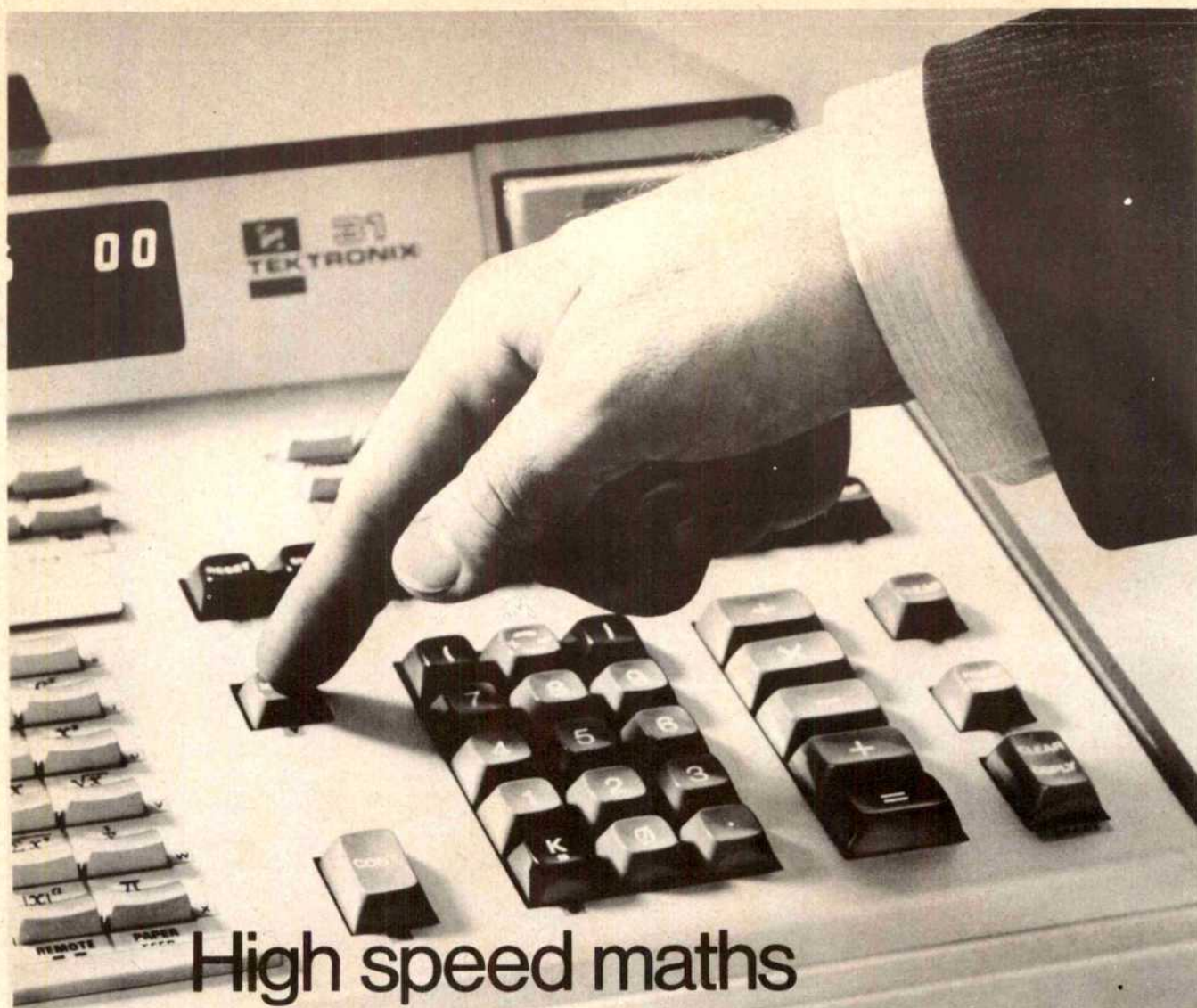


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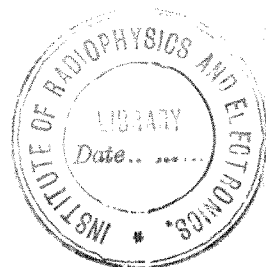
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Cover picture

A stereoscan electron micrograph of the head of a breach fly of the *Lipochaeta* genus. Their strange behaviour has led to a study, reported on page 167, which shows they are adapted to an unusual form of feeding.

nature



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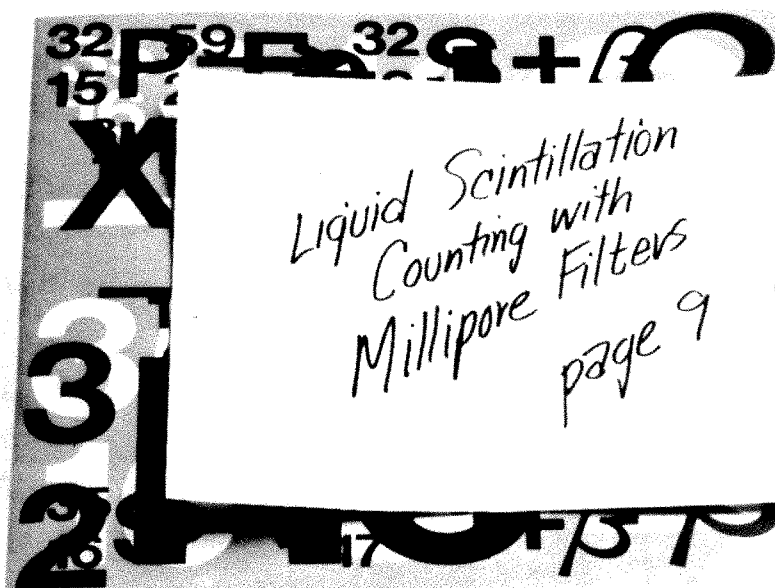
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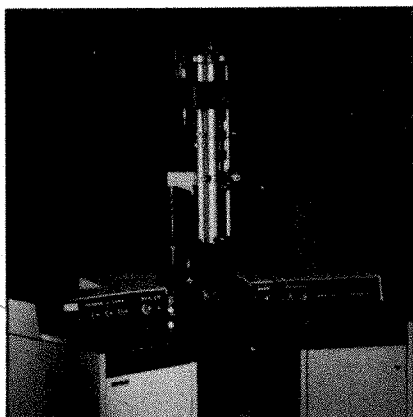
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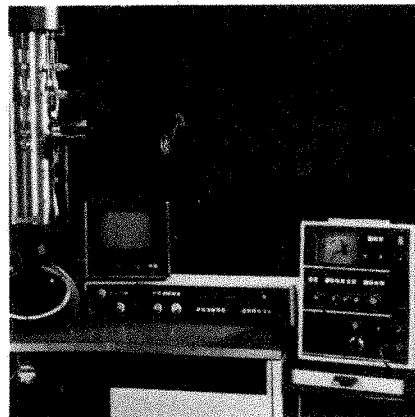
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Electron Optics Department
Eindhoven - The Netherlands



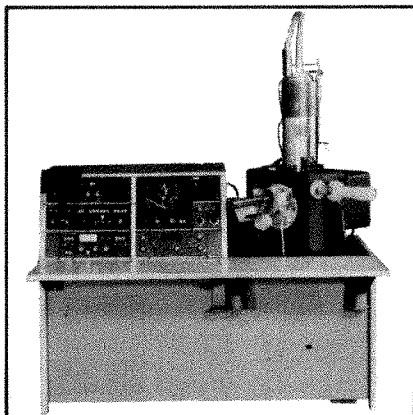
Above
EM 301G with energy dispersive X-ray analyser



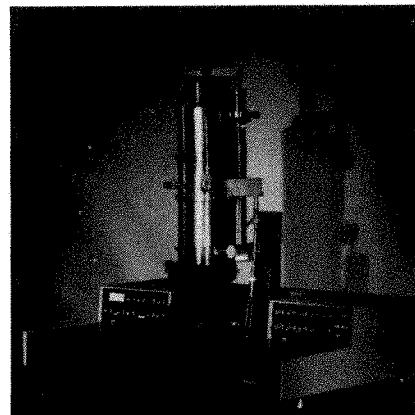
Above centre
EM 301S advanced research electron microscope



Above right
EM 301 with S(T)EM unit gives choice of TEM, STEM and SEM images



Right
PSEM 500 high performance scanning electron microscope



Far right
EM 201C high resolution (3.5 Å) electron microscope

Tomorrow's electron optics... today!



PHILIPS

Hedging & fence-sitting from the Think Tank

ONE of the prime skills of the politician is to find the right words to ensure that nothing is given away during debate. The academic politician, canniest of them all, is usually able to find the most eloquent way of keeping open all options, and even in response to the most mundane question will unearth a convoluted answer. "Will all the delegates be coming in to dinner?" the Master of a Cambridge college was asked recently. "Some . . . but perhaps by no means all" was his reply. The Think Tank's latest offering, *Energy Conservation* (HMSO, £1.00) is replete with such generalised High Table fence-sitting.

The Central Policy Review Staff (CPRS) set itself—at least nowhere is there any indication that the idea sprang from elsewhere—the task of producing a document for public discussion on how energy conservation might be achieved. A broad interpretation was put on the words 'energy conservation' and the CPRS included an analysis of methods of using replenishable forms of energy as a means of conserving fossil-fuel-generated energy. We reported last week the outlines of the report. What concerns us here is whether the CPRS does a good job when venturing into such fields and whether energy conservation is an appropriate matter for the CPRS to study in any case.

The most striking feature of the report to a scientist is the utter lack of any substantiating material for all that is said. In the report's 64 pages it roams widely over material as varied as domestic insulation and energy from sea waves. The authors (anonymous, of course) have clearly done a lot of reading and presumably have also been to see many of the scientists and technologists whose work forms the basis of the report's conclusions. Yet nowhere is there a single reference to published work, nor to a conversation with anyone. As a result it is extraordinarily difficult for this document to be a vehicle for public discussion. Beautifully manicured paragraphs that would have delighted Gowers for their style and clarity are no substitute for well documented arguments.

Consider, for instance, the Severn barrage scheme. It is described and dismissed in two paragraphs. "Significant environmental benefits are claimed by proponents [here follows a list of benefits] . . . They are not, however, easy to substantiate and would not appear to transform an apparently uneconomic project into an economic one . . . The long term ecological effects are very uncertain." This may be an elegant way of covering a lot of ground but it leaves the reader with too many unanswered questions—who are the proponents, why are the benefits not easy to substantiate, who is concerned with long term ecology? Or again in discussing energy storage schemes, " . . . nevertheless, the widening

differential between nuclear and fossil fuel costs in favour of the former may prove sufficient to make at least some of these schemes economic." Some, but perhaps by no means all.

The central question, however, is whether the CPRS should be doing this sort of study at all. There are various spurious arguments that can be raised against its involvement, such as that the staff cannot possibly be technically qualified or that the subject is more properly a departmental matter. If a Think Tank is a good thing, and we believe it to be so, then it must certainly be allowed to do what it wants and to risk offending the specialist departments. What it must not do, for its own sake, let alone that of taxpayers, is to bite off more than it can chew. This it has palpably done in this case.

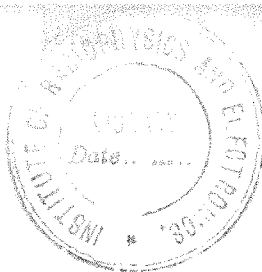
Not only is the subject of energy conservation colossal in extent, but the CPRS has expanded it even more by discussing the economy of the inexhaustible resources. With such a wide field it is almost inevitable that there will be no incisive thinking, since the staff, desiring to be comprehensive, can have had little time to develop expertise in individual areas. The overall impression that the report gives is of diligent reading—and there has been ample material to read in the last year—but no very profound thinking. The document bears no marks of being the subject of intensive discussions amongst the staff; indeed it seems instead the sort of thing that an academic might produce during a sabbatical year. This, surely, is not what the Think Tank should be all about. If, in the future, the CPRS does not want to get a reputation amongst scientists for vapid thinking it will have to concentrate its attention much more.

100 years ago



If anyone wants to see how lamentable is the absence of practical work in the examination system of the University of London, let him get "Questions in Chemistry and Natural Philosophy given at the Matriculation Examination of the University of London from the year 1864 to June 1873, classified according to the syllabus of subjects," by C. J. Woodward, B.Sc. (Simpkin, Marshall, & Co.) We say nothing against the book itself, which is a creditable compilation of its kind, but the system capable of giving birth to such a text-book must be an unmitigated encouragement to "Cram."

From *Nature*, 10, 215, July 16, 1874.



Gamma-ray astronomy: the last observational frontier

ON June 10, 11 and 12 a symposium on "The Context and Status of Gamma Ray Astronomy" was held at the ESRIN laboratory in Frascati. The rationale behind such a symposium under the ESRO banner at the present time derives partially from the forthcoming launch of COS-B (see *Nature*, **249**, 398; 1974); but quite apart from this slightly parochial incentive it turned out that gamma-ray astronomy has indeed reached something of a landmark recently, and is now likely to fulfil some of the promise which it has held for so long.

Certainly throughout the 1970s we have heard repeatedly that the big breakthrough in gamma-ray astronomy 'is about to be made'. But the difficulties of first detecting any gamma-rays from outside the Solar System and secondly deciding from exactly which direction they are coming have made some of the premature claims ring rather hollow in recent years. One result of these difficulties has been a considerable broadening of the accepted definition of gamma rays in this context; some of the data presented at Frascati would have been equally well suited to a symposium about X-ray astronomy. Such definitions are, however, intrinsically arbitrary, and as long as the active observers know what they mean by gamma rays no harm is likely to result from a broadening of the definition.

Ironically, in spite of the intensive efforts made to detect gamma ray events from outside the Solar System the most impressive data gathered so far have come from a series of satellites designed to monitor events on Earth. These, of course, are the Vela satellites, which are intended to detect radiation from nuclear explosions on Earth. Over the past four years, these satellites have not had the opportunity to discover many terrestrial nuclear explosions; but the same laws of physics apply to nuclear explosions elsewhere in space, where bursts of gamma rays are produced. As a result, the various Vela satellites have marked all of the 30 or so gamma ray bursts which have been recorded so far. And in only a couple of cases was it necessary to

A recent symposium provided an insight not only into the present status of gamma-ray astronomy, but also into the conventions of scientific symposia, as John Gribbin reports.

examine the Vela data in the light of evidence gained from other satellites before the evidence of the bursts became clear.

So gamma-ray astronomy can definitely be said to have cleared the first hurdle, of detecting something definite. The second problem has also been at least partially resolved since the proliferation of satellites equipped to detect gamma-ray bursts has made direction finding, by a kind of astronomical triangulation, a feasible proposition. And this, more or less, is where the Frascati symposium comes in. Other topics were also discussed, covering the gamma-ray background, low energy gamma-ray astronomy, and galactic emission. But to an observer from outside the gamma-ray astronomy family, the bursts and their interpretation provided the dominant interest.

I. B. Strong (Los Alamos) reviewed the observational data concerning the bursts, and other contributors elaborated on specific measurements before F. Pacini (Frascati) reviewed theories relating to their origin. In both branches of the investigation, the pioneering nature of gamma-ray astronomy was clearly apparent—but the effect of this on experimentalists and theoreticians differed greatly.

In a very real sense, gamma-ray astronomy is the last frontier of observational astronomy. It is now possible to observe every part of the electromagnetic spectrum, and the gamma-ray region is the last for which practical techniques have been developed. The observers, many of whom have a background in particle physics rather than in astronomy, seem somewhat overawed by this, and perhaps by the long struggle they have had to get hold of any worthwhile data. The result is that each observation seems to

be exhaustively analysed and picked over for significance—and the danger, of course, is that too much might thereby be read into what may well be atypical, or even incorrect, data.

The theoreticians, however, have fewer inhibitions. Indeed, a great many theoretical astronomers delight in a situation where there is just enough evidence to make model building worthwhile, but not enough to prove that their favoured model is incorrect. The study of gamma-ray bursts today provides a happy hunting ground for such theoreticians.

The fondness of the observers for smothering detail extended even to the presentation of their contributions at the conference. In the most extreme cases, we were told openly that although only a short paper was being presented, the version in the published proceedings of the conference would be much more 'complete'. That hardly seems fair on participants (some of whom had come from the United States and Japan), let alone on the humble reporter. In addition, many of the observations seemed essentially to duplicate one another, so that we had a stream of speakers standing up and saying much the same things about various events observed by different satellites, and showing graphs which seemed to be essentially interchangeable, allowing for the largish error bars.

It this kind of presentation really necessary? Clearly, each group must present its results and have them published in the proceedings of such a key conference, or the holders of the purse strings will want to know what is going on. But if each group had provided a summary of its data, circulated to participants in advance, and one or two speakers had outlined the implications then I feel sure that the symposium would have been just as fruitful, and perhaps a lot less tedious in parts.

Much the same could be said about most scientific symposia. But in the case of gamma-ray bursts such a streamlining of the proceedings would have been particularly apt, since only about three things are known about the bursts. First, they occur about seven times a year. Second, they are

very sharply defined in time with perhaps a double structure. And third, on the basis of 12 direction measurements, their distribution seems isotropic.

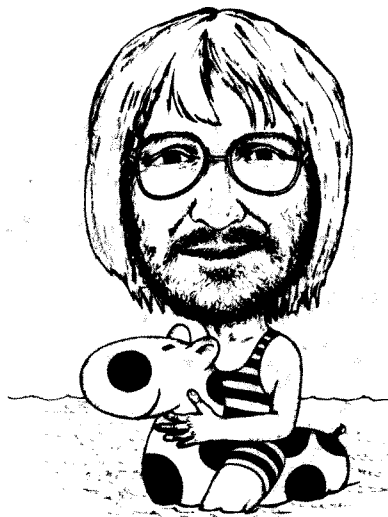
Of course, much more in the way of detailed measurements is available, and was presented. But that is just about all the evidence on which the theoreticians can build their models. Nothing daunted, the theoreticians have proceeded to do just that, as Pacini described.

The isotropy of the burst distribution provides vital information. Either the bursts originate nearby (a few hundred parsecs from the Sun), or they must be extragalactic. The intermediate case, of a distribution over a large part of our Galaxy, would reveal structure related to the structure of the Galaxy. With that proviso, Pacini happily listed the models which have been proposed: radiation from relativistic dust grains entering the Solar System; comets falling into collapsed stars; traces of "defunct" pulsars; superflares on stars; supernovae in external galaxies; birth of neutron stars; and the "first manifestation" of radio outbursts in external galaxies.

It does not really matter which, if any, of these models may turn out to be correct. What is significant is that theoreticians are prepared to toss such ideas into the melting pot, throwing them out or revising them as observations require. The observers, on the other hand, seem to wish to build complete detailed models on the basis of incomplete data, while justifying the procedure with a barrage of statistics (not always valid for small samples) and the interchangeable, large-error graphs which I have already mentioned.

Perhaps this is a result of the lack, in some cases, of astronomical training. With experiments which simply cannot be repeated and the everyday hazards of balloon and rocket observations, something more of the pioneering attitude seems to be needed in this last frontier of observational astronomy.

But on the other hand, this earnestness among the observers is really only manifested when they get up on their hind legs to address a gathering of



Glaxo awards

JOHN Gribbin, of *Nature*, is among the four recipients of Glaxo Travelling Fellowships for science journalists announced on July 9. His award, in the National category, is chiefly for a series of articles on the significance of climatic change; much of this work has appeared in *The Times Science Report* and longer articles appeared in *Nature*, *New Scientist* and *Environment and Change* during 1973.

The awards, which are sponsored by Glaxo and administered in collaboration with the Association of British Science Writers, are in the form of £500 travel grants; John Gribbin will be using his award to travel to the United States and Canada during the autumn, and will be reporting for *Nature* on research into climatic

John Gribbin (left) lives by the sea at Brighton.

change being carried out there.

John Gribbin joined the staff of *Nature* in October 1970, after completing research for a PhD in astrophysics at the University of Cambridge. In that year, he received the First Award of the Gravity Research Foundation of New Boston for work on "Using Gravity to Determine the Nature of Superluminous Astronomical Objects"; for most of the past four years he has been in charge of the *Nature-Times News Service*, which reports on developments in all the sciences. As well as the interest in climatic change which has led to this award, John Gribbin is concerned about the application of science to other global problems, and is co-author of a book on earthquakes and earthquake prediction (*The Jupiter Effect*) which is to be published by Macmillan in September.

Fellowships in the other three categories (Radio and Television; Regional; and Trade, Technical and House Magazine) go to David Wilson (Science Correspondent of BBC Television News), Judith Hann, a freelance science journalist, and Geoffrey Watts, Deputy Editor of *World Medicine*. Miss Hann is the first person to win a Glaxo Fellowship for the second time; the first occasion was in 1967.

Last year's award winners included John Maddox, then Editor of *Nature*, in the National category.

their peers. Individually or in small groups (especially after lubrication with the local Frascati wine) they appeared as intelligent people with the sense of humour needed to cope with the rigours of their trade. In some cases, they even agreed that their data were really rather vague and open to other interpretations, at least in detail.

The symptoms are, indeed, very similar to those severe cases of jargonese which result when many people try to write a "scientific paper". In the long term, the answer to both problems must lie in a basic change of attitude in the direction of clarity and honesty of communication as opposed to cunning packaging which can only be interpreted by the initiated.

In the short term, since almost every scientist is willing to communicate as a human being on a face to face basis, saving the 'scientist' mask for lecturing,

a solution might be to cut down on formal presentations at symposia, in favour of circulation of papers together with ample opportunity for informal discussion. At Frascati, more than 50 talks were on the official timetable (covering 2½ working days) and more were crammed in at short notice. Apart from the obvious benefits of a trip to Italy in June, it is difficult to see how the participants would have been less well served simply by reading the papers. The advantages of a symposium should be personal contact and the opportunity to assess the abilities of colleagues in the same field who may work on another continent; it seems that the time has certainly come when a move away from the present system, in the direction of the original "drinking party" implications of the word "symposium" could well be a good thing.

international news

EVER since the United States Supreme Court ruled last year that abortion is essentially a private matter between a woman and her doctor, a coalition of organisations campaigning under the banner of the 'Right of Life' movement has been trying to make it again a criminal matter between a woman, her doctor and the courts. The prime objective of the campaign is to get Congress to pass an amendment to the constitution to "protect the life of the unborn", but the chief casualty so far, much to the consternation of many biomedical scientists, is research of human foetuses.

The list of restrictions which have been placed on such research is impressive. Congress last month passed a bill which imposes a four-month ban on research on living foetuses while a special commission will draw up ground rules under which foetal research should be funded. The National Institutes of Health (NIH), which claims that it does not support research on live, aborted foetuses anyway, is drafting regulations governing all research of human subjects, including foetuses. An amendment has been put into the authorisation bill for the National Science Foundation (NSF) forbidding that agency to support research on live foetuses. (Backers of the amendment were evidently unmoved by protestations that NSF has never been in the business of funding foetal research, and does not intend to be.) On June 26, a bill was signed into law in Massachusetts which makes it a criminal act for anybody to carry out research on living foetuses there. And finally, a law has been on the books in Illinois for a year now which forbids research on all "aborted tissue" in that state.

Aside from the Illinois law, which imposes a blanket ban on all foetal research, the restrictions and prohibitions apply chiefly to two areas of study. First, they rule out research on foetuses aborted during mid-pregnancy which are delivered with their hearts still beating but which are insufficiently developed to remain alive for more than a few seconds or minutes. And second, they ban most research projects on foetuses while they are still in the womb. It is the latter area which is at the centre of most of the disputes, for research on foetuses *in vivo* has already produced some shortsighted and ill-founded conclusions.

At the federal level, the issue of

Foetal research aborted in United States

by Colin Norman, Washington

foetal research burst into the public spotlight last year following newspaper reports that NIH was drawing up guidelines for research on living foetuses. The day after the report was published, Dr Robert Berliner, then deputy NIH director for science, told about 200 Catholic high school students who had descended on the NIH campus in protest that "we know of no circumstances at present or in the foreseeable future which would justify NIH support of research on live, aborted human foetuses." He added that no such research was then being supported.

Nevertheless, anti-abortionists in Congress, led by representative Angelo Roncallo, a republican from New York, proposed a series of amendments to various bills which would have ruled out entirely all federal support for research on living foetuses. It was largely to head off such restrictive measures that a provision which imposes a four-month ban on such research was put into a bill dealing with biomedical ethics. The bill, which cleared Congress late last month, simply states that the Secretary of Health, Education and Welfare cannot support research on a living human foetus, "unless such research is done for the purpose of assuring the survival of such foetus". While the ban is in effect, a special Biomedical Ethics Commission will draw up rules governing such research.

As for the Massachusetts law, its implications were neatly summed up last week by Dr Elizabeth D. Hay, Professor of Embryology at Harvard and past president of the Society for Developmental Biology, who pointed out that "the federal ban cuts off funding for foetal research, but the (Massachusetts) state law will put you in prison for up to five years".

Sponsored by William Delahunt, a young state legislator, and vocally backed by the Boston Citizens' Committee for the right to life, the original bill would have ruled out most foetal research in Massachusetts, but it quickly fell foul of the Boston scientific community, which ran an effective

lobbying campaign to make the measure less restrictive. In its final form, the bill prohibits research in Massachusetts on live human foetuses, before or after abortion, but it also contains two important exemptions. First, research will be allowed if it has potential therapeutic value to the mother or the foetus and, second, it does not ban diagnostic tissue from dead foetuses, provided that the mother's consent has been obtained.

Asked for their opinions on the various federal prohibitions and the Massachusetts law last week, a number of biomedical scientists offered two chief observations. The first is that foetal research has provided many very significant findings, and a good deal of direct benefit to public health. One piece of research which is often cited in that regard is the growing of polio virus in cells cultured from foetal tissue, for which Dr John Enders and Dr Thomas Weller won the Nobel Prize in 1965. The second is that it is inadvisable to legislate an overall ban on any area of scientific research—such matters are best dealt with by ethical review committees which, according to federal regulations, must be established to guide research in institutions which receive biomedical research funds from the government.

What, exactly, is going to be ruled out by the various restrictions? The first thing to be said is that the ban on research on foetuses whose hearts are beating after they have been aborted will have little effect in the United States because there is very little of that kind of research going on there. For one thing, the usual method of abortion during the second trimester is saline injection which usually kills the foetus. Some research on foetal circulation and on the development of the immune system has, however, been carried out by American scientists in Scandinavia, where the preferred method of abortion during later stages of pregnancy is Caesarean section, and the federal ban will clearly rule out all NIH funding of such studies.

According to Dr David Nathan, a scientist at Boston's Childrens' Hospital, and a leading opponent of the Massachusetts legislation, the chief result of the various prohibitions will be "two serious losses, one in the area of foetal pharmacology, and the other in the screening of teratogens and dangerous drugs", both of which will be ruled out by the bans on research

THE launching of the space station Salyut 3 on June 24, 1974, and the successful docking with it of Soyuz 14 and the transfer of the two-man crew on July 5, marks a resumption of the Soviet programme of manned orbital space stations which was tragically interrupted in June, 1971 with the death of the three-man crew of Salyut 1 during re-entry.

In the three year moratorium, modifications have been made both to the Salyut station itself, and to the Soyuz programme which supports it. The re-designed Salyut was officially flight-tested (unmanned) during April 1973. This craft incorporated modifications to the solar panels (three panels rotatable through 180°, instead of the previous four) and to the optical ground-viewing systems. In addition to this "official" test (Salyut 2), other craft of the series have almost certainly been tested, unnamed, under the cover-all of the Kosmos programme.

The Soyuz, too, was almost certainly tested out as a number of Kosmos satellites (numbers 496, 573, 613, in particular, are widely considered to have been unmanned Soyuz craft) before manned testing was resumed with Soyuz 12 (launched September 29, 1973—a two-day flight) and Soyuz 13 (launched December 18, 1973—an eight-day flight).

The principal modification to the resumed Soyuz programme was the use of a two-man, rather than a three-man crew. In view of the cause of the Salyut 1 tragedy—the failure of a seal and loss



Cosmonauts Popovich and Artyukhin reporting ready for take-off on July 4th.

of cabin pressure during re-entry, this is almost certainly intended as an additional safety precaution, providing the additional space needed for the crew to wear spacesuits during re-entry. This policy is continued with the latest flight, and it is significant that the *Pravda* picture of cosmonauts Pavel Popovich and Yuri Artyukhin, apparently inside the Soyuz cabin, shows them in space suits.

The official aims of the test according to the Tass reports are: "Further elaboration of the improved construction of the station and also of the on-board systems and apparatus, and the conducting of scientific and technological investigations and experiments in cosmic flight". This includes observa-

tions of geomorphological features of the Earth's surface and atmospheric phenomena, investigation of the physical parameters of space, and medical and biological studies of the effect of "the factors of space flight on the human organism and the 'determination of rational regimes of the crews working' on board."

This last aim seems to be of particular significance in view of the joint Apollo-Soyuz mission planned for next year. Although the official aims make no specific mention of this, the timing of the flight, to coincide with President Nixon's visit, is surely intended to imply that the Soviet preparations for the joint United States-USSR mission are well in hand.

on foetuses before induced abortion.

Specifically prohibited will be research projects which, for example, involve the administration of drugs or vaccines to a pregnant woman scheduled to undergo an abortion, in order to see whether they cross the placental barrier and affect the foetus. Such studies have, however, produced some extremely important findings.

A study carried out in Helsinki and reported in 1972, for example (Vaheri *et al.*, *New Engl. J. Med.*, **286**, 1071; 1972) showed that virus from rubella vaccine can cross the placenta and enter the foetus. The research, which consisted of vaccinating ten women before they had abortions and analysing the foetal tissue for signs of rubella virus, was described by a Harvard scientist last week as "fantastically important", since it provides very strong evidence against vaccinating women during early stages of pregnancy. The research was particularly significant since previous studies had indicated that the virus from the rubella vaccine does not cross to the

foetus through the placenta.

It is also worth pointing out that four doctors are now awaiting trial on criminal charges arising from a research project they conducted at Boston City Hospital in 1971 and 1972. The study consisted of administering antibiotics to 33 women about to undergo abortions to see which was the most effective in crossing the placenta. The idea was to find the best drug to use in place of penicillin to clear up foetal infections. But the four doctors were charged earlier this year with infringing an obscure 19th century Massachusetts law which was originally designed to prevent grave robbing. Although Delahunt said last week that his bill was in no way connected with those indictments, it is clear that if the grave robbing law is found ineffective in preventing such research projects the new Massachusetts law will certainly do the job.

Two chief arguments have been raised against such research, for one thing, it puts the mother who has consented to take part in the study in a

position in which it would be difficult to change her mind about having an abortion. And for another, it constitutes research on a human being (the foetus) without its consent, which is contrary to every code of biomedical ethics including the Nuremberg Code, which was promulgated in response to Nazi medical experiments. Such a view point rests, however, on the assumption that life begins at conception rather than at any other stage of foetal development—an issue which was deliberately left vague by the Supreme Court when it issued its historic abortion decision last year.

Dr Michael N. Oxman, a virologist at the Children's Hospital Medical Center in Boston, argues, however, that a ban on foetal studies *in vivo* "creates a situation where children are going to be put at risk rather than foetuses which are going to be aborted". He pointed out that if a vaccine or a drug administered during pregnancy crosses the placenta and harms the foetus, it would be better to know about it through research on

What that treaty means

LAST week Mr Nixon and Mr Brezhnev signed a treaty on the Limitation of Underground Nuclear Weapons Tests. It was probably the most substantial arms-control agreement to come from Mr Nixon's visit to Moscow and it bears some scrutiny.

- It is bilateral. There is no provision for any further country to attach itself to the treaty.

- After March 31, 1976 the yield of tests may not exceed 150 kilotons. The wording indicates that the United Kingdom, for instance, would not be allowed to test more than 150 kilotons on United States property.

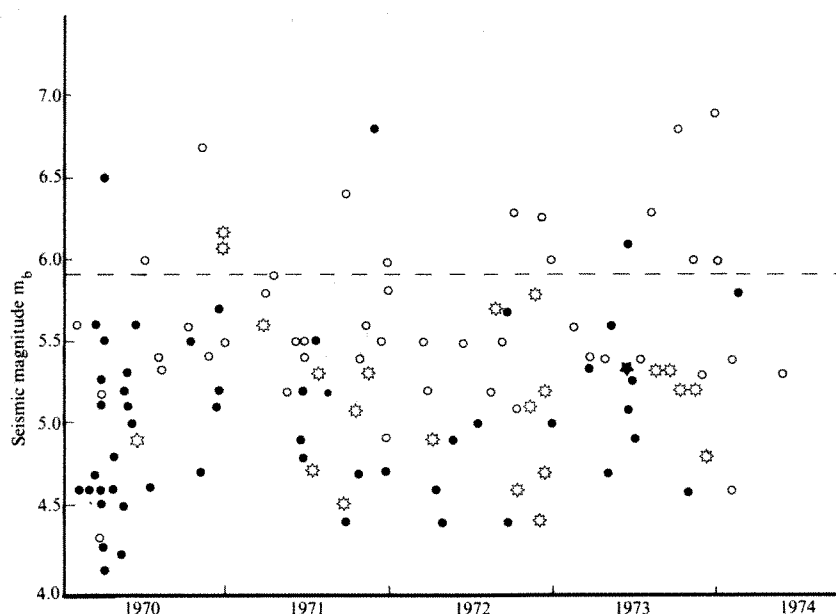
- 'National technical means' will be used for monitoring and neither party will interfere with the other party's means. This leaves much obscure, particularly the question of what could be regarded as interference. If a test of 200 kilotons is fired in an appropriate environment to reduce the apparent yield as seen by a seismometer to 150 kilotons, is this interference?

- Peaceful explosions are excluded from the ban; negotiations on these will continue.

- The treaty must be ratified.

- Data shall be exchanged on the geological and geophysical environment of present test sites and of any future site. A tall order for an area as complicated as southern Nevada.

- Locations of tests shall be exchanged after firing. But the United States, and doubtless the Soviet Union, test many devices much too small to be monitored by national technical means. Are these to be included?



Open symbols—Soviet tests. Filled symbols—U.S. tests. Circles—weapons tests. Stars—peaceful explosions. The dashed line roughly represents a yield of 150 ktons.

- Yields of two tests in each geographically distinct area will be exchanged for calibration purposes.

Reaction amongst those with a long-term interest in a test ban has been bitter. The treaty is blatantly bilateral—there is no provision that any of the data exchanged need get beyond Kremlin and Pentagon files. The extraordinary length of time before the treaty comes into force permits no end of significant tests to be performed at leisure. The threshold is much higher than anyone had believed possible. The figure shows testing

activities for the last four years. It is difficult to relate yield to seismic magnitude in a unique way, because of varying geological environments, but a value of 5.9 might roughly represent 150 kilotons. It is obvious how much testing will still be permissible. But the thing which most people are perturbed about is the way this treaty appears to close out, at least for the immediate future, any thought of a comprehensive ban. The United Nations Disarmament Conference in Geneva will hear some vigorous opinions from the other nuclear powers and the potential proliferators in the next two months.

foetuses about to be aborted rather than through damage to children.

As for the exemptions in the Massachusetts legislation, opinion is divided on how effective they will be in allowing potentially valuable research to take place. Nathan, who last week praised Delahunt's willingness to compromise on the bill, welcomes the provision which allows research on diagnostic techniques likely to be of value to either the mother or the foetus. The exemption will allow research aimed, for example, at developing a foetoscope capable of 'taking foetal samples for the diagnosis of such diseases as cystic fibrosis and Cooley's anaemia.

But other researchers have pointed out that many valuable techniques and research findings have been developed from research which at the time it was conducted had little potential benefit

for that particular pregnancy. The technique of amniocentesis—removal of fluid from the amniotic cavity—which has led to identification of more than 50 diseases before birth, for example, was used first to measure intrauterine pressure during labour, and would probably never have been developed under the terms of the Massachusetts law. That piece of research would definitely have been ruled out under the terms of the federal ban on foetal research since it was not aimed at "assuring the survival" of the foetus.

Finally, many researchers are concerned that restrictions in foetal research, particularly when they carry possible criminal penalties—the Massachusetts law makes live foetal research a crime punishable by 1–5 years in prison—will drive good scientists away

from important studies. Oxman said, for example that the Massachusetts bill "creates an unfortunate atmosphere in which a good researcher will decide to do something else. The loss could be serious."

Mark V marks time

The University of Manchester has announced "with regret" the decision by the Science Research Council that it will not be able to accommodate the cost of the proposed Mark VA telescope at Meiford, Wales, in its budget "over the next few years". Tenders for the 375-foot instrument indicate a cost of £16–£17 million; the SRC and the University will now have discussions in the near future about more modest possibilities for developing the progress of research at Jodrell Bank.

As a doctor and researcher in medicine and bioelectronics who has always stressed the human mission of medical science and the necessity for international cooperation, I am appealing to you for moral support in my apparently hopeless endeavour to return to active and creative scientific work. For four years now I have been refused all opportunities to work scientifically and professionally in my country and for this reason I applied in 1972 to our Ministry of Health for permission to work abroad. After a year's delay, permission was refused. I have recently sent a new application to the Ministry of the Interior asking for a long term permit to work at research institutes in the United States or West Germany; in the event of this permit being withheld, I have applied to emigrate.

I have been led to this decision by bitter experience with Czechoslovak officialdom and employers, and by the realisation that, as a citizen who in 1968 supported the humanistic reform of socialism undertaken under the leadership of A. Dubcek, I stand no chance in my own country of making any contribution to society according to my skills.

Following the change in leadership of the Czechoslovak Communist Party and the change in party policy, all kinds of repressive measures were, as is generally known, instituted against supporters of the democratic reforms. I suffered harsh reprisals in 1970, when the Minister of Health, without stating any grounds, ordered my immediate dismissal from the post of Director of the Research Institute for Electronics and Modelling in Medicine, the institute which I founded and built up. Then an unprecedented check-up was made in the institute in order to gather evidence against me concerning its economic management. The intentions underlying this action were obvious, and I would be willing to publish the evidence should circumstances demand. At the same time officers of State Security carried out an extensive investigation of many staff members of the institute, and I myself was subjected to repeated interrogation.

Since 1970, after dismissal from the directorship, I have not been allowed to engage in research, experimental or teaching work of any kind, and since 1971 I have even been banned from clinical work. In 1972, on returning from a spell of sick leave, I was not allowed to resume work at my place of employment, although such an order was in contravention of valid Czechoslovak law. I was also banned from publishing anything at all. Scientific papers in the press had to be thrown out, and my name had to be deleted from collective works or was deleted by the censors. Nor can my name

appear in literary references.

By degrees I was expelled from all Czechoslovak professional societies, from membership of the editorial boards of scientific and popular scientific journals, from the Collegium of Medical Sciences, from the Scientific Council of the Ministry of Health and from all technical commissions, and I was banned from lecturing at Prague and Brno universities and at the Prague Institute for Further Education of Doctors and Pharmacists. I am officially excluded from participating not only in conferences abroad but also in medical conferences and meetings at home.

For a full four years I have tried to find a place in society in accordance

**I have been subjected
not merely to social
discrimination but also
to a form of protracted
and total spiritual and
intellectual liquidation
against which I have
no defence**

This is the text of an appeal addressed to the World Federation of Scientific Workers by Bohumil Peleska, a Czech scientist

with my qualifications, I have paid dozens of visits to officials of the Communist Party, to political offices, and to government and public institutions, I have made dozens of proposals as to how I could be employed. All efforts have been in vain, and equally fruitless have been applications to employing organisations; they always cite orders from superior departmental and political bodies. No account was taken even of the fact that, in the course of twenty years of work in this field of research, I built up the Experimental Department of the Institute of Clinical and Experimental Surgery, that I founded and built up the Research Institute for Electronics and Modelling in Medicine, and that I was awarded by the President of the Republic in 1959 the Order of Merit for Services to Construction and in 1965 the Klement Gottwald State Prize for research, nor that I have published 150 scientific works.

The persecution culminated in 1974

when, in contravention of the Labour Code, I was dismissed from my employment under Article 46 on the grounds that I had committed a breach of the social order by my political attitude in 1968. Knowing that colleagues who had suffered dismissal under this Article were in financial straits and searching for jobs, I appealed against the dismissal, but my employers and the trade union refuse to rescind this illegal act, so that now I am not only barred from scientific and professional work—I am unemployed and have no prospects of being able to earn a living for my family, which includes three young children. That is how things are, although the Communist Party and the Czechoslovak government officially maintain that nobody can be made to suffer on account of their political views.

I have been subjected in recent years to a procedure which I consider to be not merely social discrimination but also a form of protracted and total spiritual and intellectual liquidation against which I have no defence at present. The feeling that the rights guaranteed by the Constitution and the Labour Code are inaccessible has convinced me that further efforts to find work in my profession would merely involve further loss of time and of the creative years of my life.

Having, for four years, been banned from scientific and professional activity, from publishing and teaching in the field of medicine and bioelectronics, having been illegally dismissed from my employment and having been repeatedly interrogated by State Security, I have lost the sense of legal and personal security; this has induced me to apply to the Czechoslovak authorities for a long term permit to work abroad, accompanied by my whole family, a step which I prefer to emigration. This demand is in accordance with the spirit of the Charter of Human Rights of the United Nations, on ratifying which the Czechoslovak government also undertook to observe and implement it.

As my previous application was rejected, I am approaching you, not only as an administrative body but also as the conscience of the world community of scientific workers; I ask for moral support in my endeavour to return to scientific life and creative work. Through the widespread and unselfish cooperation among scientists the world over, the fruits of medical research are part of the common cultural wealth of all mankind. Thus no power on Earth has the right to curb, prevent or forbid free scientific cooperation in medicine, or to isolate scientific workers and treat them as serfs merely because they wanted a little more freedom, but not nearly as much as they are entitled to as human beings.

correspondence

Synchrotron radiation facility

SIR,—After reading the article by John Gribbin (*Nature*, April 12) on the proposal for a new synchrotron radiation facility, I am wondering if he and I went to the same meeting. Certainly the meeting I attended did not show clearly that the proposal to build a new £2 million storage ring “needs careful rethinking” or that “the plan as originally envisaged seems unlikely to survive”. At the conclusion of the meeting, the physicists, chemists, biologists and metallurgists present made it overwhelmingly clear that they firmly supported the existing SRC plans.

The project is not at the early stages of planning. Indeed, the scientific case for a dedicated storage ring has been established over a considerable period of time, accepted by the Science Board of SRC and a design study costing £70,000 is already well under way.

Storage ring facilities being built in Hamburg and Paris are such that synchrotron radiation users will have limited access and remain parasitic. What is envisaged in the United Kingdom is a unique facility, with 10 access points and accommodating 30 experiments simultaneously. Many experiments which could not previously be performed due to lack of intensity now become feasible. New experiments utilising the polarisation properties of synchrotron radiation have been proposed, and the enormous flux in the 1 Å region will allow time resolution of milliseconds or better in the study of complex biological functions, such as muscle flexing. The storage ring is also expected to be a potent source for scattering experiments below 1 Å.

What the scientists present at Reading were extremely concerned about was the time scale involved in building a dedicated storage ring. The building of such a facility could in principle be started almost immediately because no major new technological advances are envisaged (I omit consideration about the “wigglers” required to reach the shorter wavelengths). It need not be built at Daresbury and therefore need not await the closure of NINA. This would ensure that the United Kingdom has its share of new and exciting physics. Is it realistic, however, to suggest such an alternative? The estimated cost of £2

million would raise, since a new building to house the storage ring, and all the services available in the NINA facility, would have to be provided. Incidentally, Professor Bleaney’s suggestion of a disastrous 5 year period between NINA closure and storage ring commissioning was refuted by Daresbury personnel present. A preliminary estimate is 12 to 18 months, given the level of funding shown in the SRC document. This could be reduced with sufficient manpower and money if SRC were to give whole-hearted support to the proposal.

It is now up to the SRC to act swiftly and decisively in this matter or the United Kingdom will once again find itself an also-ran in the race for scientific discovery. The critics have had their chance to comment on the proposal and no substantial argument was forthcoming. (For example, nobody has suggested that a tunable soft X-ray laser will turn such a facility into an under-used white elephant.) In the absence of such criticism, let us press ahead with a facility which will surely be the envy of synchrotron radiation users throughout the world.

Yours faithfully,

K. CODLING

J. J. Thomson Physical Laboratory,
Whiteknights, Reading RG6 2AF

We have also received another letter in similar vein from seventeen of those at the Reading meeting.—ED.

Penicillin

SIR,—I am grateful to Ernst Chain for his comments on the rediscovery of penicillin (*Nature*, June 14). There would, however, seem to be ample justification in the literature for the viewpoint I put forward in my earlier article (*Nature*, May 24) concerning the direction of research into antibiotics during wartime and the quest for agents of chemotherapy. Chain’s first joint paper in the field¹ refers to chemotherapy in title and substance, and his later joint publication that same year (on penicillinase) discusses the relative merits of penicillin as an agent of chemotherapy². The extensive paper on therapy using penicillin published some months later by Chain and the other members of the Oxford team³ expands on the theme. The effects of the war effort, to which I have referred elsewhere⁴ is repeatedly emphasised in Fleming’s classic volume⁵

(to which, apparently, the Oxford workers declined to contribute). Bacharach and Hems⁶ in particular, state that penicillin seemed to “merit much more attention than it had received”, a pointer to exactly the form of directed research I would like to see in oncology. It contrasts radically with Coghill’s comments⁷ that Raistrick, apparently the first to see the significance of antibiotics, “could get no clinical tests made”. Perhaps the essence of what we should say is that, though it is widely assumed that Fleming’s fortuitous discovery gave rise to the concept of antibiosis, the discovery of *Penicillium notatum*, and the first recorded examples of ‘microbial therapy’, it did not in fact originate any of these⁸. In this respect the research work of Howard Florey and Ernst Chain, with their coworkers, was of peerless importance.

Yours faithfully,

BRIAN J. FORD

Cardiff

¹ Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, ii, 226–228 (1940).

² Abraham, E. P., and Chain, E., *Nature*, **146**, 837 (1940).

³ Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G., and Jennings, M. A., *Lancet*, ii, 177–189 (1941).

⁴ Ford, Brian J., *History of the Second World War*, 2851–2857 (Purnell, London, 1968).

⁵ *Penicillin, its practical application* (edit. by Fleming, A.) (Butterworth, London, 1946).

⁶ Bacharach, A. L., and Hems, B. A., *ibid.*, 24–45.

⁷ Coghill, R. D., *Chem. Engng News*, **22**, 588.

⁸ Ford, Brian J., *The Revealing Lens: Mankind and the Microscope*, 130–135 (Harrap, London, 1973).

Wursted

SIR,—We are not in complete agreement with Neidle’s point (*Nature*, **249**, 212; 1974) that there is a cultural bias in the understanding of our “hotdog” model for repressor-operator interaction. The Germans, for example, might consider it the *Wurst* model of operator-repressor binding.

Yours faithfully,

T. A. STEITZ

B. ENGELMAN

Yale University,

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The briefer the letter the better its chance of being published. We reserve the right to cut correspondence.

news and views

Ophiolites and oceanic lithosphere

THE most obvious way of testing whether present-day ophiolites represent ancient oceanic crust and uppermost mantle is to compare the properties of such sequences with those of modern oceanic lithosphere. The scope here is wide; and comparisons have already been made in terms of geology, petrology, chemistry, magnetic properties and seismic velocities. Unfortunately, however, the results of these attempts at correlation have not been entirely conclusive because comparisons have proved to be less straightforward than had originally been hoped. No doubt it is easy to be wise after the event; but it is clear in retrospect that some of the, as yet incompletely fulfilled, hopes expressed in the early literature of the subject were based on overly-naïve expectations even if it must be admitted at the same time that some of the subsequent investigations have been afflicted by peculiarly bad luck. But be that as it may, the fact is that comparisons between ophiolite suites and modern ocean floor are likely to encounter some quite obvious difficulties even if it is assumed that present and past oceanic lithosphere were produced by comparable processes.

Problems arise partly because even if ophiolite sequences were submarine in formation they are now subaerial in fact; and so comparison with modern ocean floor means comparison between structures under quite different circumstances. The effects of differing conditions become quite evident when, for example, Matthews *et al.* (*Nature*, **231**, 200; 1971) measured seismic velocities in the outcrops of the Troodos Massif of Cyprus in the hope that they would be similar to the velocities in the layers of the oceanic crust. In fact, the velocities determined were "surprisingly low"—so low that almost all of them, including those in the ultramafics at the bottom of the sequence, were comparable to those in oceanic layer 2 alone. The conclusion that Matthews and his colleagues came to was that the low velocities were probably due to the presence of open cracks in the rocks under very low confining pressures—cracks which are presumably not present in *in situ* oceanic crust, at least in the lower layers. This view later received convincing support from Poster (*Nature phys. Sci.*, **243**, 2; 1973) who showed that ultrasonic velocities are higher in Troodos samples under pressures of up to 2 kbar.

Then there are the more direct effects associated with older material which has been uplifted and simultaneously subjected to considerable tectonic forces. Irreversible chemical changes may have taken place in some or all of the ophiolite components, making direct comparison with newer oceanic material impossible; and physical changes may have made it difficult to measure accurately even the simplest of parameters such as the thicknesses of the various ophiolite units. For example, although Poster found that the seismic velocities in Troodos samples increased with increasing pressure and thus approached more closely the velocities observed in oceanic crustal layers, he also found that even under pressure the velocities in the ultramafics forming the lowest layer of the ophiolite sequence were lower than those in the gabbros comprising the next layer up. Insofar as the velocities in modern oceanic layers in-

crease with depth, the comparison between the Troodos sequence and present-day ocean floor cannot therefore be considered entirely satisfactory. Poster's suggested explanation of this apparent anomaly was that the ultramafics have undergone a considerable degree of serpentinisation, a process known to lower grain density and consequently seismic wave velocity; and a study of the ultramafic samples in thin section confirmed that serpentinisation had indeed taken place.

So far, however, whenever there has been a discrepancy between a property of ophiolite suites and the corresponding property of oceanic lithosphere, it has seemed quite natural to suppose that the 'problem' originates with the ophiolites. Thus it was entirely reasonable for Poster to suppose that if the Troodos Massif is a remnant of Mesozoic ocean floor and yet exhibits an important difference from modern oceanic lithosphere, then something must have happened to the Troodos Massif. But what about the other half of the comparison? Up to now, it has apparently never occurred to anybody to question whether or not ophiolite sequences are actually being compared with the correct model of the ocean floor. On the face of it, of course, there is no reason why anybody should; the generalised oceanic crustal section proposed more than 10 years ago by Raitt (in *The Sea*, Interscience, 1963) has long been a part of the accepted wisdom of geophysics. But on page 136 of this issue of *Nature*, Moores and Jackson present a comparison between certain features of ophiolites and ocean floor which is apparently much more satisfactory than any proposed hitherto—and they do so on the basis not of the Raitt model but of a somewhat different structure proposed about 3 years ago in a little-noticed article by Sutton *et al.* (in *The structure and Physical Properties of the Earth's Crust*, American Geophysical Union, 1971).

What is still generally regarded as the standard model of 'typical' oceanic crust and uppermost mantle (that is, of ocean basins at normal depths away from ridges, trenches, seamounts and so on) comprises three layers below the unconsolidated sediments (layer 1). The values quoted for thickness and seismic velocity differ between authorities; but as originally given by Raitt, layer 2 has a thickness of 1.7 ± 0.8 km and a P wave velocity of 5.1 ± 0.6 km s⁻¹, layer 3 is 4.9 ± 1.4 km thick but possesses a much less variable velocity of 6.7 ± 0.3 km s⁻¹, and layer 4 (uppermost mantle) has a velocity of 8.1 ± 0.2 km s⁻¹. Layer 3 is usually regarded as the bottom layer of the crust, although from time to time some workers have interpreted marine seismic profiles as indicating the presence of a layer with a velocity in the range 7.1–7.6 km s⁻¹. This 'extra' layer has sometimes been described as layer 3 with anomalously high velocity and sometimes as mantle with anomalously low velocity (and even, on occasions, as the result of misleading data)—reasonable interpretations in most cases because either one or both of the 6.7 km s⁻¹ and 8.1 km s⁻¹ layers were apparently missing and/or the relevant profiles were obviously associated with a prominent feature such as a ridge or trench. In short, the intermediate velocities derived from marine profiles were generally dismissed as atypical and not very important.

But Sutton *et al.* came to a quite different conclusion. Using a new method of seismic refraction profiling involving a single ship, sonobuoys, a repetitive seismic energy source and continuous recording (the Asper method), they were able to identify a high velocity basal crustal layer

($7.4 \pm 0.3 \text{ km s}^{-1}$) at eight Pacific Ocean sites ranging from Fiji to the coast of California. Moreover, they were able to find in the literature reports of ten other sites in the Pacific, the Atlantic and the Mediterranean at which a layer of velocity about 7.4 km s^{-1} was sandwiched between a crustal layer of at least 6.0 km s^{-1} and an upper mantle layer of at least 7.7 km s^{-1} . In addition, there are numerous sites at which the 7.4 km s^{-1} layer had been identified but at which either layer 3 or the uppermost mantle had been missed or (in the case of the mantle) inferred from other data. As a result, Sutton and his colleagues proposed that a basal crustal layer about 3.1 km thick, far from being rare in the oceans (though quite familiar beneath continents), exists under all Pacific basins and probably under basins in other oceans as well. The reason why it is frequently missed even by more elaborate refraction techniques, they suggested, is that it appears largely as second arrivals masked by other arrivals. In other words, the situation is analogous to that in which difficulties were encountered in observing layer 2 during the early days of marine seismic refraction work; layer 2 only came to be observed regularly when conditions were arranged for a specific search.

When Sutton and his colleagues first presented their work at a conference at the University of Colorado in 1970, A. Hales praised them for their courage in reporting "some rather unusual mantle velocities" and, by implication, in proposing an important modification to a model which has come to be accepted as one of the near-certainties of geophysics. Since then, the article in which the results were described in greater detail has apparently received little attention. Moreover, even though confirmation of the basal crustal layer would, in the words of Sutton *et al.*, "have major significance for both interpretation of other geophysical data (gravity and surface waves) and speculations on the geologic processes that resulted in the formation of the Earth's crust", the possibility of such a layer has been little discussed. Presumably this is in part because the Asper technique is not yet in widespread use, so that few new results have been obtained. In the meantime, however, Sutton and his colleagues may take heart from the indirect evidence adduced by Moores and Johnson, for if the latter authors are right in their interpretation, the consistency between the Sutton model and the view of ophiolite suites as oceanic lithosphere brings support for both.

PETER J. SMITH

Picornaviruses still useful as model systems

JUST as the drift of molecular biology towards eukaryotic systems led to a return-to-*E. coli* backlash, so too the present trend of many virologists towards tumour virology led to the retort "polio is not dead" (Baltimore, *Perspectives in Virology*, 7, 1; 1971). Indeed, as model systems the picornaviruses have much to offer, and still produce regular surprises.

Porter, Carey and Fellner (*Nature*, 248, 675; 1974) have recently demonstrated the presence of a large poly(C) tract, about 90–100 residues long and with 85–90 C residues, within the RNA of encephalomyocarditis (EMC) virus. Since the molecular weight of the viral RNA exceeds by about 1,000 nucleotides that necessary to code for all the viral proteins, and because none of the known viral proteins is rich in proline, Porter *et al.* argue that the poly(C) tract is probably near to one of the termini of the molecule, in the untranslated regions. It is interesting that partially purified EMC RNA polymerase will synthesise poly(G) using exogenous poly(C) as a template (Rosenberg, Diskin,

Oron and Traub, *Proc. natn. Acad. Sci. U.S.A.*, 69, 3815; 1972) and this perhaps indicates a role for poly(C) in replication. Further support for this idea is given by the fact that bacteriophage Q β replicase will also transcribe poly(C) but cannot recognise other homopolymers. Q β RNA does not contain a poly(C) tract, however, and so the significance of these observations remains unclear.

Porter *et al.* have not been able to detect poly(A) in EMC RNA. A similar result was previously reported for the closely related cardiovirus, Mengo (Miller and Plagemann, *J. gen. Virol.*, 17, 349; 1972), but is in contrast to results with poliovirus, an enterovirus, which contains a poly(A) region of 50–100 residues at its 3' terminal (Yogo and Wimmer, *Proc. natn. Acad. Sci. U.S.A.*, 69, 1877; 1972). The reason for this difference between the RNAs of different picornavirus subgroups is puzzling, particularly since it is known that the proteins coded for by the various viral RNAs and their gene order along it are very similar. An understanding of the requirement for poly(A) in the messenger RNAs of viruses which replicate in the cytoplasm might give some clue as to the role of this structure in normal mRNA; on the other hand it is possible that the poly(A) in viruses has a different function to that of the host, for example in viral assembly.

Many plant viruses contain a transfer RNA-like structure covalently linked to the genomic RNA, which can be enzymatically aminoacylated. The tRNA-like structure is present at the 3' end of the RNA, and is different in primary sequence to the corresponding host cell tRNA molecules. The function of these structures is unknown but they are able to interact with several of the enzymes involved in protein synthesis. Salomon and Littauer (*Nature*, 249, 32; 1974) have now reported that mengovirus RNA can be acylated with histidine, by aminoacyl synthetases from mouse liver. Similar enzymes from *E. coli* were unable to produce this result. That the material acylated by the liver enzymes was not contaminating host tRNA was shown by polyacrylamide gel electrophoresis under denaturing conditions. Although cleavage of the viral RNA occurred during aminoacylation, the viral fragment containing labelled histidine was significantly larger than host cell histidyl-tRNA. It is not yet known whether cleavage of Mengo RNA is necessary for charging with histidine or is an artefact caused by contaminating RNases.

Since poliovirus RNA terminates in poly(A) at the 3' end, it cannot have a tRNA-like structure in that position, and not surprisingly previous attempts to aminoacylate polio RNA have failed (Oberg and Philipson, *Biochem. biophys. Res. Commun.*, 48, 927; 1972). This observation therefore serves to emphasise the differences that I have mentioned between the RNA of the cardio and enteroviruses within the untranslated regions.

Earlier experiments have tended to support a role for aminoacyl-viral RNA in protein synthesis. Indeed, the 3' terminal portion of turnip yellow mosaic virus (TYMV) RNA donates valine into polypeptide in a cell-free system (Haenni, Prochiantz, Bernard and Chapeville, *Nature new Biol.*, 241, 166; 1973). The physiological significance of this finding, however, has been questioned by Shih, Kaesberg and Hall (*Nature*, 249, 353; 1974) who made use of the fact that brome mosaic virus RNA can act as a messenger *in vitro* to give well-defined virus-coded products and can also be aminoacylated with tyrosine. Both these activities were examined after chemical modification of the 3' terminus of the viral RNA. As expected, such modification completely abolished the ability of the RNA to accept tyrosine, but surprisingly had virtually no effect on the protein synthetic activity of the viral RNA nor on the authenticity of the cell-free product. Thus Shih *et al.* feel that this eliminates any role for aminoacyl-viral RNA as an obligatory requirement for viral protein synthesis, and instead favour a regulatory role for the tRNA-like structure.

Since protein synthesis elongation factor 1 recognises aminoacyl-tRNA and is also involved in RNA synthesis (at least in bacteria) then perhaps the aminoacylviral RNA is somehow used to integrate protein and RNA synthesis in virus-infected cells.

Two technical problems should be borne in mind, however; first, Salomon and Littauer could only charge about 20% of their Mengo RNA with histidine, and, second, cell-free protein synthesis systems are so very inefficient compared with whole cells that any subtle effects which might be caused by tRNA 'modulation' *in vivo* may be completely lost *in vitro*.

ALAN E. SMITH

258,247 dam earthquakes

by David Davies

BUILD a large reservoir and expect earthquakes. This has been a common experience in the past 20 years. The Koyna Dam in India and the Kariba Dam in Africa both triggered off much seismic activity and there have been many other similar cases. A well-documented example in China is now presented by Shen Chung-Kang in a recent issue of *Scientia Sinica* (17 (2), 239–272; 1974).

The Hsinfengkiang Dam in Kwangtung Province was started in July 1958 and by August 1960 was producing electric power. The reservoir covers 390 km² and impounds 11,500 million m³ of water. The dam is 440 m long and has a maximum height of 105 m. The immediate vicinity was no more seismic before the construction of the dam than any other area in China, where there is, of course, much scattered seismicity.

Within a month of the first impounding of water in October 1959, seismic activity started up and as the water level rose so did the frequency of quakes. Many of the earthquakes could only be detected instrumentally, but a few were felt in the area around the dam—these generally had a surface-wave magnitude of 2 to 3. A set of seismometers was moved into the area to enable accurate locations to be produced and these still operate.

The first earthquakes were in the region of the dam itself, but progressively the activity spread to other regions. Relatively few events were located under the water itself, the majority being within a kilometre of the water's edge on the landward side. Each new rise in water level seemed to stimulate fresh activity; the depths of the earthquakes were typically 4 or 5 km. Finally in March 1962 came a magnitude 6 event within 1 km of the

dam and 5 km deep. The intensity at the epicentre was VIII—a violent shock, but the dam survived since it had been strengthened months previously on the basis of the earlier activity.

In the last 20 days before the main shock there was a marked reduction in activity everywhere in the reservoir region, and those small earthquakes that there were began to move towards the epicentre of the main shock. Since March 1962 aftershocks have continued with characteristics conventionally expected of an aftershock sequence and the activity in the area continues to this day. Up to 1972, 258,247 shocks with Ms greater than 0.2 were recorded.

What causes reservoir earthquakes? The best general explanation put forward so far, which the author also favours, is that water percolates into the underlying rocks and finds its way into groundwater channels and deep fissures. As the seepage pressure rises the water acts as a means of lubricating, as it were, the faults in the region. Thus earthquakes are facilitated as slippages along these faults. Such an explanation in terms of pore interstitial fluids, is increasingly being favoured as a general explanation of tectonic earthquakes.

Healthy carriers of hepatitis B

from Arie J. Zuckerman

Medical Virology Correspondent

NUMEROUS seroepidemiological surveys on selected groups have shown that the prevalence of hepatitis B antigen, a marker of infection with hepatitis B virus, in apparently healthy persons in Western Europe and North America is 0.1–0.6% by comparison with 5–20% in tropical Africa, South East Asia and the Far East (*Tech. Rep. Ser. Wld Hlth Org.*, No. 512; 1973). Although relatively little information is available from serial samples collected over a period of time, which would permit more precise determination of the carrier rate in defined populations, the implications are that there may be tens of millions of individuals throughout the world who carry silently in their serum the hepatitis B antigen. For practical purposes it has been agreed that a persistent carrier state exists in persons in whom the antigen has been detected repeatedly for more than 3 months. The carrier state may be life long; Zuckerman and Taylor (*Nature*, 223, 81; 1969) described the

Tidal drag cannot move plates

from Peter J. Smith

Geomagnetism Correspondent

THE idea that continents might be displaced by tidal forces was discussed at some length by Wegener in various editions of his book *The Origins of the Continents and Oceans*. But ever since Jeffreys (*The Earth*, Cambridge University Press, 1929) showed that the stress over the Earth's surface arising from tidal friction is only of the order of 10^{-4} dyn cm⁻², few people have considered tidal drag a likely driving mechanism for continental drift or plate motions. On the other hand, such a mechanism does seem to be feasible from the energy point of view. Recent calculations suggest that the energy dissipated tidally may exceed 5×10^{19} erg s⁻¹ (Rochester, *Eos*, 54, 769; 1973), of which about 2.5×10^{19} erg s⁻¹ is likely to be the maximum dissipated in shallow seas and of which less than 10^{18} erg s⁻¹ is lost in the solid Earth. Thus, in principle, as much as $2-3 \times 10^{19}$ erg s⁻¹ might be available for driving plates.

Recently, Bostrom (*Nature*, 234, 536; 1971) and Moore (*Geology*, 1, 99; 1973) have revived the tidal drag mechanism, citing in its support such phenomena as the dependence of seismicity on latitude

and the tendency of oceanic ridge segments to lie north-south (with transform faults lying east-west). But Jordan (*J. geophys. Res.*, 79, 2141; 1974) has now carried out a 'simple calculation' showing that although a plate velocity of 5 cm yr⁻¹ would involve the dissipation of energy at a rate of only 0.8×10^{17} erg s⁻¹ (well within the amount available), a torque of 10^{33} dyn cm would be required to maintain it. The actual couple exerted on the Earth by the Moon is probably less than 10^{24} dyn cm, or 9 orders of magnitude too small to allow plate motion if currently quoted values of asthenospheric viscosity are accepted. Tidal forces could produce lithospheric motion, but only if the viscosity of the asthenosphere were to be as (unbelievably) low as 10^{11} poise.

In further support of the view that tidal forces are not relevant to present-day plate tectonics, Jordan cites recent plate models based on the fixed hot spot hypothesis, which suggest that the westward displacement of the lithosphere as a whole is small compared with the relative motions of plates. In the meantime, however, all this leaves open the rather different question of what happens to the balance of the energy released by tidal friction.

case history of a well documented former blood donor who has been a healthy carrier for more than 20 years.

In high prevalence areas, for example in tropical countries, the antigen is detected in individuals of all ages, most frequently in children. The prevalence of antigen in Caucasians living in some tropical areas is higher than those in temperate zones yet it is considerably lower than in the indigenous population. In all regions the antigen is reported to be more frequent in males than in females and in urban than in rural communities. The universal problem which the carrier state poses to blood transfusion and other medical services, to morbidity from liver disease and to health in general, requires no elaboration. Yet the mechanisms leading to the formation of the carrier state are little known.

In a recent study by Gerety and his colleagues (*J. Pediat.*, **84**, 661; 1974), paired samples of serum from 200 children in the United States and 1,165 children from Upper Volta in West Africa and from two Pacific islands (Guam and Tol, Truk Islands) were tested for hepatitis B antigen by radio-immunoassay and for the homologous antibody by passive haemagglutination. The antigen was not detected in any of 100 normal children in the United States but it was found in 5% of 100 children resident in institutions in that country. The antigen was found in 2–10.9% of sera from normal children from overseas. Hepatitis B antibody was not detected in normal American children, but it was present in 9% of American children in institutions, 3% in Guam, 20.3% in Tol and 7% in Upper Volta.

Gerety *et al.* calculated the 'per cent. carriers' according to the formula: antigen carriers/infected group \times 100, where the infected group is defined as children with hepatitis B antigen or antibody, or both. The figures obtained were nil for normal American children, 35.7% for children in institutions, 40% for children in Guam, 35.2% for children in Tol and 28.6% in Upper Volta. The respective figure for American adults, calculated from published reports, is 1.8–3.5%.

Several different mechanisms may predispose to persistent carriage of hepatitis B antigen. It has been suggested that the establishment of a complete or at least a partial immunological tolerance to this antigen is the most likely explanation. A relative immune deficiency, associated with slow maturation of cellular immunity in newborn infants, is one possibility. This mechanism may play an important part in carriers resulting from vertical transmission of hepatitis B virus (*Nature*, **249**, 105; 1974). Gerety and associates (*loc. cit.*) found, however, that the risk

Channelled scablands of Washington



THIS picture shows part of the 40,000 km² 'channelled scablands' of eastern Washington, and is from a new booklet produced by the US Geological Survey (*The Channelled Scablands of Eastern Washington*, US Government Printing Office, Washington DC, 1974, 65c). The geological events leading to the formation of this unique landscape began during the Miocene with the extrusion of up to 3,000 m of basalt lava flows which later tilted and warped and acquired a

30–60 m cover of loess. Then beginning about 100,000 years ago ice lobes from the northern hemisphere glaciation encroached on the lava field, damming rivers with glacial ice and debris and forming large lakes. Finally, 10,000–20,000 years ago the dam containing the largest lake broke and more than 2,000 km³ of water and debris were released within a day or two, carving the surface as observed today.

P.J.S.

of becoming an antigen carrier seemed to be uniform among children ranging in age from 1 month to 15 years. They considered that factors of exposure such as the route and amount of virus may contribute to the disparate chronic infection rates between children and adults. For example, means of transmission of low dose of virus in children, other than by direct skin penetration, might influence the severity of the infection and the immune response, favouring the development of the carrier state, but the available information is incomplete. An intensive immune response may be essential for the elimination of the virus and the prevention of the establishment of an equilibrium between the host and the infecting agent. There are reports in the literature which suggest that adults seem to become carriers more frequently after a mild attack of hepatitis (Barker and Murrey, *J. Am. med. Ass.*, **216**, 1970; 1971; Aach *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1656; 1971; Gocke, *J. Am. med. Ass.*, **219**, 1165; 1972). And, in general, children experience a milder disease with many sub-clinical infections.

Carriage of hepatitis B antigen has been consistently reported to be more

frequent in males than in females and evidence has been obtained which indicated sex as an independent primary variable factor responsible for a higher prevalence of the antigen in males than in females. Washburn and colleagues (*Pediatrics*, **35**, 57; 1965) studied the sex difference in susceptibility to a number of infections. A significant preponderance of males was found and this was most marked in infancy. The sex difference in susceptibility was postulated to be consistent with a gene locus on the X chromosome which is involved in the synthesis of immunoglobulins. Small differences in the amounts or rates of synthesis of antibody might be responsible for a slightly greater susceptibility to infection among members of one sex. Gerety and his colleagues found that the likelihood of the development of the carrier state in the groups of children they studied did not seem to correlate with the sex or with the geographical origin of the children or with the subtype of hepatitis B virus. What the results did show clearly was that children became persistent carriers more frequently after infection with hepatitis B virus than adults in the United States.

Identification of HO₂

from our Chemical Physics Correspondent

Most chemists and chemical engineers would feel that HO₂ is one of the commoner and better known of the gas phase free radicals which are common intermediates in so many reactions. Hydrocarbon combustion, both in heating plants and in motor engines, is a major process in which HO₂ is a significant intermediate and many important kinetic rate constants are concerned with its creation and subsequent destruction. However clear cut, physical identification and study have proved remarkably difficult. Even the powerful tools of photolysis and of matrix isolation have found HO₂ to be a slippery customer and ultraviolet, infrared and electron resonance spectra of only indifferent quality and resolution have been obtained. More informative gas phase spectroscopy has been, hitherto, remarkably unsuccessful and this is not for want of much hard and difficult work.

Now, however, the powerful team of Radford, Evenson and Howard (*J. Chem. Phys.*, **60**, 3178; 1974) has obtained a remarkably clear spectrum which must be attributed to HO₂, unless even now this radical has sent a Doppelgänger to confuse us. The authors believe it relates to HO₂ but the evidence is indirect. The spectrum appears from more than twenty likely reaction mixtures including O₂+C₂H₄, O₂+C₂H₂, H₂O₂+F, H+O₂ and from discharges in water vapour. The spectrum near 130 cm⁻¹ vanishes when the H is exchanged for D or ¹⁸O by ¹⁸O, but it remains unaltered when ¹³C is used instead of ¹²C in the reagents. The species is certainly paramagnetic, probably uncharged and seems to contain an odd number of nuclei like H with a spin of 1/2. The evidence is certainly sufficient to convince most readers, including your correspondent, but is not quite at the level of absence of all reasonable doubt that would be required by a murder jury. A particularly important aspect of the present spectrum is its availability as a monitor during the maximisation of the concentration of the concentration and life-time of the HO₂ in future experiments. This will be of interest to research workers in combustion and further details of the rotational spectrum will be eagerly awaited by astrophysicists who would seek HO₂ in interstellar gas by means of its microwave spectrum.

The spectrum was observed with a powerful technique which uses the absorption of the H₂O and D₂O laser lines near 119, 126 and 139 cm⁻¹ (84, 79 and 72 μm). Since these lines do not coincide perfectly with the HO₂ absorption, the latter is modified by a magnetic field up to 2 T (20 k gauss) using the Zeeman effect. This technique, called laser magnetic resonance, is sensitive only to paramagnetic species, so that the many dia-

magnetic species in the reaction stream do not interfere. Using magnetic field modulation and a phase sensitive detection system about twenty lines are easily detected with each laser frequency using separately the laser electric field parallel and perpendicular to the magnetic field, a change which modifies the selection rule on the Zeeman sublevels. Indeed the signal to noise ratio is such that the doublet nature of the lines, due to the proton magnetic moment coupling with the unpaired electron, is clearly resolved with a separation of 1 mT (10 gauss). The radical is close to a prolate symmetric top and has $A \sim 19.4$ cm⁻¹ and $(B+C)/2 \sim 1.26$ cm⁻¹. The rotational quantum numbers N and K provide useful state labels and the transition near 139 cm⁻¹ is assigned to the $K \leftarrow 2$ and $N \leftarrow 18$ transition although $N \leftarrow 19$ is not completely excluded by the evidence. There is a zero field splitting between the $J=N+1/2$ and the $J=N-1/2$ states which is 0.605 cm⁻¹ for $K=2$ and 1.139 cm⁻¹ for the $K=3$ level. The transitions are for the $J=N+1/2$ states.

Vegetation disturbance in arctic tundra

from Peter D. Moore
Plant Ecology Correspondent

THE slow recovery rate of alpine tundra following the disturbance of surface vegetation (see *Nature*, **249**, 690; 1974) is causing concern. A similar, but more complex problem confronts conservationists in arctic tundra regions, where the existence of permafrost within the soil means that thermal equilibrium is a critical factor in determining ecosystem stability.

Dingman and Koutz (*Arctic Alpine Res.*, **6**, 37; 1974) have studied the pattern of frozen subsoil distribution in a region of central Alaska where permafrost is discontinuous. They were concerned in particular with microtopography and the way in which such factors as aspect can influence the net potential radiation input and hence the depth of the active layer (seasonal thaw zone). A rather rough, non-linear correlation emerges between their potential insolation index and active layer depth, and their figures suggest that permafrost occurs where the average annual solar radiation falls below 265 cal cm⁻² d⁻¹.

The anomalous points on the graphs of Dingman and Koutz, which distort some of their regressions, frequently correspond to locations of unusual vegetation type, such as forest cover or exceptionally thick moss hummocks. Evidently the influence of surface vegetation on ecosystem energy balance, particularly on albedo and on latent

heat losses associated with transpiration, must be taken into account.

This aspect has been studied by Haag and Bliss (*J. appl. Ecol.*, **11**, 355; 1974) who have conducted experiments to test the effect of vegetation disturbance upon tundra energy budgets. Damage to surface vegetation and peat on areas used as roads during the winter months resulted in increased surface moisture and therefore in considerable albedo changes (6.1%, compared with 15.2% in control sites); in consequence there was an increase in net radiation input in the road site. In addition, the compaction or disturbance of the surface peat layer led to an increase in thermal diffusivity, as a result of which higher soil temperatures were recorded during the day even at a depth of 50 cm in the road profile. Latent heat losses were greater in the control site, especially in the growing season when actively transpiring vegetation was fully developed and deeper reserves of water were being tapped.

One of the important conclusions of this work is that the use of tundra as a winter road alters the soil thermal régime, resulting in a deeper active layer in summer (about 56 cm compared with 36 cm in control). Similarly other types of disturbance such as fire and oil spills produced deeper active layers (46 cm and 42 cm respectively). Such conditions are conducive to surface instability and erosion. A possible solution to the problem would be the reseeded of disturbed areas to assist surface healing.

Wein and MacLean (*Can. J. Bot.*, **51**, 2509; 1973) investigated the potential of *Eriophorum vaginatum* (cotton sedge) as a recolonist of bare peat and mineral soil and came to the conclusion that the species could be of particular value where mineral soils were exposed and where the soil is permanently saturated. This is precisely the set of conditions found on parts of the winter roads. The seeds germinated best at 25–30° C and had no dormancy problems; when sown in July they were found to be strong enough to withstand the subsequent winter.

Re seeding experiments in disturbed tundra areas are now being conducted, but the initial data of Haag and Bliss suggest that the development of a vegetation cover will be slow; only 20–50% surface cover has been attained after three seasons' growth. Albedo has risen in the reseeded plots, leading to a decrease in the net radiation input, but active layer depth has decreased by only 2 cm (from 52 cm to 50 cm). It would seem that the loss of thermal insulation provided by a peat cover is even more vital for the maintenance of a shallow active layer than is high albedo. The redevelopment of a peat blanket will obviously take a very long time.

Seven years of L-dopa therapy

by Miranda Robertson

NOMINALLY, the symposium held on June 28 by the Parkinson's Disease Society was to mark the fact that it is now 150 years since the death of James Parkinson. What was most clearly reflected in its content, however, was that it is now seven years since the advent of L-dopa therapy, which seemed at one time to promise freedom from parkinsonism but has turned out merely to suspend sentence. This has left pharmacologists to try and find out what to do next and clinicians with the problem of what best to do in the meantime; and that, apart from an historical excursion by J. D. Parkes (Institute of Psychiatry, London), was what the symposium was about.

There has been no radical change in the therapeutic approach to parkinsonism since Charcot first used anticholinergic drugs a century ago. The aim is still to redress the balance between cholinergic and dopaminergic agonists in the basal ganglia, but with the emphasis on supplying the missing dopamine rather than blocking acetylcholine reception. K. Fuxe (Karolinska Institute, Stockholm) favoured ergot derivatives and in particular 2-bromo-alpha-ergocryptine (CB154) as future alternatives to dopamine derived from L-dopa. The advantages of these dopaminergic agonists over L-dopa would be that they act directly on the dopamine receptor without having to be converted to the active form by enzymes which may be deficient as a result of the same degenerative process that produces the dopamine deficiency. Fuxe, who has tried CB154 on rats with nigro-striatal lesions, is optimistic about the compound whose effects, though weaker than those of L-dopa, are more prolonged. His hope, however, that CB154 might also be free of some of its side effects is not substantiated by preliminary clinical trials, reported by D. Calne (Hammersmith Hospital, London). Functional improvement was assessed blind at between 9% and 18% on average for 20 patients on the drug, the degree of improvement being positively correlated with the severity of the disability. But adverse reactions seemed to parallel those seen with L-dopa therapy.

Amplification of post-synaptic effects through the adenylyl cyclase system, which is also under investigation at the Karolinska Institute, may conceivably help with the separation of therapeutic from side effects, although as C. D. Marsden (King's College Hospital, London) pointed out, it is not clear how different the receptors mediating the one are from those mediating the other. In Fuxe's rat model, however,

theophylline (which inhibits phosphodiesterase and thus increases the concentration of cyclic AMP) does seem to increase activity in the denervated striatal neurones, and he believes that it may be possible to use the drug to potentiate the effects of CB154.

All this, however, assumes a functional post-synaptic receptor mechanism, and as M. Sandler (Queen Charlotte's Hospital, London) pointed out, receptor pathology is by no means ruled out. Sandler, who has long held that the dopamine replacement theory of L-dopa therapy is an oversimplification, devoted much of his talk to hauling skeletons out of cupboards. One of them is ignorance of the exact cause of Parkinson's disease, and of the contribution of deficiencies in chemicals other than dopamine to the syndrome. The other is the failure to explain either how L-dopa works or why it stops working. Neither the disease nor dopa metabolism, in short, is understood.

What seems to be missing is a really good animal model for parkinsonism. Sandler stressed that drugs do not always have the same effect on lesioned rats as they do on parkinsonian humans. This is hardly surprising if only because the parkinsonian lesion is most unlikely to be as simple as the destruction of the nigro-striatal pathway, besides which, autopsy brains of parkinsonian patients show evidence (quoted by M. Yahr, Mount Sinai School of Medicine) of continuing degeneration under L-dopa therapy; whereas the surgical lesion does not evolve.

There is also the question of the effect of prolonged administration of drugs. Clinicians repeatedly brought up the need at the very least for studies on the effects of anti-parkinsonian agents over long periods of time. Progressive disease may explain why parkinsonism in due course overcomes L-dopa therapy; but what is the explanation of the simultaneous increase in the dyskinetic side effects of the drug?

Some recent work of H. Klawans (University of Chicago) suggests that the answer may lie in the post-synaptic dopamine receptors. He used amphetamine to stimulate the dopamine receptors of guinea pigs to the point at which the drug produced stereotyped movements, and found that after regular treatment over a period of time, the movements could be elicited not only by progressively lower doses of amphetamine but also by the dopaminergic agonist apomorphine.

The meeting ended with a review lecture by A. Carlson (University of Goteborg), whose efforts in establishing dopamine as a neurotransmitter in the late 1950s helped to make possible the introduction of dopaminergic antagonists in the treatment of parkinsonism.

An amoeba becomes a flagellate

from F. E. G. Cox
Parasitology Correspondent

PARASITOLOGISTS are tidy people and like to know how parasites should be classified. The understanding of the correct taxonomic status of a parasite is more than the provision of a handy slot in which to file information; it is the basis of an understanding of the biology of the organism and a point to which related organisms can be referred. From such a reference point stems a knowledge of basic biochemistry, physiology and epidemiology and on these studies the control of the parasite can be based. Parasites, particularly protozoa, are often very specialised and it is not always easy to see their basic structures with the light microscope. During the past decade, however, the electron microscope has been used to solve several taxonomic problems. Partially as a result of such studies the piroplasms and *Toxoplasma* have taken their rightful position within the sporozoan class Coccidia, and the number of discrete taxonomic groups containing a few parasites of uncertain taxonomic position has been diminished.

One of the outstanding taxonomic problems is concerned with an amoeba, *Dientamoeba fragilis*, which lives in the gut of man. It has long been recognised that this binucleate amoeba could be a flagellate but absolute proof has been lacking. Two independent papers in the *Journal of Protozoology* have now supplied this proof. Honigberg and his colleagues (Camp, Mattern and Honigberg, *J. Protozool.*, **21**, 69; 1974) now report that the fine structure of *Dientamoeba* places it among the trichomonad flagellates and this conclusion is also reached by Dwyer (*ibid.*, 139) using immunoelectrophoretic methods to analyse the antigens of *Trichomonas*, *Dientamoeba*, *Histomonas* and *Entamoeba*. This latter work is a culmination of Dwyer's earlier immunological studies on these parasites (*ibid.*, **19**, 316 and 326; 1972). All the evidence now points to common structures and common antigens linking *Dientamoeba* with the flagellates *Trichomonas* and *Histomonas* and there is a clear evolutionary sequence in which the number of flagella are reduced in *Histomonas* and disappear altogether in *Dientamoeba*. Honigberg suggests a revised classification of the Order Trichomonadida in which *Dientamoeba* is placed in the same family as *Histomonas* and this is likely to be generally accepted by protozoologists and parasitologists.

The implications of these studies lie in an understanding of the mode of transmission of parasitic members of the order Trichomonadida, Protective

cysts are unknown in these forms and the parasites have had to evolve unusual ways to ensure transmission. *Histomonas meleagridis*, the causative organism of blackhead in poultry, enters the egg of the nematode *Heterakis gallinarum* (Lee, *Parasitology*, **59**, 877; 1969) and there is abundant evidence to suggest that *Dientamoeba fragilis* of man uses the nematode *Enterobius vermicularis* (Ockert, *J. Hyg. Epidem. Microbiol. Immunol.*, **16**, 213 and 222; 1972; *Abstracts 4th Int. Cong. Protozool.*, Clermont-Ferrand, 1973). As very little is known about the affinities or the transmission of the three species of *Trichomonas* in man there may be some surprises for those who suggest infection as a result of direct contamination.

Structure of nuclear single-particle states

from Peter E. Hodgson
Nuclear Theory Correspondent

MANY features of nuclear structure can be understood by considering the individual motions of the constituent nucleons. For most of the time they move on independent orbits, and occasionally collide with each other. The energies and quantum numbers of these orbits can be determined by reactions that remove a nucleon from a nucleus or add one to it.

The single-particle states near the Fermi surface—those with the highest energies—can be most easily studied by one-nucleon transfer reactions like the (d, p) stripping reaction. Deeper states can best be studied by nucleon knock-out reactions like (p, 2p).

Many studies of single-particle states have now been made, and their systematic behaviour from one nucleus to another is becoming clearer. It is found that most states are split into a number of fragments by the residual interactions that are not taken into account in the simple shell model. Thus instead of a single state several states of the same structure and quantum numbers are spread over one or two MeV of energy. The energy of the state, however, can still be defined as the centroid of the fragments, weighted by their spectroscopic factors.

A very important question is then whether all the fragments of a particular state have been found, for if some fragments far away from the main strength have been missed this could produce serious errors in the centroid energies. It is not easy to detect small fragments experimentally, so other methods must be tried.

If one could rely on the theory of the interaction removing the nucleon there would be no difficulty, for one could simply calculate the sum of the

spectroscopic factors and see if they added up to unity, the value corresponding to a pure single-particle state. Unfortunately, even in favourable circumstances the distorted wave theories cannot be relied upon to better them about 10–20%, and one is concerned with the loss of small fragments constituting around 5–10% of the total strength.

Another approach is to use a sum rule with all the single-particle states in a particular nucleus. Koltun (*Phys. Rev. Lett.*, **28**, 182; 1972) has shown that provided only two-body forces are present in the nucleus, the total energy of the nucleus may be expressed as a sum of the kinetic and removal energies of the constituent nucleons.

$$E = \frac{1}{2} \sum_i (T_i + E_{iR})$$

The total energy E is known accurately from the nuclear masses, and the kinetic energies T_i can be calculated quite easily from a simple nuclear model. The removal energies E_{iR} are obtained directly from the centroid energies. The sum rule thus provides a sensitive test of the completeness of the sets of fragments assigned to each single-particle state. It does, however, refer to all the nucleons, so the set of single-particle states is tested as a whole, and not individually.

Since the most complete sets of data are available for the proton single-particle states, it is useful to split the above sum rule into the corresponding relations for protons and neutrons separately. Thus the mean binding energy per proton becomes

$$E/Z = \frac{1}{2} \{ \langle T \rangle + \langle E_R \rangle - m_p \langle T \rangle / M_{A-1} \}$$

where the angular brackets denote averages only over the proton single-particle states. The extra term is a recoil correction that must be included in accurate work.

This sum rule has recently been tested by a group at Saclay (Bernheim *et al.*, *Phys. Rev. Lett.*, **32**, 898; 1974) using the (e, e'p) reaction at 497 MeV. This reaction is preferable to the (p, 2p) reaction because the electrons are less likely than protons to interact with other nucleons before or after their main quasi-elastic interaction with one of the nuclear protons. For the same reason the cross section for the knockout reaction is reduced, but improvements in the experimental apparatus have so increased the incident intensity that it is possible to obtain significant results in a practicable time.

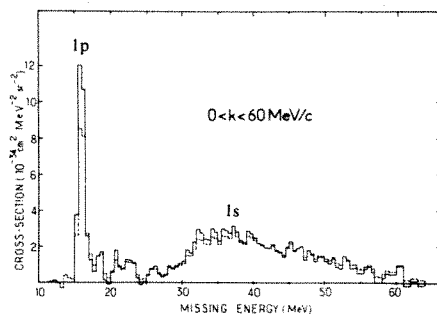
The single-particle states are detected by measuring the energies of the inelastically-scattered electron and of the ejected proton. Application of the conservation laws then gives the energy required to remove the proton, which is just the required energy of the

single-particle state. If the distribution of this energy is plotted for a large number of knockout events the single-particle states are evident as peaks, and the angular distributions of these peaks enable the quantum numbers of the corresponding single-particle states to be found.

The results for $^{12}\text{C}(e, e'p)$ are shown in the figure, and clearly show the peaks corresponding to proton removal from the 1s and 1p states. The widths of these peaks are due partly to the inaccuracies in the measurements and partly to the energy spread of the fragments which are not resolved individually.

These data were used to test the proton sum rule. After correcting for distortion of the outgoing proton waves in the nuclear field they found -4.0 ± 0.5 MeV for the mean binding energy per proton, compared with the value -6.93 MeV obtained from the nuclear masses with Coulomb corrections. This is a very significant discrepancy. One possible explanation is that there are some small fragments many MeV away from the main peaks. Bernheim *et al.* estimate that 5% of the strength associated with average separation energies of 150 MeV would be sufficient to account for the discrepancy. The rate of fall-off of the 1s peak with increasing energy is such, however, that if it continues to higher energies this explanation is very unlikely. On the other hand if there is a discontinuity in the fall-off this constitutes a new phenomenon requiring explanation.

Another possible explanation of the discrepancy is the presence of three-body forces, whose absence was assumed in deriving the sum rule. In order to account for the data, the contribution of three-body forces to the binding energy per proton would have to be $\langle H_3 \rangle / Z = 5.9$ MeV, which at present seems unlikely. If this possibility is confirmed, it would be of



Spectrum of the 'missing energy' in the reaction $^{12}\text{C}(e, e'p)$ with (—) and without (---) radiative corrections. The 'missing energy' is equivalent to the energy required to remove a proton from ^{12}C , and the peaks at about 16 and 35–40 MeV correspond to removal from the 1p and 1s states respectively.

great importance for the study of nuclear structure. It is also possible that the discrepancy would be reduced or even eliminated by a full relativistic analysis, but this has not yet been done.

Sun-shuttling lizards

from our *Animal Ecology Correspondent*

LIZARDS are distinctive by their relative absence from temperate forests. This situation, on the face of it, seems anomalous in view of the great abundance of insect prey species in this sort of habitat. Huey (*Science*, **184**, 1001; 1974) now points out that this anomaly may be resolved by considering the ecological difficulties encountered by poikilothermic carnivores.

Unable to thermoregulate physiologically, most lizards move about from sunshine to shade or modify their body posture to alter surface areas exposed to heat sources and thus thermoregulate behaviourally (Cowles and Bogert, *Bull. Am. Mus. nat. Hist.*, **83**, 261; 1944; Heath, *Univ. Calif. Publ. Zool.*, **64**, 97; 1965). Their thermal strategy can be described as heliothermy. It is theoretically possible to predict the amount of behavioural thermoregulation that would maximise net profits by calculating the 'costs' of a physical movement into or out of sunshine as against the 'gain' involved in the attainment and maintenance of a specific body temperature. In Huey's study on *Anolis cristatellus* in Puerto Rico the energetic cost of physical movement from shade into sunshine was estimated by measuring the shortest practicable distance from a perch in the shade to one in the sunshine. Anoles occupy a variety of habitats. Huey chose to study two adjacent populations, one in a shaded forest and the other in an open meadowland. If a tree or other perch was totally shaded and offered no sunlit patches, this fact was noted. Many more lizards in the forest were unable to reach full sun without changing perches (which usually meant changing trees) than were those in the meadow. Furthermore, those lizards in the forest that could reach the sunshine were significantly further from it than were those in the open. Thus there seemed little doubt that the energetic 'cost' of heliotactic thermoregulation was far higher in the forest population.

During the early morning the cloacal temperature of anoles in the forest increased significantly slower than that of meadow lizards and was strongly correlated with ambient air temperature. Such a strong relation did not exist between lizards living in the open and the surrounding air; body temperature of lizards in the open fluctuated by only 1.6° C during the day as compared with 4.9° C in the forest.

The interpretation of these observations is that behavioural thermoregulation occurs only where sun and shade conditions are juxtaposed. Where 'costs' are high, as in the three-dimensional environment of the forest, anoles are passive to ambient conditions. Under these conditions the rewards of sun-shuttling are insufficient and this is reflected in very low population densities. What is the evolutionary significance of this exploitative strategy?

A. cristatellus is a remarkable reptile in having such a plastic ecology. The genus is known for its ability to colonise islands *de novo* as well as to form sympatric species on islands already colonised (Williams, *Q. Rev. Biol.*, **44**, 345; 1969), and its degree of thermal tolerance possibly accounts for its widespread success in the Caribbean. Those populations of *A. cristatellus* that occupy open habitats have an energy budget from which reproduction can have a larger share than is possible in forest populations. Although lizards in the two habitats are indistinguishable in terms of dewlap colour, snout-vent length or midbody scale rows, and do not differ with respect to preferred body temperature in a laboratory gradient, it would seem likely that their populations are in the process of active divergence. Perhaps sun-shuttling is the key to two questions: first, the absence of lizards in forests; and second, the complexity of *Anolis* speciation.

Magnetospheres of Earth and Jupiter

from N. A. Heard and A. R. L. Tatnell

THE tragic death of Neil Brice earlier this year in an air crash over the Pacific led to the renaming of the symposium in Frascati (May 28–31) on the magnetospheres of Earth and Jupiter, the Neil Brice Memorial Symposium. After V. Formisano (Spazio Plasma Laboratory, Frascati) had opened the meeting, C. Kennel (University of California) described the valuable contribution of Neil Brice to the study of the Earth's magnetosphere.

The symposium fell naturally into two parts. In the first half the Earth's magnetosphere was reviewed and results from several satellites were presented. K. Schindler (Ruhr University, Bochum) and V. Vasylinas (Massachusetts Institute of Technology) presented theoretical calculations based on models of the magnetosphere; some attempt was made to extend the model of the Earth's magnetosphere to that of Jupiter.

It has been known for some time that Jupiter is a very intense planetary radio source but, as D. Gurnett (University of Iowa) showed, the Earth is not to be outdone in this respect. Imp-6 and 8

satellites have revealed that the Earth is a comparable radio source and in the frequency range from 50 kHz to 500 kHz, at peak intensity, the total power emitted is about 10^9 W. This radio emission is associated with discrete auroral arcs and seems to originate from altitudes of about 2,000 km. It was suggested that a satellite at this altitude might provide some interesting results.

As R. W. Fredericks (TRW Systems, California) pointed out the value of studies of wave-particle interactions to the understanding of the Earth's magnetospheric processes; it was generally agreed that similar studies of the wave-particle interactions in Jupiter's magnetosphere are essential.

Motion of the Earth's bow shock—the shock caused by the supersonic solar wind impinging on the Earth's magnetic field—was discussed by several speakers. The causes of shock motion are not understood but, from data from Heos 1, Formisano and G. Mastrantonio (Frascati) showed that a satellite is likely to encounter the bow shock in motion if tangential discontinuities are the main cause of motion.

E. J. Smith (Jet Propulsion Laboratory, California) confirmed that the outer boundary of Jupiter's magnetosphere fluctuates considerably, a possibility mentioned by several speakers. The vector helium magnetometer results have revealed that the magnetopause is crossed twice on the inbound pass and a number of times on the outbound pass. An interesting feature of Jupiter's magnetic dipole field is that it is directed opposite to that of the Earth. This dipole field is inclined 15° to the rotation axis and offset about $0.1R_J$ north of the equatorial plane and about $0.2R_J$ towards longitude 170°. From this estimated dipole moment the surface field is between 2.3 and 11.7 gauss compared with the Earth's field of between 0.3 and 0.6 gauss. The dipole approximation for Jupiter is even less valid than in the case for Earth, because of the considerable contribution from current sheets around the planet. Pioneer 10 has also confirmed the presence of helium on Jupiter and the existence of an internal energy source in the planet because the excess radiation is 2 to $2.5 \times$ the solar input.

The role of the Galilean moons is still uncertain but, as several speakers showed, Io in particular seems to influence the particle flux considerably. Io is about the same size as Earth's moon and occultation experiments have shown that it has an ionosphere and a surface pressure of 10^{-6} bar.

•The fly-by of a planet obviously leaves a number of unanswered questions and all speakers emphasised the difficulty in distinguishing between spatial and temporal variations.

Health costs associated with the mining, transport and combustion of coal in the steam-electric industry

L. A. Sagan

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By assigning an arbitrary cost to a human life, Dr Sagan arrives at rough estimates of the health costs associated with power generation. The costs of miners' accidents and diseases are shown to be heavy compared with those of air pollution.

CHOICE between energy sources rarely takes into account factors other than the market value of the commodities^{1,2}. The costs of such decisions to society in general are rarely considered. Some of these costs are environmental: for example, disposal of industrial waste has often been free. Others involve the health, both of the general population³ and of the persons employed in the industry of interest. In my recent study of the nuclear fuel cycle⁴ I showed that the health effects incurred by occupational personnel are far greater than those suffered by the public.

In this study I evaluate the health costs of generating electricity from the combustion of coal. The health costs of coal production and combustion should be considered in energy planning, but it would be presumptuous to imply that choices among alternate fuels will be made solely on the basis of health. Even so, such quantitative data as are available, as well as an understanding of the deficiencies of the data, should be available to planners and decision makers.

I decided to consider the use of coal for the generation of electricity for the following reasons. Although energy demands in the United States are generally doubling every 14 yr, demand for electricity is now doubling every 7 yr. Although nuclear fuel is expected to increase its share of electrical generation to 66% by the year 2000, coal production for steam generation will continue to increase (Table 1).

It is difficult to consider together such diverse health effects as accidental death and injury, and chronic disease caused by industrial chemicals, dusts and gases. The latter are elusive, and their existence and magnitude is difficult to demonstrate. The technique I adopted was the assignment of dollar losses associated with disability and death. This requires an arbitrary assignment of a cost per human life. The figure I have chosen is \$300,000 which is the multiple of an assumed productive value of \$50 d⁻¹ and a loss for accidental death of 6,000 working days as suggested by the American National Standards Institute⁵. No attempt is made to put this estimate on an annual basis by future discounting. I must emphasise that this is not an attempt to make moral judgment about the value of life. Such a valuation of life is implicit in decisions concerning

health and safety expenditures. In a study for the Office of Science and Technology⁶, a life value of \$140,000 was assumed with which to measure the cost effectiveness of automobile safety features. This was arrived at simply by multiplying average American per capita income (\$3,786) by the average age of those who died in automobile accidents (36.9 yr). It is also of interest that in a recent analysis of risks and benefits of United States technology, C. Starr (personal communication) has found that the implicit value of life in public acceptance of technology is \$300,000.

My evaluation is limited to effects which investigators have tried to quantify. In general, I have adopted pessimistic assumptions regarding health hazards in order to assess worst possible effects rather than to ignore effects for which there may be some evidence but not demonstrated proof. For example, the possibility of threshold levels of toxicity are ignored in favour of the assumption of the same proportionate effect at low levels of exposure as found at higher levels. This assumption is often made, particularly for radiation effects, but has recently been criticised as being at variance with the available biological evidence⁷. Health costs are evaluated in relation to measures of true or real value rather than in terms of out of pocket expenditures since these health costs have rarely been paid by the industry responsible for the injuries. A cost of \$50 d⁻¹ is assessed for lost time due to accident, and an equal amount for medical costs incurred. This latter assumption is based on an estimate of medical costs of all accidental injuries⁸.

So I have estimated the health costs of the 180 GW generated by coal-fired power stations in the United States. Those costs are then reduced by a factor of 180, to represent the equivalent of a single 1,000 MW (electric) plant which is the size of a modern plant supplying a city of 1 million people.

For a 1,000 MWe electrical generating plant of modern design, about 2 million tons per year of coal are required, assuming a heat efficiency of 8,500 BTU per KW h, a 67% load factor and the use of a medium grade bituminous coal of 12,000 BTU per pound. In 1970, by the Census of Mineral Industries Report, 140,000 persons were employed in production of bituminous and lignite coals⁹. Production was 3,350 tons coal per employee, so to supply a 1,000 MWe plant for one year with coal would require the labour of 600 persons.

Morbidity and mortality

In 1969 the underground coal mining accident frequency rate was the highest among all industries—nearly 32 disabling injuries per million man hours compared to the average for all industries of only about eight per million man hours¹⁰. As productivity has increased with increasing mechanisation, the risk of coal mining, as measured in fatalities per million tons mined has fallen, but coal mining still remains more than twice as hazardous as metal mining and almost three times as hazardous as non-metal mining. In the five years 1966–1970, 966 officially reported underground miners lost their lives, an average of about 200 men annually. Although surface or strip

Table 1 Predicted use of fossil fuels

	Total energy ($\times 10^{15}$ BTU)	Electricity ($\times 10^{12}$ KW h)	Fossil Fuels for electricity ($\times 10^{15}$ BYU)
1970	69	1.5	14
1985	133	4.5	20
2000	191	9.0	24

mining is considerably less hazardous than underground mining, there were also 31 fatal accidents in surface mining in 1969 and 33 in 1970, the total yearly average thus being approximately 230 men. In 1970, 48.3% of coal was mined from the surface and of this 75% was used by electric utilities. Thirty-nine per cent of deep mined coal was used by electric utilities (US Bureau of Mines, personal communication). Based on these estimates, 103 fatalities can be attributed yearly to the 180,000 MWe of coal generating stations. This is equivalent to slightly more than a half a fatality per 1,000 MW electric steam plant of \$171,000 yr.

In addition to fatal injuries, there were about 2,000 d lost per million man hours worked in 1969 and 1970 (ref. 9). Assuming an 8 hour working day, 1 d in 62.5 is lost to accident. For a 1,000 MW plant requiring the labour of 600 men for its fuel supply, 2,880 d at a cost of \$288,000 would be incurred.

The Bureau of Mines has recently initiated a study of cost-effectiveness of mine safety measures which, if not definitive, at least provides a model for future research and development¹¹. A summary of their recommendations with estimates of cost to the industry together with an estimate of lives saved annually is seen in Table 2. These recommendations are not, however, mutually exclusive nor additive. They also ignore savings that might accrue from increased productivity of new techniques, reduced compensation insurance costs, and reductions in non-fatal accidents.

Pulmonary disease

The Federal Coal Mine Health and Safety Act of 1969 establishes pneumoconiosis as a compensable disease occupationally related to coal mining, based on the observation that both morbidity and mortality from pulmonary disease, regardless of cause, is very significantly increased among coal miners¹². Though the relationship between coal dust inhalation and pulmonary disability remains uncertain¹³⁻¹⁵, some estimate of pulmonary disability from coal mining seems in order.

Since no single objective measure of coal-workers pneumoconiosis relates well with clinical disability, miners' complaints of shortness of breath (dyspnea), are the best measure available. Summing the percentage of men in all age groups with severe dyspnea, the increased frequency of disability is approximately $\frac{1}{2}$ % per year of underground mining experience¹⁴. Most men over 45 with severe dyspnea are disabled, that is, non-working, whereas most men with no or slight dyspnea, both under and over 45, are active. Although this estimate does not fit the data with precision, it does not seem to be an extreme view.

Applying the 0.5% yr formula to the 600 men providing coal to a 1,000 MWe electrical station, 3 men per year would be disabled. Since the Social Security Administration estimates the median age at first application for disability for this disease among miners as 57.1 yr¹⁶, and assuming a normal working life to age 65, then an average of 7.9 yr of working life are lost per man afflicted. Calculating 300 working days per year, each day valued at \$50 d⁻¹, total cost per electrical station per year would be \$355,000.

Table 2 Cost-effectiveness of various improvements in coal mine engineering

Equipment	Annual cost ($\times \$ 10^6$)	Lives saved (yr ⁻¹)
Temporary roof support system	1.62	22
Permanent roof support system	1.5	6
Maintenance and repair	15.2	10
Operator protection on face equipment	1.306	10
Shuttle haulage	40.0	12
Machine loading roof fall accidents	0.045	5
Alternative equipment modifications	(6.611)*	11
Auger-type continuous mining accidents	0.150	6
Performance of miscellaneous hand operations—roof scaling	0.180	8
Surveying	0	1
Brattice maintenance	0.240	3

* Source: abstracted from data in reference 11.

Table 3 Emissions from coal-fired electrical generating stations ranked by effect

Pollutant	Total US emissions (10 ⁶ tons)	Coal-fired steam plants (10 ⁶ tons)	Effect factor		
		(A)	(B)	(A \times B)	%
SO ₂	33.4	15.8	15.3	242.0	62.0
Aerosol	35.2	3.7	21.5	79.5	20.3
NO ₂	23.8	3.1	22.4	69.4	17.7
CO	115.4	0.2	1	0.2	0.1
Total	207.8	23.8		391.1	100.0

Source: ref. 25.

These estimates are based on experience with technology and regulatory standards for dust control which are now replaced by newer mining conditions. But because those characteristics of coal dust which produce pulmonary disease are unknown, there can be no confidence that current dust control standards and procedures will be effective in reducing disease. Furthermore, modern coal mining equipment tends to create more dust, not less.

The Bureau of Labor Statistics does not list accident rates for steam plant operation as a separate category. Information is available, however, from the Tennessee Valley Authority's Colbert and Gallatin plants (personal communication) which both approximate the 1,000 MW plant which serves as my model. Averaging the experience for 1967-71, 6.65×10^6 man-hours were worked annually at each plant with 388.5 d lost because of accidents. There were no fatalities.

Transport of coal

At present 2,300 people are killed annually in the United States by railroad trains, and more than 20,000 persons injured, mostly at grade crossings¹⁷. There were 27,015,000 carloads of freight hauled by railroads in 1970 and 5,296,000 of these were coal. Since half of the coal consumed in the country is destined for steam stations, it can be calculated that about one tenth of all railroad cars are hauling coal to electrical generating plants. If that assumption is correct, then about 230 people are now being killed annually and 2,000 persons injured from transport needs for electrical generation from coal. Since this generation capacity represents the equivalent of approximately 180 stations of 10³ MWe each, 1.3 deaths would occur for each plant. In the absence of any data on the magnitude of disability associated with non-fatal accidents, no charge can reliably be assessed for these losses. Nor is there any charge for other modes of transportation, that is, barge or truck.

Combustion of coal

Goldsmith has reviewed episodes¹⁸ of intense smog which are generally associated with a transient increase in the incidence of death from cardiopulmonary disease among the elderly and chronically ill. This may only reflect a life shortening of a few weeks or months in those already ill, since analysis over several years does not show correlative relationships with air pollution levels. It is more difficult to assess effects on induction of chronic disease from more moderate concentrations of pollutants. Unfortunately, too many other variables have effects which cannot be distinguished from those of the combustion products of fossil fuels (see, for example, ref. 19). There is also the difficulty of identifying the relative effect of each of the air pollutants.

Most attention has focused on sulphur dioxide which can produce acute toxicity in both human and animal populations²⁰. The necessary concentrations are, however, far greater than those found in urban air. Acute smog episodes, such as those of London, 1952 (ref. 2) and Donora, Pennsylvania, 1948 (ref. 22) have produced increased mortality with SO₂ levels which cannot be demonstrated to produce unequivocal human disease.

The earliest published attempt at estimating the cost of health from air pollution is that of Ridker²³ who estimated that 18 to 20% of the approximately \$2 billion in national health costs for respiratory diseases are due to air pollution. Thus the damage to health from air pollution in 1958 was between \$360 and \$400 million.

The Lave-Seskin study³ expands the scope of disease covered by Ridker. In particular they take into account air pollution damages to health in the forms of heart disease and of cancers of the stomach, oesophagus and bladder. Approximately half the lost income and current medical expenses associated with morbidity and mortality from bronchitis are ascribed to air pollution. The coefficient for lung cancer is estimated to be 0.25. In other disease categories air pollution is held responsible for an estimated 15% of the damages associated with non-respiratory lung cancers, 25% of all respiratory diseases and 10% of cardiovascular diseases. These coefficients were estimated by regressions that were run on data from published epidemiological studies.

The total annual reduction in health costs which Lave and Seskin anticipate from a 50% reduction in air pollution is \$2,080 million. Scaling up to 100% implies a total cost of \$4,160 million annually. An Environmental Protection Agency evaluation²⁴ has updated this figure calculated in 1963, to \$6,060 million to account for inflation.

The method of regression analysis as a technique for assessing health effects of air pollutants is not definitive; but in the absence of other data I shall use this value of \$6,060 million for evaluating health costs of coal-combustion products.

Of the total tonnage of United States air pollutants shown in Table 3, coal burned for electrical generation is responsible for approximately 8% (ref. 25). To apply this percentage to the total air pollution health cost of \$6.06 billion would be to treat all pollutants as having equally damaging effects on a weight basis. Walther²⁶ has calculated an 'effect factor' for each pollutant on the basis of its toxicity as reflected by primary air quality standards. The technique is obviously crude, but the health impact of any pollution source can be roughly estimated by multiplying the annual emission of each pollutant by the effect factor. This (Table 3) produces an annual estimate of \$357.5 million for the nation as a whole or approximately \$2 million per 1,000 MW plant. Such an evaluation would clearly be high because of the implicit assumption that all emission sources contribute equally to ground level concentrations, and ignores the fact that the use of high stacks at steam plants very much increases dispersion and therefore reduces ground level concentration.

British studies demonstrate that even in the neighbourhood of a modern 1,000 MW electric plant less than 5% of ground level SO₂ can be attributed to plant emissions. This was equivalent to adding only 0.1 to 0.2 parts per hundred million (p.p.h.m.) SO₂ to average background levels measured at the worst point. At greater distances where population density would usually be greater than in the immediate plant area, ground concentrations of SO₂ contributed by the plant would be in the range of 0.01–0.02 p.p.h.m., which is in the order of 1% of background levels. SO₂ generated by steam plants

cannot be detected beyond 20 mile even under the worst meteorological conditions. Nevertheless, I shall arbitrarily accept the value of 1% of the \$357.5 million cost estimated from the tonnage of pollutants emitted from coal-steam plants. This produces an estimated total cost for the 180,000 MW of coal-generated electric power of \$3.6 million (\$20 thousand per 1,000 MW electric plant).

Wilson has estimated from Norwegian data a damage function for ambient SO₂ levels of 10,000 deaths per million "man-concentrations" (a man concentration is equal to number of persons exposed, multiplied by average exposure to SO₂ in p.p.m. averaged over one week)²⁸. Estimating the population within 20 miles of a steam plant from recent environmental impact statements, it would appear that 400,000 people live within this vicinity. Exposure to 0.02 p.p.h.m. for one year would produce, on the basis of the Wilson estimate, 0.8 death.

Although the evidence relating the health effects of air pollution to its sulphur dioxide content is frail, environmental control efforts focusing on reduction of ambient sulphur dioxide levels have been adopted with little opposition. Techniques for the removal of SO₂ from stack gases are still relatively unproven; however, the US Bureau of Mines estimates that for the technique receiving the greatest interest commercially, lime/limestone scrubbing, costs (including capital charges) are in the range of 1.0 to 0.25 cents per kW h. This system has not yet proved capable of removing more than 30 to 50% of the sulphur dioxide present. At best, stack gas removal systems are not expected to remove more than 75% of the SO₂ present. For most United States coal, this degree of removal would not be sufficient to reach current ambient air standards²⁹. Clarke, Lucas and Ross³⁰ have calculated costs of reducing ground level concentrations from a coal-burning steam plant (Fig. 1). They conclude that stacks are far more economical than sulphur removal systems for reducing ground level concentration.

Adopting the meteorological and cost assumptions³⁰, shown in Fig. 1, and the damage function of Wilson (1 death per 100 man-concentrations) Table 4 was constructed. A population density of 320 persons km⁻² was also assumed producing an expectation of 42 deaths annually with a stack height of 30 m. Intuitively, this result clearly seems unlikely, probably implying a highly inflated risk factor.

With ground level concentrations converted to mortality data, an assumed value of life can be applied to evaluate cost-effectiveness of reducing sulphur dioxide concentrations. The precision of the numbers may give the illusion of precise knowledge which is, in fact, not available.

Accepting the life value used in this study (\$300,000) or even a far smaller one, tall chimneys are clearly cost-effective, whereas sulphur removal processes seem relatively cost-ineffective except in the unlikely case of sources near ground level. This implies that scarce, low-sulphur fuels should be reserved for ground level sources whereas tall stacks should be used for major industrial sources such as steam plants. This conclusion ignores questions of property damage or possible benefits to agriculture from sulphur contamination.

Total human costs of operating a 1,000 MW station are summarised in Table 5. Although these costs are indeed large

Table 4 Cost effectiveness of stacks and sulphur removal in reducing mortality

Chimney height (m)	Capital cost (\$)	Chimney alone		Plus 50% S removal (\$5,000,000)		Plus 75% S removal (\$10,000,000)	
		Annual deaths	Implicit life value* (\$)	Additional lives saved	Implicit life value (\$)	Additional lives saved	Implicit life value (\$)
30	0	42	0	21	238,000	31.5	318,000
70	120,000	23	222	11.5	434,000	17.3	578,000
100	333,000	11	358	5.5	910,000	8.25	1,220,000
120	575,000	4	504	2	2,500,000	3	3,330,000
170	1,600,000	0	1,270	0	infinite	0	infinite

* Implicit life value = total reduction in mortality over 30 yr plant life divided by capital cost.

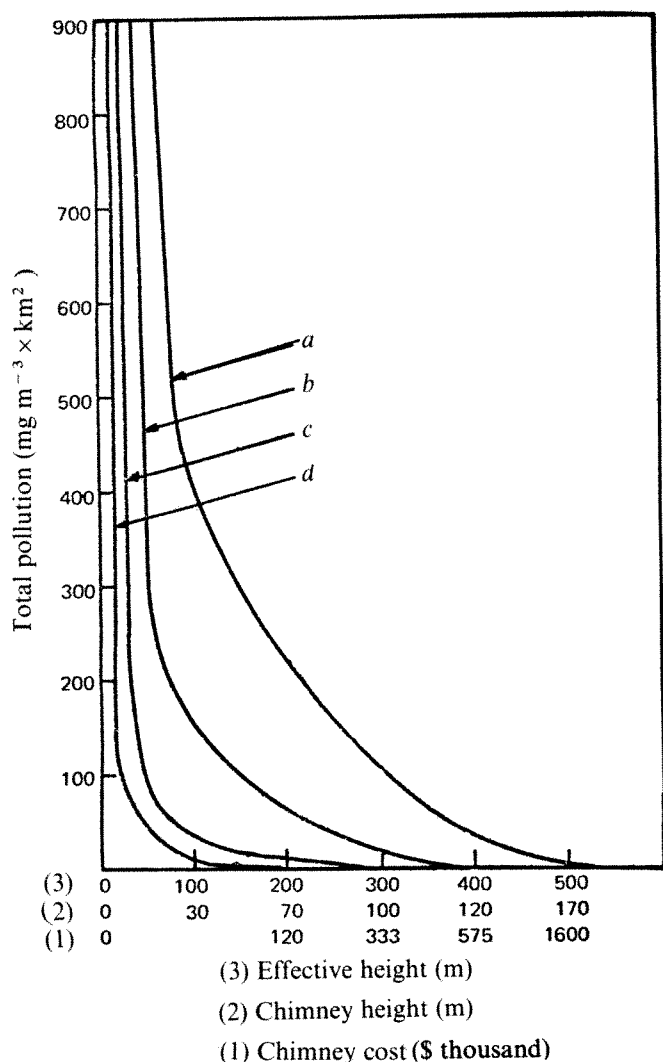


Fig. 1 Dependence of total pollution on source strength and chimney height. Threshold: $100 \mu\text{g m}^{-3}$; wind speed: 5 m s^{-1} . Source strength of SO_2 (kg s^{-1}) and capitalised cost of its removal: a, 1, \$0; b, 5, \$ 5 million; c, 25, \$ 10 million; d, 125, \$ 15 million.

in human terms, they are small compared to annual revenues generated by such a plant. Assuming a load factor of 67% and sales at 2 cents per kW h, annual revenue from such a plant would be about \$29 million.

Mining of coal is seen to account for approximately 50% of all health costs associated with the generation of electric energy from coal. Strip mining is only about 15% as likely to cause death and probably provides a similar advantage in terms of coal workers' pneumoconiosis. This phenomenon receives little attention from those who are attempting to limit or eliminate surface mining in favour of the far more hazardous

underground mining. Still, opportunities for accident reduction in underground mining which are highly cost-effective seem to be available but are so far unexploited.

On the other hand, air pollution costs seem to be small compared to other health costs of coal-steam plants—less than 5%. Sulphur removal techniques at their present state of development cannot be shown to be cost-effective, particularly in contrast to stacks and other operational control procedures. Because of the greater effectiveness of the latter, regulatory control would rationally relate to ambient air standards where the operating utility could avail itself of cost-effective techniques rather than to effluent standards where the utility must resort to technology which is clearly cost-ineffective. Furthermore, ambient controls are more likely to reduce levels of other pollutants a corresponding amount whereas effluent controls are likely to be specific to SO_2 . Since the health hazard of air pollution from the combustion of coal is not known to be related to SO_2 exclusively, the importance of this effect is obvious.

It may be useful to compare this estimate of health costs with my estimates for a nuclear power plant of equal power rating⁴. Total costs for 10,030 MWe of the current United States nuclear industry were evaluated at \$2,175,520. For a single 1,000 MWe nuclear plant, cost would be \$217,552 or less than 20% of the annual cost of the coal fuel plant. Although there are uncertainties in both estimates, it would seem likely that the nuclear plant is clearly less hazardous than the coal-fired plant. An official Swedish study³¹ comparing nuclear plants with oil fuel plants reached essentially the same conclusion although the spread between the two fuel sources was found to be considerably greater. Lave has reached a similar result³².

These conclusions refer to normal operating conditions and ignore possible differences in accident potential, for which precise data are not available. Recently Starr *et al.*³³ have analysed and compared accident probabilities for nuclear plants and fossil-fuelled generating plants. They conclude that the probability of accidental death from the nuclear plant is smaller than from a similar fossil-fuelled plant.

The greatest weakness in this analysis is the estimate of health costs of air pollution. Ultimately, the most significant estimates will follow establishment of damage functions for each pollutant and the ability to evaluate total human impact of such exposures from known meteorological and chemical behaviour in the environment.

¹ Solow, R. M., *Science*, **173**, 498 (1971).

² Netschert, B., *Bull. atomic Sci.*, **27**, 13 (1971).

³ Lave, L. B., and Seskin, E. P., *Science*, **169**, 723 (1970).

⁴ Sagan, L. A., *Science*, **177**, 487 (1972).

⁵ *USA Standard*, **716**, 1 (1967).

⁶ *Cumulative Regulatory Effects on the Cost of Automotive Transportation (RECAT)* (Office of Science and Technology, DC 20506, February 28, 1972).

⁷ Dinman, B. D., *Science*, **175**, 495 (1972).

⁸ Rice, D. P., *Public Health Service Publication* 947-6 (US Department of Health, Education and Welfare, Washington, D.C., May, 1966).

⁹ *Injury Experience and Worktime in the Mineral Industries, 1969-70* (Mineral Industry Surveys, US Dept. Interior, Bureau of Mines, Washington, DC (1971)).

¹⁰ Scott, J. J., *Nat. Safety Congress Trans.*, **7**, 9 (1971).

¹¹ *U.S. Bureau of Mines Contract Report* 50110601 (Department of Interior, Bureau of Mines, National Technical Information Service, Springfield, Virginia, 1971).

¹² Enterline, P. E., *Ann. N.Y. Acad. Sci.*, **200**, 260 (1972).

¹³ Rossiter, C. E., *Trans. Soc. Occup. Med.*, **22**, 83 (1972).

¹⁴ Lainhart, W. S., and Morgan, K. C., in *Pulmonary Reactions to Coal Dust: A Review of US Experience*, (edit. by Key, M.M., Kerr, L. E., and Bundy, M.) 30-58 Academic Press, N.Y. (1971).

¹⁵ Morgan, W. K. C., Reger, R. B., and Handelsman, L., *J. Am. Med. Ass.*, **223**, 922 (1973).

¹⁶ *Public Health Service Publication No. 1531* (U.S. Government Printing Office, Washington, D.C., 1967).

¹⁷ *United States Census*, U.S. Bureau of the Census (Washington, D.C., 1971).

Table 5 Summary of annual health costs for 1,000 MW coal-fired electrical station

		\$
Miners	Deaths	171,000
	Accidents	288,000
	Pneumoconiosis	355,000
Transport	Deaths	380,000
	Accidents	*
	Station operation	38,850
	Air pollution	20,000
	Total	1,252,000

* No estimates available.

- ¹⁸ Goldsmith, J. R., in *Air Pollution*, (edit. by Stern, A. C.), 534 (Academic Press, New York, 1968).
- ¹⁹ *Air quality for particulates* (U.S. Department of Health, Education and Welfare, National Air Pollution Control Administration, December, 1968).
- ²⁰ Stokinger, H. E., and Coffin, D. L., in *Air Pollution*, (edit. by Stern, A. C.), 446–533 (Academic Press, New York, 1968).
- ²¹ *Ministry of Health Reports on Public Health and Related Subjects*, No. 95 (H.M. Stationery Office, London, 1954).
- ²² Schrenik, H. H., Heinmann, H., Clayton, G. D., Gafefer, W. M., and Wexler, H., *U.S. Public Health Service Bull.*, **306** (U.S. Public Health Service, 1949).
- ²³ Ridker, R. G. *Economic Costs of Air Pollution; Studies in Measurement* (Frederick and Praeger, New York, 1967).
- ²⁴ Barrett, T. B., and Waddell, T. E., *The Cost of Air Pollution Damage: A Status Report* (Appendix I–J of ref. 6).
- ²⁵ Environmental Protection Agency, Office of Air Programs (Division of Applied Technology, May, 1971).
- ²⁶ Walther, E. G., *J. Air Pollution Control Ass.*, **22**, 352 (1972).
- ²⁷ Stone, C. N., and Clarke, A. J. (Presented at American Power Conference, Selinor's Institute of Technology, April 27, 1967).
- ²⁸ Wilson, R., *Science*, **178**, 182 (1972).
- ²⁹ Mills, G. A., and Perry, H. (Presented before the Division of Petroleum Chemistry, American Chemical Society, N.Y. Meeting August, 1972).
- ³⁰ Clarke, A. J., Lucas, D. H., and Ross, F. F. (Presented at Second International Clean Air Conference, Washington, D.C., December, 1970).
- ³¹ Bergstrom, S. O. W., in *Proc. Fifth Annual Health Physics Society, Midyear Symposium*, 29–54 (Burton R. Baldwin, Idaho Falls, Idaho, 1970).
- ³² Lave, L. B. (Presented at Sierra Club Conference on Environmental Effects of Electricity Generation, Johnson, Vermont, January, 1972).
- ³³ Starr, C., and Greenfield, M. A. (Report prepared for the Resources Agency of California, University of California, Los Angeles, 1972).

Are BL Lac-type objects nearby black holes?

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Isolated black holes accreting interstellar gas can account for the salient properties of the BL Lac-type objects. The observed frequency spectra in the visible and infrared, the rapid variations in intensity and polarisation, and the absence of discrete features in the optical spectra are all consequences of the adopted black hole accretion model.

STRITTMATTER *et al.*¹ have discussed the possible existence of an entirely new class of astronomical objects with the following properties: (1) extremely rapid variations in the radio, infrared, and optical intensity; (2) most of the energy is emitted at infrared wavelengths; (3) absence of emission or absorption lines in their optical spectra; and (4) substantial and variable polarisation at optical and radio wavelengths. The prototype and most extensively studied object in this class is the variable star BL Lacertae (VRO42.22.01). Other Lacertids, as members of this class are now known, include OJ287 (VRO20.08.01), ON231 (W COM), ON325 (B2 1215+30) and PKS1514-24 (AP Lib).

Optical and near infrared spectrophotometry of the objects¹⁻⁴ reveal a steepening decline in the flux density from 10^{14} to 10^{15} Hz. The spectral shape, together with the observed rapid variations in intensity and polarisation, indicates that the observed radiation from the Lacertids results from nonthermal processes. The rapid variations further suggest that the emission originates from a very small region ($\leq 10^{17}$ cm). As a consequence of their nonthermal spectra, compactness and variability, these objects have been likened to quasistellar objects. But no redshifts can be measured because of the absence of spectral lines; so their distances remain unknown. Moreover, in the case of BL Lac, the very steep slope of the spectral-energy distribution above $10^{14.5}$ Hz suggests that this object is not a QSO (refs 3, 5).

As an alternative, we wish to point out that isolated black holes accreting interstellar gas can account for the characteristic properties of the Lacertids. The suggestion that these compact nonthermal sources may be massive ($\geq 10^4 M_\odot$) black holes has already been proposed by Pringle *et al.*⁶. Here we discuss the possibility that the BL Lac-type objects are nearby, stellar-mass black holes ($1 M_\odot \leq M \leq 100 M_\odot$) in the galactic disk.

Basic model

The total luminosity and frequency spectrum of radiation emitted by interstellar gas accreting on to a black hole have recently been calculated for spherically symmetric, steady state accretion^{7,8}. In this case, if there is a finely tangled and randomly oriented magnetic field in the interstellar gas, the rapid reconnection of oppositely directed radial field lines promotes an equipartition of magnetic and gravitational energy throughout the infalling plasma, ($B^2/8\pi \approx GM\rho/r$). Synchrotron radiation by relativistic electrons gyrating around the magnetic field lines is the principal radiation mechanism in the gas, resulting in a total observed luminosity

$$L_s \approx 5 \times 10^{30} (T_0/10,000 \text{ K})^{-3} (M/10 M_\odot)^3 (n_0/10 \text{ cm}^{-3})^2 \text{ erg s}^{-1} \quad (1)$$

provided $\beta = (T_0/10,000 \text{ K})^{-3/2} (M/10 M_\odot) (n_0/10 \text{ cm}^{-3}) \ll 10$. Here, n_0 and T_0 are the density and temperature, respectively, of the ambient gas and M is the mass of the black hole. If the black hole is moving supersonically through a uniform medium with a velocity V , the results of the calculations still apply provided we set $kT_0 \approx m_i V^2$, where k is Boltzmann's constant and m_i is the ion mass. The calculated spectral energy distribution falls sharply with frequency throughout the range $v_1 \leq \nu \leq v_2$, where

$$v_2 \approx 7 \times 10^{15} (n_0/10 \text{ cm}^{-3})^{1/2} (T_0/10,000 \text{ K})^{-3/4} \text{ Hz} \quad (2)$$

is the peak synchrotron emission frequency in the region immediately outside of the Schwarzschild radius at $r = 2m$ = $2.95 \times 10^6 (M/10 M_\odot) \text{ cm}$ and

$$v_1 \approx 2 \times 10^{13} (n_0/10 \text{ cm}^{-3})^{40/49} (T_0/10,000 \text{ K})^{60/49} (M/10 M_\odot)^{31/49} \text{ Hz} \quad (3)$$

is the characteristic frequency below which synchrotron radiation is self-absorbed by the gas.

Comparison with observations

The continuous emission spectrum is plotted in Fig. 1 for the accretion of ionised hydrogen with $T_0 = 10,000 \text{ K}$ (corresponding to $V \approx 10 \text{ km s}^{-1}$) on to a single black hole. Emission spectra are shown for various interstellar gas densities and black hole masses and the results are compared with the data plotted by Strittmatter *et al.* for the BL Lac-type objects. Each theoretical curve has been fitted to the observed data for BL Lac at approximately $10 \mu\text{m}$. This corresponds to placing the black hole at a distance.

$$d \simeq 10 \text{ pc } (n_0/10 \text{ cm}^{-3}) (M/10M_\odot)^{3/2} \quad (4)$$

For all curves most of the energy is emitted in the infrared part of the spectrum in agreement with the observed spectra. The theoretical curve computed for $n_0 = 10 \text{ cm}^{-3}$ and $M \simeq 10 M_\odot$ is in reasonable agreement with the observed spectral energy distribution of BL Lac in the visual and infrared to $10 \mu\text{m}$.

Irregular time variations in intensity and polarisation are a natural consequence of an accretion model. Inhomogeneities in the ambient medium as well as instabilities and turbulence associated with the flow will inevitably lead to significant fluctuations in the total emission from the gas. These fluctuations are expected to occur over a variety of time scales. For example, intensity variations may result when the hole moves out of one turbulent cell of interstellar gas and into another⁹. The time scale for such variations is roughly

$$\Delta t_{\text{urb}} \simeq r_c/u_0 \simeq (10 \text{ yr}) (M/10M_\odot) (u_0/10 \text{ km s}^{-1})^{-3} \quad (5)$$

where $r_c = GM/u_0^2 \simeq 5 \times 10^{14} (M/10M_\odot) (u_0/10 \text{ km s}^{-1})^{-2} \text{ cm}$ is the capture radius of the black hole and $u_0 = (a_0^2 + V^2)^{1/2}$, where a_0 is the ambient sound velocity. On shorter time scales magnetic field reconnection, which is believed to be responsible for solar flares (see ref. 10 and references therein) may lead to strong flaring in the luminosity of the accreting gas. In the proposed accretion model the largest scale size associated with magnetic reconnection is roughly r^* , where r^* is the radius at which the frozen-in magnetic field has been sufficiently amplified by the compression of the infalling fluid that $B^{*2}/8\pi \approx GM\rho^*/r^*$. In the zone $r_c \geq r \geq r^*$, lying just above the reconnecting region, the field is frozen in, requiring that $B \simeq B_0(r_c/r)^2$; so the reconnection rate at r^* , is given by

$$\Delta t_{\text{rec}}^* \simeq r^*/v_A^* \simeq (0.01 \Delta t_{\text{urb}}) (B_0/3 \times 10^{-6} \text{ gauss})^2 (n_0/10 \text{ cm}^{-3})^{-1} (u_0/10 \text{ km s}^{-1})^{-2} \quad (6)$$

where v_A^* is the Alfvén speed at r^* and B_0 is the ambient field strength. Magnetic reconnection will occur throughout the region $r^* \geq r \geq 2m$, maintaining an equipartition of magnetic and gravitational energy in the plasma. The longest timescale associated with magnetic flaring is $\Delta t_{\text{mag}} \approx \Delta t_{\text{rec}}^*$ or

$$\Delta t_{\text{mag}} \simeq (0.1 \text{ yr}) (B_0/3 \times 10^{-6} \text{ gauss})^2 (n_0/10 \text{ cm}^{-3})^{-1} (M/10M_\odot) (u_0/10 \text{ km s}^{-1})^{-5} \quad (7)$$

A sudden release of magnetic field energy and magnetic pressure should accompany each flare, causing an enhanced flow of plasma toward the hole and a corresponding burst of luminosity lasting a period Δt_{mag} . Finally, the shortest timescale for luminosity fluctuations is presumably associated with the infall rate (\simeq reconnection rate) near the Schwarzschild radius,

$$\Delta t_{\text{min}} \simeq 2GM/c^3 \simeq (10^{-4} \text{ s}) (M/10M_\odot) \quad (8)$$

The random outbursts lasting ~ 1 month which occur on BL Lac and OJ 287 (refs 11–14, 18) agree with the predicted duration of a magnetic flare, assuming stellar-mass black holes and typical interstellar conditions (see equation (7)). BL Lac also exhibits more gradual luminosity modulations lasting about 10 yr (ref. 12). This long period variability may be associated with inhomogeneities in the interstellar medium in accordance with equation (5). Similar optical variations, occurring on a timescale of years, have been observed in OJ287 (refs 14, 15). Short period variability ($\ll 1$ d) has also been observed for these two objects^{3,14–17}, as would be expected from an accretion model.

A net polarisation of the emitted synchrotron radiation would not occur in the case of spherically symmetric flow and finely tangled and randomly oriented fields. But inhomogeneities in the ambient medium, angular momentum of the gas, instabilities and turbulence may all lead to a highly variable, random net polarisation. The polarisation variations would then be correlated with the flaring activity of the source, a result consistent with the observations^{5,18}.

As shown by the dotted lines of Fig. 1, the synchrotron radiation is strongly self absorbed below $\nu \sim \nu_1$; so another radiation mechanism must be invoked to explain the radio emission from the objects. In the infalling plasma, conditions are favourable for the generation of radio emission through

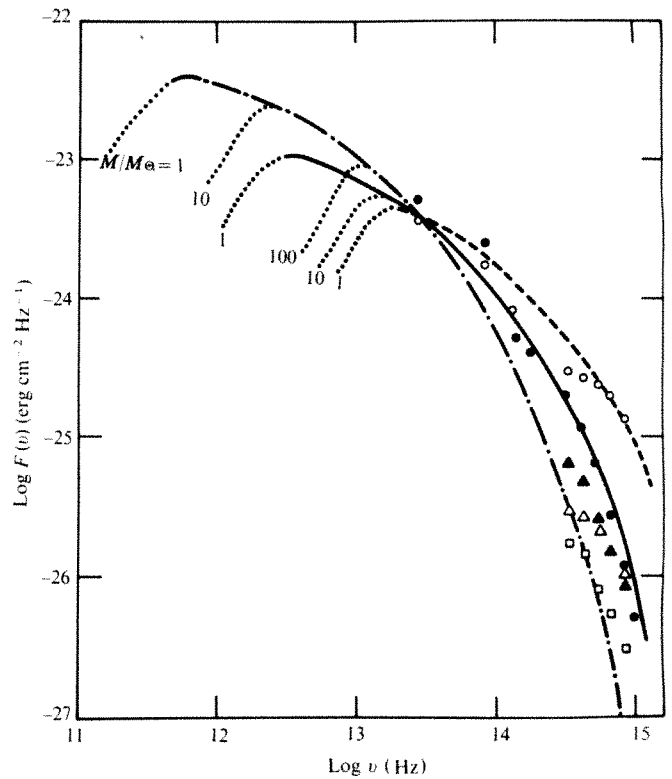


Fig. 1 The continuous emission spectrum from interstellar gas accreting on to a black hole compared with the observed spectra of the BL Lac-type objects as plotted in Strittmatter *et al.*¹. The gas consists of pure hydrogen with $T_0 = 10,000 \text{ K}$ ($V \approx 10 \text{ km s}^{-1}$); each curve is labelled by the corresponding value of black hole mass M . The dotted lines indicate synchrotron self-absorption. ●, BL Lac; ○, OJ287; □, ON231; △, B2 1215+30; ▲, AP Lib. — · — ·, $n_0 = 1 \text{ cm}^{-3}$; —, $n_0 = 10 \text{ cm}^{-3}$; — — —, $n_0 = 100 \text{ cm}^{-3}$.

coherent processes. Because of steep density and temperature gradients, longitudinal plasma waves and transverse electromagnetic waves will be coupled in the plasma. The possibility then exists for converting some of the electrostatic energy contained in plasma oscillations into radiation, a process which has been proposed as the origin of the enhanced radio emission from active regions on the Sun^{19,20}. The emission from a particular region is confined to the narrow frequency band $\nu_p < \nu < 1.4 \nu_p$, where $\nu_p = (e^2 n / \pi m_p)^{1/2}$ is the plasma frequency. In the model, the radio emission in a given frequency band centred about ν originates from the thin spherical shell of gas at r in which $\nu \simeq \nu(r)$, where

$$\nu_p(r) \simeq 2 \times 10^{10} (2M/r)^{3/4} (n_0/10 \text{ cm}^{-3})^{1/2} (u_0/10 \text{ km s}^{-1})^{-3/2} \text{ Hz} \quad (9)$$

If the emission is proportional to the available flux of gravitational potential energy passing across each shell ($L \propto GM\dot{m}/r$, where \dot{m} is the accretion rate in gm s^{-1}) then the radio emission spectrum will vary as

$$L_\nu \propto \nu^{1/3}, \nu \leq \nu_p(2m) \quad (10)$$

in rough agreement with observations below 10^{11} Hz (ref. 1). For each of the BL Lac-type objects the radio emission is only a small fraction of the total luminosity. In the case of BL Lac, $L_{\text{radio}}/L_{\text{IR}} < 0.04$. Since the proposed synchrotron emission process itself converts only a small fraction of the available gravitational energy into radiation ($\sim 0.01\beta$), the conversion of gravitational energy into radiation by plasma oscillations can be quite inefficient and still produce the required flux.

Predictions

Model calculations⁷ predict that, in addition to infrared and optical radiation, a high energy tail of γ -ray radiation between 1 and 10 MeV will be emitted due to electron-proton and electron-electron bremsstrahlung in the plasma. The total bremsstrahlung luminosity is

$$L_B \simeq 2 \times 10^{26} (T_0/10,000 \text{ K})^{-3} (M/10M_\odot)^3 (n_0/10 \text{ cm}^{-3})^2 \text{ erg s}^{-1} \quad (11)$$

Unfortunately, for typical interstellar conditions and stellar-mass black holes, this γ -ray flux is far below the detectability limit of modern devices, even for nearby objects.

Rough estimates indicate that there may indeed be a finite number of stellar-mass black holes close to the Earth as required by the theory. From the known stellar birth rate function in the disk, stellar evolution time scales, and numerical computations of supernova explosions, it has been estimated⁹ that as much as 1% of the mass of the Galaxy may now be in the form of black holes with masses $\geq 10M_\odot$. This implies a local space density of black holes

$$n_{bh} \sim 2 \times 10^{-3} \text{ pc}^{-3}, \quad (12)$$

indicating that there could be eight such objects within 10 pc of the Earth.

A proper evaluation of the proposed model is not possible without a reliable determination of the distances to the objects. According to Pigg and Cohen²¹, the 21-cm absorption profile of BL Lac suggests that the object is at least 200 pc away and may be extragalactic. This lower limit would be substantially overestimated if a dense interstellar cloud is associated with BL Lac. If BL Lac and the other such objects are located within a few tens of parsecs of the Earth, their proper motions could be detected by both optical and radio techniques.

If it is determined that the BL Lac-type objects do indeed lie outside of the galactic disk, a black hole accretion model may still apply if any of the following are true:

(1) The infalling material possesses sufficient angular momentum to form an accretion disk about the black hole. In this case, the conversion of gravitational energy into infrared radiation *via* synchrotron emission may be far more efficient than in the case of spherical accretion, even for stellar-mass objects and typical interstellar conditions.^{6,22}

(2) The Lacertids are clusters or loose associations of stellar-mass black holes.

(3) The Lacertids are supermassive black holes situated in the galactic halo or at greater distances⁶.

These three possibilities clearly require further theoretical investigation.

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- ¹ Strittmatter, P. A., Serkowski, K., Carswell, R., Stein, W. A., Merrill, K. M., and Burbidge, E. M., *Astrophys. J. Lett.*, **175**, L7 (1972).
- ² Visvanathan, N., *Astrophys. J. Lett.*, **155**, L133 (1969).
- ³ Oke, J. B., Neugebauer, G., and Becklin, E. E., *Astrophys. J. Lett.*, **156**, L41 (1969).
- ⁴ Visvanathan, N., *Astrophys. J.*, **185**, 145 (1973).
- ⁵ Visvanathan, N., *Astrophys. J.*, **179**, 1 (1973).
- ⁶ Pringle, J. E., Rees, M. J., and Pacholczyk, A. G., *Astr. Astrophys.*, **29**, 179 (1973).
- ⁷ Shapiro, S. L., *Astrophys. J.*, **180**, 531 (1973).
- ⁸ Shapiro, S. L., *Astrophys. J.*, **185**, 69 (1973).
- ⁹ Novikov, I. D., and Thorne, K. S., in *Black Holes: Les Houches* (edit. by DeWitt, C.) 243 (Gordon and Breach, London, 1973).
- ¹⁰ Parker, E. N., *Astrophys. J.*, **180**, 247 (1973).
- ¹¹ Tritton, K. P., and Brett, R. A., *Observatory*, **90**, 110 (1970).
- ¹² Shen, B. S. P., and Usher, P. D., *Nature*, **228**, 1070 (1970).
- ¹³ MacLeod, J. M., Andrew, B. H., Medd, W. J., and Olsen, E. T., *Astrophys. Lett.*, **9**, 19 (1971).
- ¹⁴ Visvanathan, N., and Elliot, J. L., *Astrophys. J.*, **179**, 721 (1973).
- ¹⁵ Craine, E. R., and Warner, J. W., *Astrophys. J. Lett.*, **179**, L53 (1973).
- ¹⁶ Bertaud, Ch., Dumortier, B., Veron, P., Wlenick, G., Adam, G., Bigay, J., Garnier, R., and Durvy, M., *Astr. Astrophys.*, **3**, 436 (1969).
- ¹⁷ Racine, R., *Astrophys. J. Lett.*, **159**, L99 (1970).
- ¹⁸ Williams, W., Rich, A., Kupferman, P., Ionson, J., and Hiltner, W., *Astrophys. J. Lett.*, **174**, L63 (1972).
- ¹⁹ Shklovsky, I. S., *Astr. J. USSR*, **23**, 333 (1946).
- ²⁰ Field, G. B., *Astrophys. J.*, **124**, 555 (1956).
- ²¹ Pigg, J. C., and Cohen, M. H., *Publ. astr. Soc. Pacif.*, **83**, 680 (1971).
- ²² Bisnovatyi-Kogan, G. S., and Ruzmaikin, A. A., *Inst. Appl. Math. Acad. Sci., USSR*, preprint 43 (1973).

Restoration of specific immunological virginity

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The specific abrogation of an established immune response was studied by removing in vivo most of the immunocompetent lymphocytes reactive against the specific antigen, thus restoring the animal to a state of specific immunological virginity.

THE specific abrogation of the allergic response *in vivo* has been described in various experimental systems. In the induction of immunological tolerance^{1,2}, there is a central failure of the allergic response either as a result of clonal elimination or inactivation of specific antigen-sensitive cells. The existence of serum blocking factors which prevent the expression of an established allergic response has recently been described^{3,4}. In this case antigen-specific lymphocytes exist *in vivo* but fail to express their specific reactivity on account of serum blocking factors thought to be immune complexes⁵.

Here we describe another approach to the specific abrogation of an established immune response. This has been done by removing *in vivo* most of the immunocompetent lymphocytes reactive against a given antigen thereby restoring a primed animal to a state of specific immunological virginity.

Model system

The system makes use of a model^{6,7} involving cannulation of the efferent lymphatic from a single lymph node in sheep and subsequent drainage of lymph from the cannulated node. In this model it has been shown⁷ that macromolecules and lymphocytes which enter the parenchyma of a lymph node, leave the node only by way of the efferent lymphatic and cannot re-enter the peripheral blood directly from the lymph node.

We wondered whether there is an antigen-specific, selective mechanism in stimulated lymph nodes which enhances the entry into the node of lymphocytes specific for the antigen. If this is so, then by draining the efferent lymph from an antigen stimulated node, it should be possible to specifically abrogate the response of a primed animal, restoring it to a state of specific immunological virginity.

Random bred ewes from 1-4 yr old were injected with 2.5 human doses of BCG (Bacillus Calmette Guerin) and then primed to fluorodinitrobenzene (FDNB) by skin painting both ears with 100 μ l of FDNB in a 1:1 solution of acetone and olive oil. The animals were injected 10 d later with 10 mg of rabbit gamma globulin (RGG) and finally with 10 mg of NIP chicken globulin (NIP-CG). The injection schedule was phased to avoid antigenic competition⁸ and all injections were

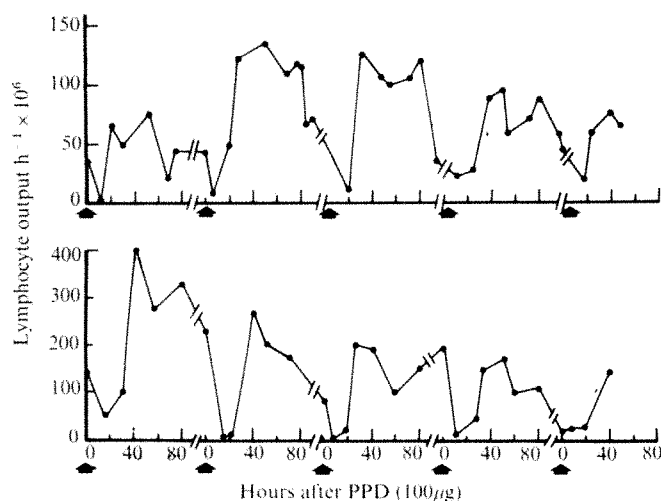


Fig. 1 Kinetics of lymphocyte output in the efferent lymph of BCG sensitive sheep after repeated stimulation of the cannulated lymph node with PPD. Heavy arrows indicate injection of 100 μ g PPD in saline.

given intramuscularly in the shoulder region. The hapten NIP (4-hydroxyl-5-iodo-3-nitrophenacetyl-) was conjugated to CG as described⁹, the resulting molar ratio being 15 NIP groups per CG molecule. Both RGG and NIP-CG were given as alum precipitates together with 4×10^8 heat killed *B. pertussis*. The sheep were thus primed to two haptens and several protein carriers.

The popliteal lymph node was then cannulated and both lymph and lymphocytes collected twice a day for 3–4 weeks. During this time PPD (purified protein derivative of tuberculin, Batch 288, Central Veterinary Laboratories, Weybridge, Surrey) was injected repeatedly into the drainage area of the node. The second local challenge with PPD was given as NIP-PPD (mean conjugation ratio 0.5 NIP groups per PPD molecule) and the anti-NIP response measured in both efferent lymph and serum. This assays for PPD-specific 'helper' T cells.

Response

After 3–4 weeks cannulation was stopped and the systemic T cell reactivity of the whole animal to PPD and other antigens was measured both by skin testing and the 'helper' T cell assay using hapten-carrier conjugates. Antibody to the hapten (NIP) is formed only if there are helper T cells present which recognise the carrier, PPD or CG (ref. 10). The hapten-binding

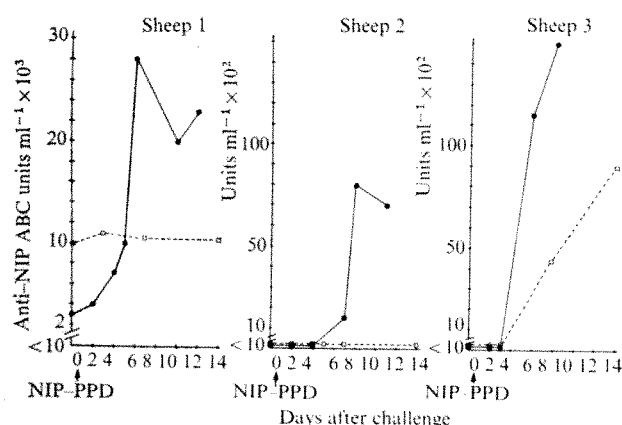


Fig. 2 Challenge of cannulated lymph nodes with NIP-PPD. Anti-NIP response in blood and efferent lymph following local challenge of the cannulated lymph nodes with NIP-PPD. Antigen binding capacity (ABC) in serum or lymph expressed as units of antibody ml^{-1} of neat serum or lymph. ●, Lymph ABC; □, serum ABC.

capacity (ABC) of the serum or lymph was measured by the Farr Assay¹¹ using 10^{-8} M N^{125}I caproic acid as hapten¹². The antigen binding capacity was calculated from the linear portion of the binding curve between 3% binding (1 unit of antibody ml^{-1}) and 30% binding (10 units of antibody ml^{-1}). Sera or lymphs were serially diluted such that two dilutions lay between 3 and 30% binding.

The kinetics of lymphocyte output in the efferent lymph of two cannulated sheep are shown in Fig. 1. In each case local challenge of the node with PPD produced a typical response in lymphocyte output characterised by a period of cell shutdown lasting 20 h followed by a large increase which is nearly always biphasic. The magnitude of the response decreased with repeated challenge.

During this response the second injection of PPD was given as NIP-PPD and the anti-NIP responses measured in both efferent lymph and blood. The first two sheep (Fig. 2) showed a brisk response to the hapten in the efferent lymph of the stimulated node. There was, however, no concurrent antibody response in the serum of these sheep, indicating that neither antigen nor antibody reaches the general circulation from the stimulated node. Thus PPD-specific T cells are present in the cannulated sheep at this stage and cooperate with hapten-specific B cells to initiate antibody formation to NIP.

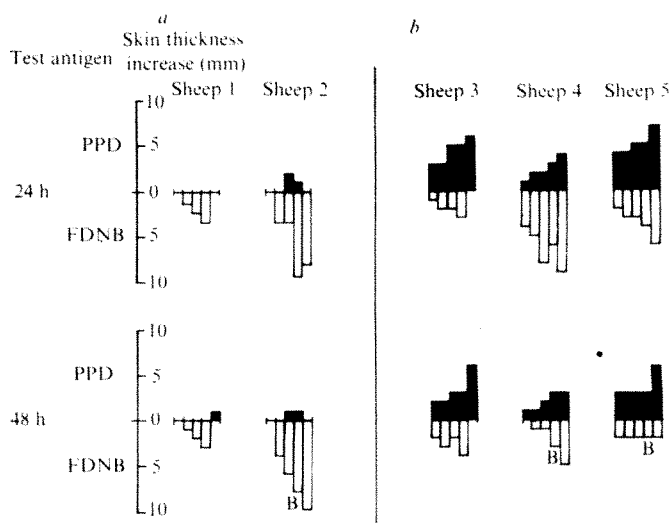


Fig. 3 Delayed hypersensitivity skin reactions to intradermal challenge with PPD and FDNB in cannulated (a) and control (b) sheep. PPD used at 6, 12, 25, 50 and 100 μ g. FDNB (100 μ l) used at concentrations 0.1, 0.2, 0.5, 1 and 2%; B, biopsy.

In the third sheep an anti-NIP response was detected in the serum as well as in the lymph. This cannulation stopped spontaneously after 8 d so it is likely that either collateral efferent lymphatics or lymphatic-venous connections had developed, affording systemic antigen escape and stimulation.

After five injections each of 100 μ g PPD into the drainage area of the popliteal lymph node, the cannulations were stopped and the sheep tested systemically for delayed hypersensitivity skin reactions to PPD and FDNB by intradermal challenge on the flank. In contrast to the control sheep (Fig. 3) the cannulated sheep were virtually skin-test negative at 24 and 48 h after intradermal challenge to concentrations of PPD ranging from 6 to 100 μ g. Two of the control sheep (3 and 4) were challenged five times at approximately 100 h intervals with 100 μ g of PPD into the drainage area of the right popliteal lymph node, before intradermal challenge. Other control sheep were not injected with PPD before intradermal challenge and produced in each case similar reactions to PPD (sheep 5).

To check the specificity of this unresponsiveness the same sheep were similarly skin tested with FDNB (Fig. 3). In each

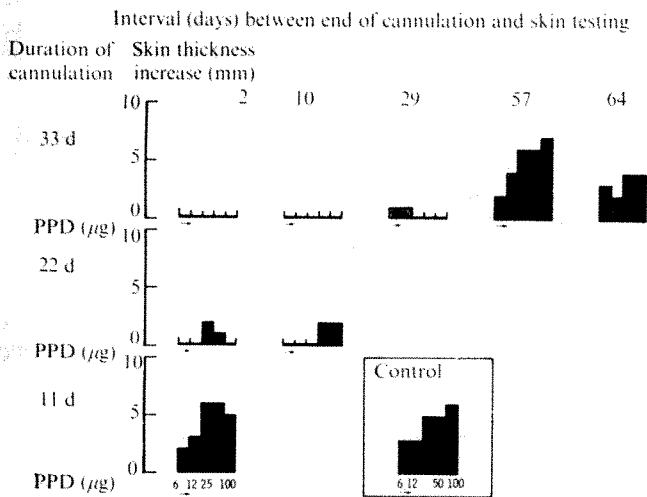


Fig. 4 Delayed hypersensitivity skin reactions to PPD in sheep cannulated for intervals of 33, 22 and 11 d and tested at several intervals after cannulation had stopped. PPD concentration as for Fig. 3.

case 100 μl of FDNB at concentrations ranging from 0.1–2% in 4:1, acetone:olive oil was painted on the left flank. As shown in Fig. 3, there was little difference between cannulated and control sheep with respect to their delayed reactions measured at 24, 48 and 62 h after challenge. In non-FDNB primed sheep (sheep 5), challenge with FDNB produced a marked inflammatory response with skin thickening which is largely due to oedema and infiltration of the site with polymorphonuclear leukocytes. This nonspecific reaction, can obscure the specific response particularly at 24 h, but by 48 and even 62 h later the inflammatory response decays whereas in primed sheep the delayed reaction persists. Skin biopsies of the reaction to 1% FDNB in primed sheep showed typical mononuclear infiltration and histologically both cannulated and control sheep were indistinguishable. Although the cannulated sheep were not skin tested with PPD before the start of cannulation it was noted that the first few injections of PPD below the lymph node produced a strong delayed reaction in the tissues surrounding the injection site.

The delayed hypersensitivity reactions to PPD of sheep cannulated for various days and then tested at frequent intervals after cannulation had stopped are shown in Fig. 4. It seems that sheep have to be cannulated for at least 21 d before they

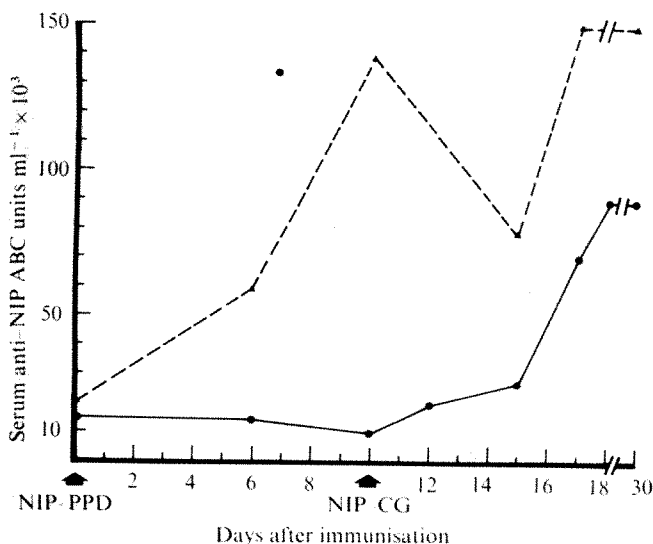


Fig. 5 Serum antihapten response in cannulated and control sheep after intravenous challenge with NIP-PPD and then NIP-CG. Serum ABC are expressed as units of antibody ml^{-1} neat serum. ●, Cannulated; ▲, control.

become specifically unresponsive to the antigen used. A sheep cannulated for only 11 d and injected three times with PPD showed little diminution in its delayed reaction to PPD. The reappearance of PPD reactivity in the sheep cannulated for 33 d is presumably due to the fact that BCG vaccination is a living infection and antigen persisting within the macrophages of the cannulated sheep stimulates virgin T lymphocytes as they emerge from the thymus.

Delayed hypersensitivity skin reactions are only one measure of T cell function. Attempts were also made to determine whether this specific unresponsiveness was also reflected at the level of specific 'helper' T cells, by challenging the sheep

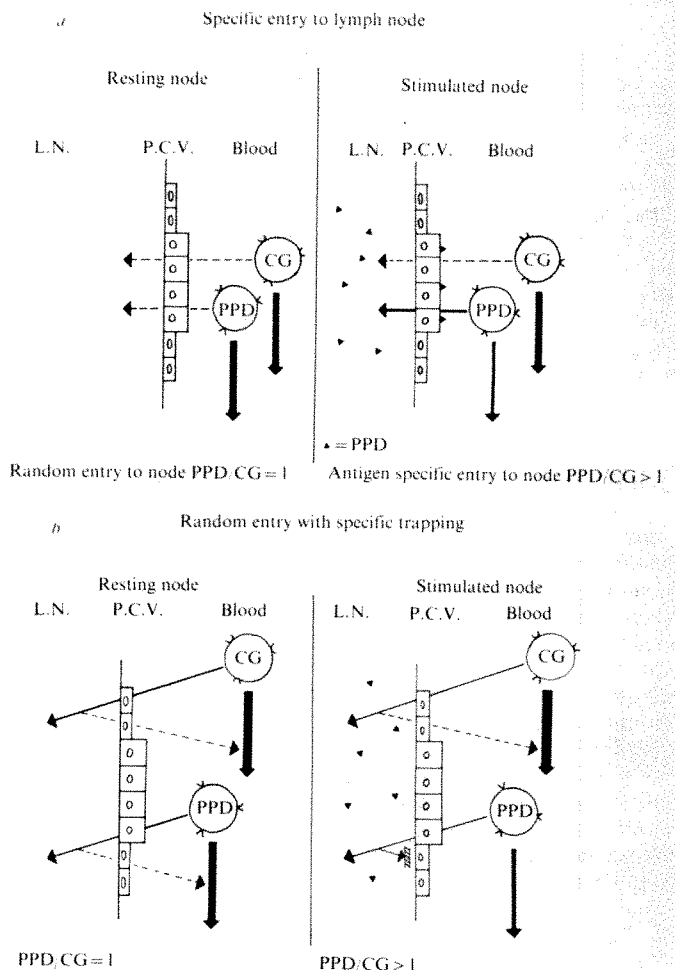


Fig. 6 Hypothetical models to explain specific depletion of antigen-reactive lymphocytes from the whole animal. *a*, Specific entry to lymph nodes; *b*, random entry with specific trapping. Explanation in text. PPD, PPD-reactive lymphocytes. CG, CG-reactive lymphocytes. LN, Lymph node. PCV, Post capillary venule. PPD/CG, ratio of specific cell types in lymph node.

with NIP-PPD at a time when they were still unresponsive to PPD. This has been done in two sheep with the same result, the typical response in one of them being shown in Fig. 5. Compared with the control, cannulated sheep fail to make any serum anti-NIP response on intravenous challenge with NIP-PPD. This unresponsiveness of 'helper' T cells was also found to be specific since challenge with NIP-CG 10 d later when the animals are still unresponsive to PPD (see Fig. 4) does result in an anti-NIP response, which was approximately 70% of that seen in control sheep.

Using these two criteria of specific T cell function, the results indicate that cannulation of the efferent lymphatic from a single lymph node of primed sheep, together with repeated stimulation of the cannulated node results in specific abrogation of the allergic response to the stimulating antigen. This

phenomenon is similar to that in which the lymphocytes involved in the mixed lymphocyte reaction can be specifically depleted from the total recirculating pool when allogeneic lymphocytes are injected into a cannulated lymph node (ref. 13 and R. S. Cahill, H. Frost, and Z. Trnka, in preparation).

Removal of antigen-reactive lymphocytes?

Our explanation for this phenomenon is that there is an antigen specific selective mechanism operating at the level of stimulated lymph nodes which specifically removes antigen-reactive lymphocytes from the total recirculating pool. Since T cells are predominantly a mobile population of cells the net result of this mechanism is to specifically deplete antigen-reactive T lymphocytes from the entire animal.

Other explanations based on some form of tolerance induction² or specific desensitisation¹⁴ would demand that either antigen or blocking factors entered the systemic circulation from the lymph node. Failure to produce a serum antibody response following local challenge of the node with NIP-PPD whilst sustaining an anti-NIP response in the efferent lymph is evidence that neither antigen nor antibody reaches the systemic circulation from the lymph node and exits only through the cannulated efferent lymphatic. In two similarly injected control animals the skin reactions to PPD in one were slightly weaker than noninjected controls but there was no difference in their anti-hapten response after challenge with NIP-PPD. This indicates a slight degree of desensitisation in such animals with regard to delayed hypersensitivity skin reactions, but this is not reflected at the level of 'helper' T lymphocytes.

At least two mechanisms can be proposed to explain antigen specific selection (Fig. 6). Assuming that the proportion of PPD-reactive and CG-reactive lymphocytes are the same in peripheral blood (PPD/CG=1) then if lymphocytes only crossed the high endothelial cells of the post-capillary venule (PCV) randomly this ratio would remain the same and systemic depletion, when it did occur, would be non-specific. To explain specific depletion it could be argued that over and above random entry into stimulated lymph nodes there is a specific entry mediated by antigen attached to the luminal surface of the high endothelial cells of the PCV which preferentially selects PPD-reactive lymphocytes into the node (Fig. 6a). The net result is that within the node the ratio of PPD-specific: CG-specific lymphocytes is greater than 1 and specific depletion from the peripheral blood gradually occurs. It seems likely that at each passage through the lymph node only a fraction of the specific cells would be removed at any one time.

There is a precedent for membrane-bound antigen attached to the surface of macrophages¹⁵ in lymph nodes but not as yet for endothelial cells. Since there is ample functional evidence to show that antigen never escapes systemically from a stimulated node^{7,16} such a model would demand that the antigen once bound to the surface of the high endothelial cell was rarely released into the circulation.

The alternative explanation is one which challenges the accepted fact that lymphocytes only move from blood to lymph and never in the reverse direction¹⁷. In this model (Fig. 6b) a proportion of the cells which enter the node then travel in the reverse direction from lymph node to blood. In stimulated nodes, antigen within the node specifically blocks the return of PPD reactive lymphocytes but not of CG-specific cells and again the net result in time, is a specific, systemic depletion of PPD reactive lymphocytes. The specific trapping of lymphocytes by antigen and their subsequent disappearance from the thoracic duct lymph has been described in other systems¹⁸⁻²⁰.

Our results at this stage do not enable a choice to be made regarding mechanisms but they do raise some interesting questions concerning the physiology of lymphocyte traffic (or circulation).

Finally, we point out that the experimental system described here affords a simple surgical means of specifically abrogating the allergic response of a primed animal to a given antigen. It remains to be seen whether this approach can be exploited in organ transplantation or in the specific abrogation of autoallergic disease.

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- ¹ Mitchison, N. A., *Transplant Proc.*, **3**, 953 (1971).
- ² Brent, L., *Transplant Proc.*, **4**, 363 (1972).
- ³ Feldman, J. D., *Adv. Immun.*, **15**, 167 (1972).
- ⁴ Hellstrom, I., and Hellstrom, K. E., *Nature*, **230**, 49 (1971).
- ⁵ Sjögren, H. O., Hellstrom, I., Bansal, S. C., and Hellstrom, K. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1372 (1971).
- ⁶ Lascelles, A. K., and Morris, B., *Q. Jl exp. Physiol.*, **46**, 199 (1961).
- ⁷ Hall, J. G., Morris, B., Moreno, G. D., and Bessis, M., *J. exp. Med.*, **125**, 91 (1967).
- ⁸ Taussig, M. J., and Lachmann, P. J., *Immunology*, **22**, 185 (1972).
- ⁹ Iverson, G. M., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.), 2nd ed., **29**, (1973).
- ¹⁰ Mitchison, N. A., *Eur. J. Immun.*, **1**, 10 (1971).
- ¹¹ Farr, R. S., *J. infect. Dis.*, **103**, 239 (1958).
- ¹² Brownstone, A., Mitchison, N. A., and Pitt-Rivers, R., *Immunology*, **10**, 465 (1966).
- ¹³ Hay, J. B., Cahill, R. S., and Trnka, Z., *Cellular Immunology* (in the press).
- ¹⁴ Uhr, J. W., and Pappenheimer, A. M. jun., *J. exp. Med.*, **108**, 891 (1958).
- ¹⁵ Unanue, E. R., and Cerrotini, J. C., *J. exp. Med.*, **131**, 711 (1970).
- ¹⁶ Smith, J. B., and Morris, B., *Austr. J. exp. Biol. med. Sci.*, **48**, 33 (1970).
- ¹⁷ Marchesi, V. P., and Gowans, J. L., *Proc. R. Soc.*, **B159**, 283 (1963).
- ¹⁸ Ford, W. L., and Atkins, R. C., *Nature new Biol.*, **234**, 178 (1972).
- ¹⁹ Sprent, J., and Miller, J. F. A. P., *J. exp. Med.*, **138**, 143 (1973).
- ²⁰ Rowley, D. A., Gowans, J. L., Atkins, R. C., Ford, W. L., and Smith, M. E., *J. exp. Med.*, **136**, 499 (1972).

Some new concepts in immunological phylogeny

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Immunocompetence is first evident at the invertebrate level in coelenterates. Primordial cell-mediated immunity revealed by specific allograft reactions with at least short term memory is demonstrable in advanced invertebrates, whereas integrated cell-mediated and humoral immunity evolved progressively with the vertebrates.

SPECIFIC immunological competence with long lived memory is found in all vertebrate immune systems, associated with lymphoid cells, a thymus equivalent and the ability to produce serum antibodies. Two major pathways determining cellular (T lymphocyte) and humoral (B lymphocyte) immunity can be distinguished in all classes of vertebrates. Though most primitive among vertebrates, hagfish and lampreys (Cyclostomes) reject skin allografts

with concomitant development of specific immunological memory and produce IgM-type antibodies to potent xenogeneic antigens'. Given such impressive capacity at this level of phylogeny, one must look to the invertebrates for the origins and early mechanisms of immunocompetence. Until recently, little was known about the molecular defence mechanisms of invertebrates beyond nonspecific phagocytosis. We now know that rudimentary immune responsiveness with at least short term memory exists among certain advanced invertebrates. At still lower levels of phylogeny, immunorecognition and immunoincompatibility reactions with impressive specificity are demonstrable, but a memory component is doubtful². In spite of information gaps, a new conception of immunoevolution is emerging: essential cellular (T-cell) immunocompetence evolved progressively among metazoan invertebrates long before the additional (B-cell) vertebrate capacity to produce immunoglobulin antibodies.

The best studied and highest level of immunoevolution characteristic of all vertebrates can be designated 'integrated cell-mediated and humoral antibody immunity'. The cytoarchitecture of the lymphoid system and the molecular diversity of antibodies produced attain progressively higher levels of complexity in the evolution from fishes to mammals²⁻⁴. Circulating immunoglobulin antibodies of one or more molecular classes are inducible in vertebrates (B-cell pathways) in addition to immunorecognition and cell-mediated immunity reactions (T-cell pathways) also evident in invertebrates². Elaborate homeostatic regulation, including still poorly understood T and B-cell cooperation and suppression, is operative in birds and mammals. This can be regarded as the 'Rolls-Royce model' of immunoresponsiveness with numerous interdependent pathways (Fig. 1).

Important details of immunoregulation, especially tolerance, immunological blocking, memory and mechanisms of specific cell-mediated immunity, remain controversial or unknown. To pursue the Rolls-Royce analogy, a less elaborate Land Rover model of essential immunocompetence is found among fishes and amphibians, while bicycle models now appear characteristic of invertebrates. Depending on the environmental circumstances of course, the bicycle or Land Rover may be superior to the Rolls-Royce. One looks then for adaptive specialisation of the defence mechanisms among distinctive groups which have survival value or assure the integrity of the body. Among vertebrates, progressive diversification in molecular classes and subclasses of immunoglobulins is evident in the transitions from primitive fishes to mammals^{4,5}.

The ancestral immunoglobulin gene probably coded for a polypeptide of 110-112 amino acids corresponding to the

separate constant and variable region domains of antibody molecules⁶. By gene duplication and diversification of heavy and light polypeptide chains presumably guided by adaptive selection, the IgM immunoglobulin found in all vertebrates had already evolved in Cyclostomes several hundred million years ago. Why this complex antibody protein should be primitive is still puzzling, although its polymeric structure and multiple antigen-binding sites obviously increase the probability of combination with any antigen that has entered the body. Even at this level, further adaptive specialisation is apparent: IgM is tetrameric in bony fishes, hexameric in anuran amphibians and pentameric in certain sharks and mammals. Moreover, both lymphocyte and granulocyte-series of immunocytes are found in advanced invertebrates and all vertebrates, but the cytoarchitecture of the lymphoid system varies greatly at different levels of phylogeny⁷. Clearly different groups of animals have evolved different immunological equipment to meet their needs.

Primordial cell-mediated immunity

The capacity to develop specific transplantation immunity with concomitant immunological memory has been demonstrated in two phyla of advanced invertebrates—annelid worms^{8,9} and echinoderms^{2,10}. Echinoderms are more immediately ancestral to the vertebrates whereas annelids represent a line of evolution which diverged early from the progression leading to vertebrates. Slow but specific rejection of orthotopic integumentary allografts has been found in several species of earthworms, sea cucumbers and sea stars. The earthworm story is complicated by the unexplained intrapopulation compatibility *versus* incompatibility between geographically separate populations. However, integumentary xenografts between earthworm species are invariably rejected after a compatible healing-in period of considerable duration. At least short term immunological memory is evident in earthworms as revealed by accelerated rejection of repeat grafts placed at the time of or soon after initial rejection. However, the memory situation is more complex as indicated by prolonged survival of repeat grafts placed some time after initial allograft or xenograft rejection. Apparently, memory can be either short or long lived and positive or negative in its effect on foreign tissue survival in worms. This is an intriguing problem of specific immunoregulation. The observed transplantation immunity in worms is inherent in populations of leukocytes (coelomocytes) as shown by adoptive transfer experiments. Passive transfer of immune serum is without effect and immunoglobulins have not been detected in annelids.

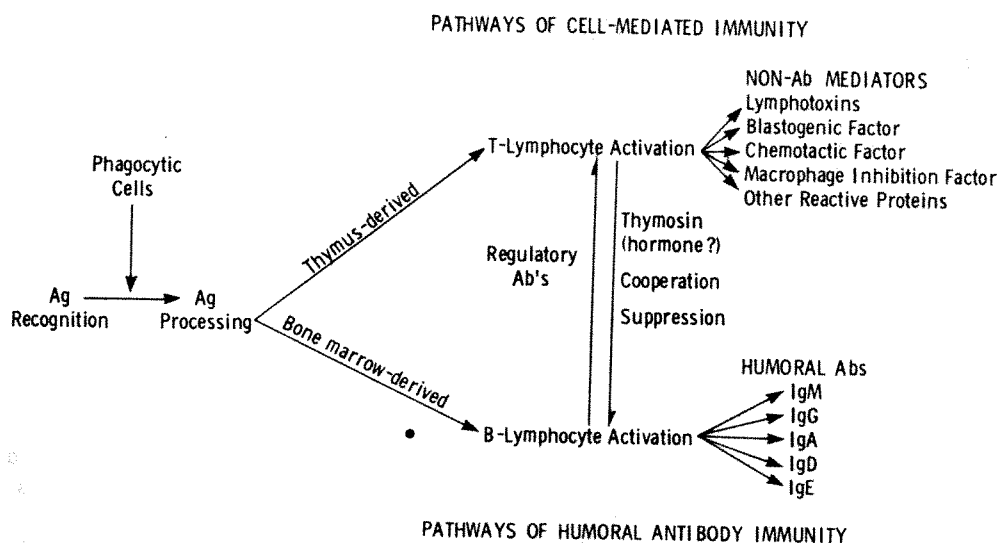


Fig. 1 Integrated pathways of cell-mediated and humoral antibody immunity at the mammalian level of immunoevolution. Elaborate and as yet poorly understood regulatory mechanisms are involved.

Echinoderms, even more than annelids, possess a rich assortment of leukocyte types, including distinctive lymphocytes, granulocytes and macrophages. Thus, mammal-oriented immunologists should not be surprised at the mounting evidence of immunological competence in the form of primordial cell-mediated immunity (PCMI) in these advanced invertebrates. Specific allograft immunity with at least short term memory was found in a sea cucumber (*Cucumaria tricolor*)¹⁰. Similar allogeneic incompatibility has now been found in two sea stars, *Protoreaster nodosus* from Australia¹⁰ and *Dermasterias imbricata* from California (Karp and Hildemann, unpublished). Leukocytes of multiple types, including macrophages, small lymphocytes (haemocytes) and eosinophilic granulocytes, were observed to infiltrate echinoderm allografts. Although the histological picture and chronic inflammatory reactions accompanying allograft rejection in these species are similar to those seen in vertebrates, the memory component may be distinctive. Experiments now in progress in our laboratory to compare the kinetics and consequences of short term compared with long term memory could be decisive in defining possible limitations of PCMI.

The lore of cell-mediated immunity (CMI) in mammals has expanded rapidly in recent years. The responsible cells are thought to be thymus-derived or T lymphocytes which originate in bone marrow. However, macrophages, granulocytes or equivalent cells cooperate critically with T lymphocytes, especially in the killing of foreign target cells. Moreover, T lymphocytes have a long life span and constitute the predominant small lymphocyte in the circulation as shown by their distinctive antigenic markers. Also T cells are the reservoir of long term immunological memory and are generally required as so-called helper T cells for the activation of antibody-producing B lymphocytes. Mammalian T cells are also distinguished by their characteristic responses to mitogens, such as phytohaemagglutinin (PHA), concanavalin A and *E. coli* lipopolysaccharide, reactivity in allogeneic mixed lymphocyte culture (MLC) reactions, and ability to mediate allograft reactions or delayed hypersensitivity reactions. The extent to which these properties of T cells persist as such throughout phylogeny remains to be determined.

Karp, Reddy, Tam and I (unpublished work) are variously investigating T cell type responsiveness in an echinoderm (*Dermasterias*), a tunicate (*Ciona*), a hagfish (*Eptatreus*) and a shark (*Heterodontis*) which together represent a transition group between the advanced invertebrates and lower vertebrates. Although all show similar patterns of chronic allograft rejection even at elevated water temperatures as evidence of PCMI, not even the hagfish and sharks seem capable of serum alloantibody responses. Blood leukocytes from these four species in culture respond distinctively to certain mitogens. Specific cell-surface recognition units or ancestral immunoglobulin receptors could be detectable on echinoderm or protochordate lymphocytes and pertinent results should be forthcoming. In short, although we are a long way from understanding cellular immune functions in phylogenetic perspective, the fact of cell-mediated immunity in advanced invertebrates is unassailable¹. Such CMI is sharply discriminating at the level of antigen recognition, but could be primordial in lacking diversification of effector functions or a long term memory component.

Immunorecognition and immunoincompatibility

Allogenic transplants in ciliate protozoans and free living amoebae remain viable, but intergenus or xenogeneic transplants have always resulted in eventual cell death¹. No immunological component is evident in the incompatible combinations. Interspecies incompatibilities in sponge cell aggregation seem to depend on binding affinities of cell-surface glycoproteins rather than any immunological

surveillance system¹¹. Species-specific adherence of sponge cells is analogous to tissue-specific cell aggregation in higher animals. No cell damage or killing has been reported as a result of incompatible sponge cell reactions, although this possibility deserves closer inspection. However, immunorecognition or capacity for non-self recognition of allogeneic tissue followed by incompatibility reactions seems characteristic of the Coelenterata or Cnidaria. Indeed, allogeneic incompatibility is now well documented in colonial hydroids¹², gorgonians¹³ and hard corals¹⁴. Straightforward enzyme-substrate or biochemical incompatibilities are not responsible, because allografts and even xenografts heal compatibly for days or weeks before tissue destruction occurs. I would now designate this 'immunorecognition/immunoincompatibility' because of the apparent specificity of the antagonistic reactions and the compatible lag period preceding manifestations of incompatibility.

The experimental evidence concerning allogeneic incompatibilities in coelenterates detailed in the references cited above can be summarised briefly. Allogeneic colonies of the hydroid *Hydractinia echinata* fail to fuse when grown in contact, whereas colonies derived asexually from single colonies merge compatibly. Hyperplastic growth occurs in areas of direct contact between two incompatible colonies and regression of the overgrown colony usually ensues. A hierarchy of hyperplastic potential was found among diverse strains of *Hydractinia* and breeding experiments revealed complex genetic control of histocompatibility¹². Both allografts and xenografts in gorgonian corals are subject to rejection, but control autografts survive indefinitely. Ectodermal as well as ectodermal-mesogial grafts are reported to behave similarly. Incompatibility reactions were scored as greater with xenografts (*Eunicella stricta* \rightleftharpoons *Lophogorgia sarmentosa*) than with allografts¹³. Surprisingly, disintegration of foreign transplants began after 4–5 d, even at the low temperature of 10°–15° C. Tests of specificity of rejection or immune memory have not been reported.

Staghorn corals of *Acropora* species are also amenable to decisive tissue transplantation experiments¹⁴. Branches from the same colony or clone fuse compatibly after 6–7 d in contact at 25° C and persist indefinitely with no sign of antagonism. Intercolony allografts generally show moderate incompatibility leading to soft tissue (coenenchyme) destruction in the contact zone, several to many weeks after initial functional fusion. Probable xenografts between *Acropora* species of uncertain taxonomic status yield detectable soft tissue death at the interface as early as a week after contact is made. Functional graft survival is easily tested by focal probing of polyp tentacles, which induces a withdrawal response instantly transmitted across a viable graft-recipient interface through the nerve net. Compatible coral grafts exhibit intact polyps and persistence of pigmented zooanthellae in the coenenchyme in zones of contact just as found in normal tissue. To what extent sharp immunological specificity or possible memory is involved in chronic rejection of allografts and subacute rejection of xenografts among these various coelenterates remains to be ascertained.

Tunicates of the protochordate group are generally held to be immediately ancestral to the vertebrates, thus being advanced invertebrates far removed from the coelenterates. As might be expected, tunicates have differentiated leukocytic cells including abundant lymphocytes. Immunoincompatibility or transplantation immunity at the level of colony specificity is common in colonial tunicates such as *Botryllus* species¹⁵, *Perophora viridis*¹⁶ and *Amaroecium* species¹⁷. The tunic and vascular stolon of compatible colonies placed in contact fuse to form a continuous tube with exchange of blood cells. With histoincompatible colonies the tunic at the site of contact thickens without fusion and local necrosis

may subsequently occur similar to the allogeneic reactions characteristic of coelenterates. Our tests for allogeneic immunoincompatibility in the solitary tunicate, *Ciona intestinalis*, show capacity to recognise and reject orthotopic allografts of integument in contrast to persistent survival of control autografts (Reddy, Bryan and Hildemann, unpublished). *Ciona* lymphocytes also respond to PHA in a manner similar to mammalian T lymphocytes. Tests with other mitogens and of MLC reactivity are underway. Existence of PCMI with memory as found in echinoderms remains to be shown in tunicates, but the precedent capacity for immunorecognition/immunoincompatibility is surely well developed. The latter capacity then may be predicted for all invertebrates amenable to testing.

MLC-type reactions in mammals, and, one would predict, other vertebrate classes, display essentially the same properties of inducible incompatibility that are found in coelenterates and tunicates. Positive reactions between allogeneic lymphocytes are reflected in new DNA synthesis and appearance of blast cells suggestive of the hyperplastic contact reactions seen in coelenterates. In mice, rats and humans, the capacity of lymphocytes to respond in MLC can be separated from the CMI capacity to kill foreign cells or reject allografts^{18,19}. Since the MLC reaction requires the two populations of cells to be alive and antigenically disparate, active immunorecognition is involved. Preimmunisation is unnecessary and a memory component is either not involved or has a short term effect on the degree of MLC responsiveness²⁰. The whole range of antigenic disparities from strong to weak can evoke positive MLC reactions¹⁹, although strong histocompatibility antigens yield the highest MLC responses in mammals²⁰. So MLC-type reactivity probably represents an ancient immunosurveillance mechanism which has persisted in phylogeny since the appearance of eumetazoan invertebrates. At lower levels of invertebrate phylogeny, immunorecognition precedes the occurrence of lymphoid cells as such, but the recognition function could be inherent in surface receptors of epithelial-type cells²¹. Allogeneic stimulation of the MLC-type is not a cytotoxic reaction. Subsequent generation of cells or molecules able to mediate the killing of foreign cells in close proximity, however, usually follows *in vivo*²².

Conflicting interpretations

All the evidence, taken together, suggests certain major sequential steps in the phylogeny of immunological reactivity (Fig. 2). The first level of cell-specific or species-specific aggregation is fundamental to all colonial or multicellular organisms. Epithelial or other cell surface proteins govern compatible tissue formation in plants, protozoans and sponges, but these, often exquisitely specific, reactions lack an immunological character in the strict sense that incompatible cell killing or heightened responsiveness are not inducible. Specific immunorecogni-

tion/immunoincompatibility is first evident at the invertebrate level of coelenterates, while cell-mediated immunity with at least short term memory has been detected initially in advanced invertebrates, notably annelids and echinoderms. There are obviously many information gaps in terms of invertebrate phyla not yet studied decisively or at all. I suggest that immunoincompatibility as regularly shown by allogeneic contact reactions is primitive and has persisted as an effective surveillance mechanism throughout metazoan phylogeny. MLC reactivity in modern birds and mammals is essentially similar to the allogeneic proliferative antagonism seen in coelenterates and tunicates. One may assume that progressively more differentiated leukocytic cells came to subserve this second level function as adaptive specialisation continued during phylogeny. Primordial cell-mediated immunity with memory constitutes a third level associated with cooperation of granulocytes and T lymphocytes in allograft rejection. This function becomes well developed in primitive fishes (cyclostomes and elasmobranchs) associated with longer-lived memory.

Integrated cell and humoral antibody immunity can be regarded as a fourth level development (Fig. 2) peculiar to vertebrates and perhaps restricted to advanced bony fishes and higher vertebrates. At this level helper T and B cells able to produce two or more molecular classes of antibodies are demonstrable. The cooperation of thymus-dependent lymphocytes (thymic hormone?) and certain B cells seems essential in mammals for the switch-over from specific IgM to IgG production²³. IgM production to quite potent immunogens may not require T and B-cell cooperation. Primitive fishes are capable only of IgM production to various antigens. Complex immunoregulation in birds and mammals (level 5) involves multiple molecular classes and subclasses of immunoglobulins in ways just beginning to be understood. The immunoregulatory mechanisms of primitive fishes making only IgM and of invertebrates with neither immunoglobulins nor a thymus gland are unknown.

Some immunologists are reluctant to accept the view that any adaptive immunity exists below the level of primitive fishes. Burnet²⁴ has considered that incompatibility in colonial tunicates and coelenterates as well as self-incompatibility in flowering plants are examples of self *versus* not-self recognition which are not analogous to the immunological processes of vertebrates. Similarly, Dausset *et al.*²⁵ regard the invertebrate incompatibilities as well as the MLC reaction in mammals as a non-immunological recognition system because a specific memory component is weak or absent. Other immunologists, however, view the allogeneic MLC reaction as an immunocompetent function and not simply the proliferative response of antigen-sensitive cells to contact with foreign cell membrane antigen^{20,22,26}. The point is that allogeneic stimulation may lead to the generation of cytotoxic lymphocytes with specific reactivity. The conflict

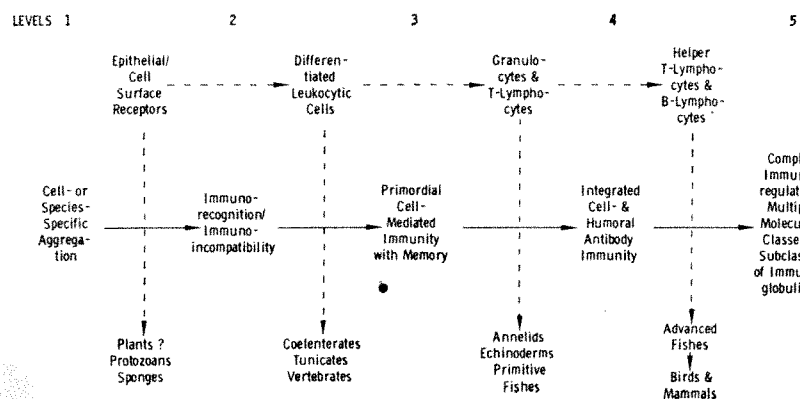


Fig. 2 Major steps in phylogeny of immunological reactivity. The suggested pathway is based on much new experimental evidence accumulated in recent years. Specific immunocompetence is already evident at the level of coelenterates, while cell-mediated immunity with memory is first detected in advanced invertebrates. Earlier manifestations of immunoreactivity seem to have been retained during progressive evolution and diversification of immunocyte functions.

is only partly one of definitions.

Extensive studies cited here indicate that immunorecognition/immunoincompatibility in coelenterates and tunicates involves not only non-self recognition of allogeneic tissue after initially compatible fusion; but also subsequent antagonistic reactions leading to localised cell death. In my view this constitutes the essence of specific, sharply discriminating, immune reactivity. Immunological memory associated with lymphoid cells may indeed be absent at the coelenterate level, but addition of these adaptive ingredients is clearly evident in echinoderms and probably also in tunicates. Available evidence suggests that cell-mediated immunity (T-cell function) evolved in invertebrates long before the additional vertebrate capacity for humoral antibody production (B-cell function). Among the vertebrates, progressive diversification of immunocyte functions appears correlated with increasing interdependence of the T and B-cell pathways. The supposition that immunological capacity paralleled leukocyte differentiation and evolved from no memory to short term to finally long term memory is attractive but unproved. Although the long known recognition system of nonspecific phagocytosis associated with the pioneering studies of Metchnikoff²⁷ is ubiquitous in metazoan phylogeny, granulocytes and other types of macrophages now appear to be critical participants in specific immune pathways and especially in cell-mediated immunity.

In essence, five levels of recognition and reaction to foreignness (Fig. 2) are now discernible in phylogenetic progression. All effective in maintaining the integrity of the organism and the species. If one demands both specificity and memory as qualifications for true immunological reactivity, then immunocompetence first appeared among the advanced invertebrates. But if the specific memory requirement is viewed flexibly also as an evolving characteristic, then immunocompetence is already manifest at the lower invertebrate level of coelenterates.

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- ¹ Hildemann, W. H., in *Transplantation antigens* (edit. by Kahan, B. D., and Reisfeld, R. A.), 3-73 (Academic Press, New York, 1972).
- ² Hildemann, W. H., and Reddy, A. L., *Fedn Proc.*, **32**, 2188-2194 (1973).
- ³ Clem, L. W., and Leslie, G. A., in *Immunology and development* (edit. by Adinolfi, M.), 62-88 (Spastics International Medical Publications, London, 1969).
- ⁴ Marchalonis, J. J., and Cone, R. E., *Aust. J. exp. Biol. Med. Sci.*, **51**, 461-488 (1973).
- ⁵ Hildemann, W. H., *A. Rev. Genet.*, **7**, 19-36 (1973).
- ⁶ Hood, L., in *Stadler symp.*, **5**, 74-142 (University of Missouri, Columbia, 1973).
- ⁷ Kampmeier, O. F., *Evolution and comparative morphology of the lymphatic system*, 620 (Thomas, Springfield, Illinois, 1969).
- ⁸ Cooper, E. L., *Transplantn Proc.*, **2**, 216-221 (1970).
- ⁹ Duprat, P. C., *Transplantn Proc.*, **2**, 222-225 (1970).
- ¹⁰ Hildeman, W. H., and Dix, T. G., *Transplantation*, **15**, 624-633 (1972).
- ¹¹ Humphreys, T. D., *Transplantn Proc.*, **2**, 194-198 (1970).
- ¹² Ivker, F. B., *Biol. Bull.*, **143**, 162-174 (1972).
- ¹³ Theodor, J. L., *Nature*, **227**, 690-692 (1970).
- ¹⁴ Hildemann, W. H., Dix, T. G., and Collins, J. D., in *Current topics in immunobiology*, **4** (Plenum, New York and London, in the press).
- ¹⁵ Tanaka, K., and Watanabe, H., *Cell. Immun.*, **7**, 410-426 (1973).
- ¹⁶ Freeman, G., *Transplantn Proc.*, **2**, 236-239 (1970).
- ¹⁷ Scott, F. M., and Schuh, J. E., *Acta Embryol. Morph. Exp.*, **6**, 39 (1963).
- ¹⁸ Abbasi, K., and Festenstein, H., *Eur. J. Immun.*, **3**, 430-435 (1973).
- ¹⁹ Ling, N. R., *Immun. Commun.*, **2**, 119-127 (1973).
- ²⁰ Oppenheim, J. J., in *Transplantation antigens* (edit. by Kahan, B. D., and Reisfeld, R. A.), 357-383 (Academic Press, New York, 1972).
- ²¹ Fichtelius, K. E., *Lymphology*, **1**, 50-59 (1970).
- ²² Lafferty, K. J., Walker, K. Z., Scollay, R. G., and Killby, V. A. A., *Transplantn Rev.*, **12**, 198-228 (1972).
- ²³ Grumet, F. C., *J. exp. Med.*, **135**, 110-125 (1972).
- ²⁴ Burnet, F. M., *Nature*, **232**, 230-235 (1971).
- ²⁵ Dausset, J., Le Brun, A., and Sasportes, M., *C. r. heb. Séanc. Acad. Sci.*, **275**, 2279-2282 (1972).
- ²⁶ Wilson, D. B., Howard, J. C., and Nowell, P. C., *Transplantn Rev.*, **12**, 3-29 (1972).
- ²⁷ Metchnikoff, E., *Lectures on the comparative pathology of inflammation*, **224** (Dover, New York, 1968 edition of 1891 monograph).

Three-dimensional structure of adenyl kinase

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A combination of X-ray data at 3 Å resolution and sequence results has yielded the atomic structure of adenyl kinase, a ubiquitous enzyme which catalyses the phosphorylation of AMP by ATP. The abundant secondary structures of the protein and the probable binding sites for ATP and AMP are described.

ADENYL kinase (adenylate kinase, EC 2.7.4.3) catalyses the reaction¹ $ATP + AMP \xrightarrow{Mg^{2+}} 2 ADP$. This interconversion of the adenine nucleotides seems to be of particular importance in organelles which have a high turnover of chemical energy, for example in chloroplasts², mitochondria³, or myofibrils⁴. With a molecular weight of 22,000 muscle adenyl kinase (myokinase) is the smallest of the known phosphoryl transferases⁵. Other kinases presently studied by X-ray

analysis have molecular weights of 40,000-100,000⁶⁻¹⁰.

A low resolution model of porcine muscle adenyl kinase¹¹ and its amino acid sequence¹² have been described recently. The X-ray analysis has now been pursued to higher resolution. A combination of 3 Å X-ray data and the sequence information yielded the atomic structure of the enzyme.

X-ray diffraction

It has been shown¹³ that porcine muscle adenyl kinase crystallises in one of the enantiomorphic space groups $P3_121$ or $P3_221$ with one molecule in the asymmetric unit. To find the correct alternative we made a separate measurement of 81 independent noncentric Friedel pairs with spacings above 10 Å (limit for negative Bragg angles on Siemens diffractometer) from the highly isomorphous CH_3HgNO_3 derivative. The observed structure factor amplitude difference within each Friedel pair was compared with the corresponding difference as calculated¹⁴ from the known protein structure factor¹¹ in conjunction with the known heavy atom structure factor¹¹. Correlation coefficients¹⁵ between observed and calculated differences were determined for both possible

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space groups. They turned out to be +0.80 and -0.80 for space groups P3₂21 and P3₁21, respectively, indicating that the true space group is P3₂21. By chance, this space group had been selected when building the low resolution balsa wood model described earlier¹¹. Therefore, this model has the correct chirality.

The present structure analysis includes the 4,200 independent reflections that have spacings above 3 Å. For phase determination corresponding data sets were measured from the four heavy atom derivatives listed in Table 1. Reflection intensities were determined on an automated diffractometer following a procedure that has been described earlier¹¹.

Data were collected in shells of about 600 reflections each, that is, a complete data set contained seven shells. A separate measurement of about 500 reflections distributed over all shells was made for the protein and for every derivative. It overlapped with each shell in about 70 reflections. We used these overlaps to put the seven shells of a data set on a common scale. All data sets were then put on an absolute scale as derived from a Wilson plot¹⁸. This scale turned out to be 12% lower than the corresponding scale determined at low resolution¹¹.

All derivative data shells were corrected for systematic errors in the measurement before scaling and merging them to data sets. This correction was based on the assumption that the differences between derivative and protein structure factor amplitudes should be evenly distributed within a given shell. We checked for systematic deviations along the longitudinal and along the latitudinal coordinates on the surface of the shell. Such deviations were reduced by applying a correction factor to the derivative structure factor amplitude that was linear in both of these coordinates. The average correction on each amplitude was about ±2%. This is not negligible because the phase analysis is based on the rather small differences between derivative and protein structure factor amplitudes¹⁹.

In the refinement of heavy atom parameters and in the Fourier synthesis we omitted those reflections that have intensities less than twice the estimated error of the measurement. This reduced the amount of data by 8%. In the refinement we followed a procedure given by Dickerson *et al.*¹⁷. It alternates between evaluation of the best phases and parameter shifts. The refined parameters are listed in Table 1.

The accuracy of the analysis as a function of resolution is illustrated in Fig. 1. Most of the phase information is derived from the CH₃HgNO₃ and the K₂Pt(SCN)₆ derivatives because their heavy atom structure factors are much

higher than their lack of closure errors. The K₂Pt(NO₂)₄ derivative has a lower radial fall-off factor than the protein (see Table 1) and it is less radiation sensitive. Its contribution to the phase information, however, is rather poor. The lack of closure error exceeds the heavy atom structure factor at higher resolution. A second CH₃HgNO₃ derivative could be prepared by blocking the thiol groups asymmetrically with 6-thioinosine 5'-*o*-monophosphate (6-SH AMP) prior to soaking with CH₃HgNO₃, so that effective substitution numbers changed (Table 1). As judged from Fig. 1 a considerable amount of phase information is obtained from this derivative. One has to bear in mind, however, that this derivative is not very different from the other mercury derivative. Therefore, their contributions to the phase information are to some extent coupled. As shown in Fig. 1 the figure of merit for phases drops 96% at low resolution to 77% at high resolution, the average being 82%. These values indicate that the electron density map is of reasonable quality.

Electron density map

The 'best' electron density distribution¹⁹ was calculated using the phased structure factors weighted with the corresponding figures of merit. The electron density map was computed in sections 0.94 Å (=c/150) apart perpendicular to the c-axis. These sections were plotted on microfilm and subsequently redrawn on perspex sheets at a scale 1 Å=4 mm. The resulting small map was very useful for chain tracing as well as for assigning side chains and preliminary Cα-positions.

With the amino acid sequence of the protein¹² at hand, chain tracing was not a great problem. All 22 markers: 14 aromatic side chains, six methionine and two cysteine residues could be found in the map and fitted. There is no density that has not been accounted for. Other chain tracings which become possible if one concedes large errors in electron density invariably lead to contradictions. Therefore, the fit of the amino acid sequence into the electron density map seems to be unambiguous.

A comparison of the resulting molecular boundaries with those determined at low resolution¹¹ showed no difference except that about five residues at the N-terminus had been incorrectly assigned to a neighbouring molecule, that is, they are at a wrong place (around residue 50, see Fig. 2) in the low resolution balsa wood model¹¹.

Table 1 Heavy atom parameters

Heavy atom compound	Soaking concentration (mM)	Soaking time (d)	R*	K†	β†	x‡	y‡	z‡	Z§	B
CH ₃ HgNO ₃	0.05	5	0.35	0.99	-2.9	0.480 0.198 0.503	0.857 0.718 0.737	0.115 0.111 0.121	70.0 21.0 12.0	8.0 4.0 22.0
K ₂ Pt(SCN) ₆	2.0	8	0.40	0.96	-4.8	0.565 0.145 0.221 0.162	0.711 0.993 0.977 0.703	0.111 0.074 0.103 0.097	90.0 38.0 27.0 19.0	11.0 33.0 28.0 33.0
K ₂ Pt(NO ₂) ₄	2.0	68	0.59	0.96	+1.3	0.336 0.546 0.145	0.149 0.698 0.705	0.044 0.109 0.094	7.0 40.0 17.0	45.0 11.0 22.0
6-SH AMP and then CH ₃ HgNO ₃	2.0 0.05	18 4	0.53	1.01	-2.9	0.480 0.203	0.858 0.728	0.115 0.113	38.0 3.0	1.0 10.0

* Centric reliability factor¹⁶.

† Scaling parameters: Initially all data sets were scaled to equal ΣF at low resolution. In the refinement a scale factor 1/K exp βS² with S=sin θ/λ was allowed for each derivative data set.

‡ Heavy atom positions in fractional coordinates.

§ Effective substitution number. Units are in numbers of electrons based on an absolute scale.

|| Radial fall-off factor¹⁷. The heavy atom structure factor is defined as $f_H = \sum_m Z_m \exp(-B_m S^2) \exp 2\pi i(hx_m + ky_m + lz_m)$ with the sum taken over all sites *m* of a derivative. The radial fall-off factor for the protein structure factor amplitudes is 9 Å².

The main sites of the K₂Pt(SCN)₆ and the K₂Pt(NO₂)₄ derivatives as well as the third site of the CH₃HgNO₃ and the only site of a HAuCl₄ derivative¹¹ are located at the His-36 side chain in the cleft region. The first and second CH₃HgNO₃ sites are at Cys-25 and Cys-187, respectively.

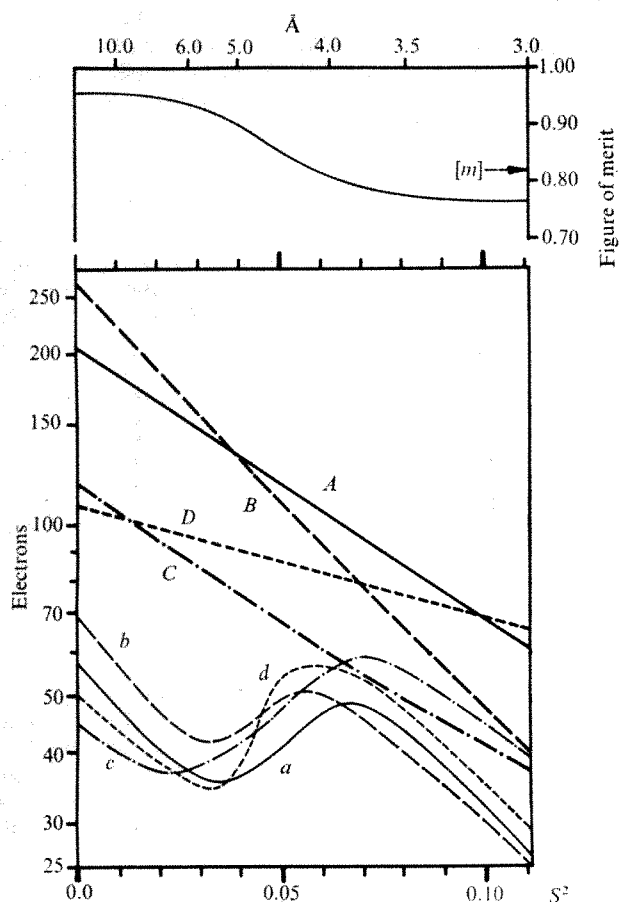


Fig. 1 Variation of root mean square (r.m.s.) heavy atom structure factors, r.m.s. lack of closure errors, and the figure of merit of the phases with resolution. Curves A, B, C, D are the r.m.s. heavy atom structure factors and curves a, b, c, d are the corresponding r.m.s. lack of closure errors for the derivatives: CH_3HgNO_3 , $\text{K}_2\text{Pt}(\text{SCN})_6$, $\text{K}_2\text{Pt}(\text{NO}_3)_4$, and $[\text{6-SH AMP} + \text{CH}_3\text{HgNO}_3]$, respectively. The plot is logarithmic with the resolution taken as $S^2 = (2 \sin \theta / \lambda)^2$. All r.m.s. lack of closure error curves have a trough at 5 Å corresponding to a similar minimum of the mean protein structure factor in this region. The overall mean figure of merit is 82%.

Description of the molecule

A stereo drawing of the polypeptide chain is given in Fig. 2. As with the low resolution balsa wood model¹¹ we selected the view that exposes the deep cleft. This cleft divides the molecule into two domains: The domain on the right side consists of three helical rods which enclose a

small hydrophobic core. The domain on the left side of Fig. 2 contains a parallel pleated sheet formed by five strands, each about five residues long. It has a right-handed twist like the pleated sheets in many other proteins²⁰. The sheet is the architectural centre of this domain. There are two hydrophobic cores, one on each side of the sheet. They are formed between the sheet and covering helices. About 55% of all residues are in right-handed helical configuration and 13% are in the sheet. Thus, about two thirds of all residues are involved in secondary structures. This probably accounts for the exceptional acid stability of adenylyl kinase⁴. Several parts of the helices seem to be distorted from the α -helical configuration²¹. The relation between primary, secondary and tertiary structure is illustrated in Fig. 3.

All charged side chains are on the surface of the molecule. Being situated deep in the cleft aspartate-93 is an exception. There is an unusual piece of chain containing five glycines and one proline between residues 15 and 22 (Fig. 3). As shown in Fig. 2 it is situated in the cleft forming a crooked loop as expected from such a sequence²³. This loop envelopes a dense narrow spherical peak that has double the height of the average backbone density. It is also higher and narrower than would be expected from a rigidly bound sulphate ion. Based upon height and width of the peak it seems to be due to a metal ion with an atomic number approximating those of Mn, Fe, Co, Ni, Cu, or Zn. Atomic absorption measurements on freshly prepared active adenylyl kinase, however, revealed none of these metals (Gastner, personal communication). We suspect that the enzyme has picked up a metal ion during the preparation of the crystals; possibly this metal ion indicates the location of the Mg^{2+} or Mn^{2+} during catalysis^{24,25}.

Location of active centre

Adenylyl kinase binds both AMP and ATP at specific sites¹. All attempts to bind substrates or substrate analogues to the crystals failed¹¹, and we must at this time rely upon indirect evidence to locate the active centre.

NMR data show that one of the two histidines in porcine muscle adenylyl kinase is involved in catalysis²⁶. His-36 is located in the cleft region and binds many heavy atom compounds (see Table 1) whereas His-189 lies flat on a very smooth surface of the molecule. Carp muscle adenylyl kinase contains only one histidine residue, and this is equivalent to His-36 in the porcine enzyme (von Zubern and Noda, personal communication). Since it is very likely that a residue involved in catalysis is conserved during species evolution²² we may assume that His-36 is at the active site, that is, that the active site is in the cleft region.

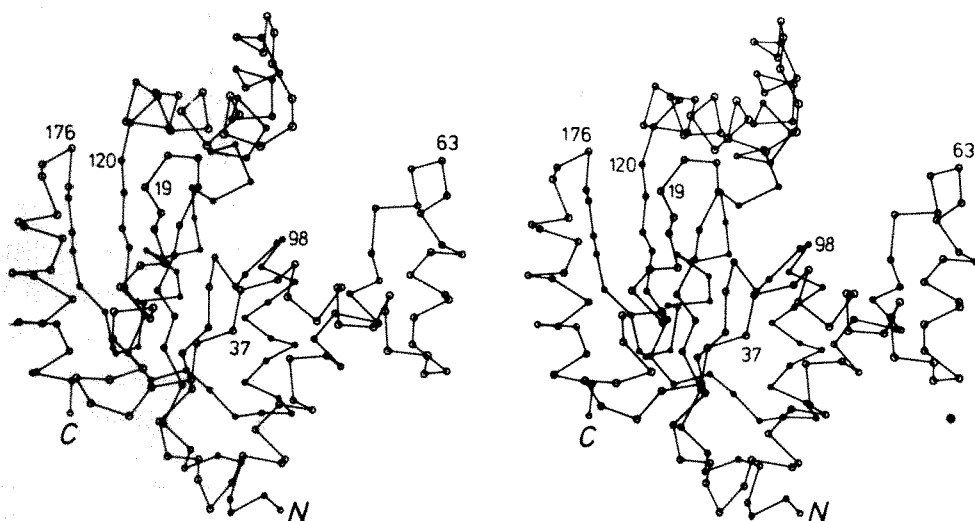


Fig. 2 Stereo drawing of polypeptide chain of adenylyl kinase. C α -atom positions are marked with circles. N and C termini as well as some residue numbers are inserted. In the electron density map the chain fades out between residues 137 and 141 in an exposed region on the surface of the molecule. This stretch of chain, however, is strongly connected in the 6 Å map and in an intermediate 4 Å map.

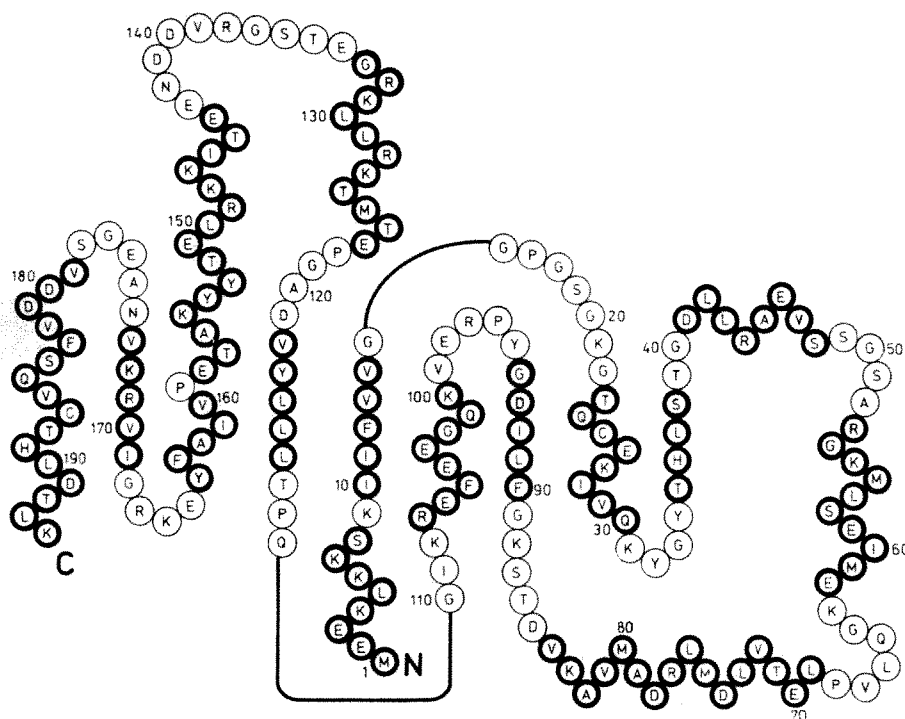


Fig. 3 Sequence of adenyl kinase in one letter notation²². Residues involved in helical structures and in the pleated sheet are drawn with heavier circles. The diagram indicates roughly the chain fold. The orientation of the molecule corresponds to that shown in Fig. 2. Helix 23-30 is above the plane of the paper and helices 100-107 and 144-164 are below.

The region around His-36 is rather conspicuous. Cys-25 is about 5 Å away. Such a close His-Cys-pair is probably also present in the active centres of creatine kinase²⁷ and of the Na-K-transport ATPase²⁸. Asp-93 is almost buried in the cleft. It may be hydrogen-bonded to His-36 and/or Cys-25. His-36, Asp-93, and Cys-25 are also present in the carp enzyme (von Zabern and Noda, personal communication). Ser-38 is very close to His-36 and Asp-93. The spatial relationship between these three residues, however, is that of an equilateral triangle and not that of a straight line as in the charge relay system of the proteases²⁹. The distances between the centre of the postulated metal ion and the centres of the side chains of His-36 and Asp-93 are 10 Å and 5 Å, respectively. There is sufficient space between His-36 and the postulated metal ion to accommodate a phosphoryl group. This group would also touch Asp-93. Moreover, there are several basic side chains in the cleft region. They might serve to neutralise the negative charges on the substrates.

There are two hydrophobic pockets nearby. One is formed in the region between Gly-20 and Val-179 (see Fig. 2), the other one between Gly-94 and Tyr-153, that is, in the cleft region. It seems geometrically possible to fill the first pocket with the adenine moiety of AMP and the second one with that of ATP in such a way that the phosphate group of AMP and the γ-phosphoryl group of ATP meet each other between His-36 and the postulated metal ion, that is, at Asp-93. This localisation of the substrates is comparable with the topology of the nucleotide binding sites in other proteins³⁰.

In the crystal the second pocket is partly covered by a neighbouring molecule. This might be one of the reasons why we have not succeeded in binding substrate to the crystalline enzyme so far. In future substrate binding studies we intend to concentrate on crystal forms of adenyl kinase¹¹ which have not yet been analysed.

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¹ Noda, L., *The Enzymes* (edit. by Boyer, P. D.), **8**, 279-305 (Academic Press, New York, 1973).

- ² Mazelis, M., *Plant Physiol.*, **31**, 37-43 (1956).
- ³ Markland, F. S., and Wadkins, C. L., *J. biochem.*, **241**, 4124-4135 (1966).
- ⁴ Colowick, S. P., and Kalckar, H. M., *J. biochem.*, **148**, 117-126 (1943).
- ⁵ Morrison, J. F., and Heyde, E., *A. Rev. Biochem.*, **41**, 29-54 (1972).
- ⁶ Steitz, T. A., Fletterick, R. J., and Hwang, S. J., *J. molec. Biol.*, **78**, 551-561 (1973).
- ⁷ Bryant, T. N., Watson, H. C., and Wendell, P. L., *Nature*, **247**, 14-17 (1974).
- ⁸ Blake, C. C. F., Evans, P. R., and Scopes, R. K., *Nature new Biol.*, **235**, 195-198 (1972).
- ⁹ Blake, C. C. F., and Evans, P. R., *J. molec. Biol.*, **84**, 585-601 (1974).
- ¹⁰ McPherson, jun., A., *J. molec. Biol.*, **81**, 8-86 (1973).
- ¹¹ Schulz, G. E., Biedermann, K., Kabsch, U., and Schirmer, R. H., *J. molec. Biol.*, **80**, 857-864 (1973).
- ¹² Heil, A., Müller, G., Noda, L. H., Pinder, T., Schirmer, I., Schirmer, R. H., and von Zabern, I., *Er. J. Biochem.*, **43**, 131-144 (1974).
- ¹³ Schirmer, I., Schirmer, R. H., Schulz, G. E., and Thuma, E., *FEBS Lett.*, **10**, 333-338 (1970).
- ¹⁴ North, A. C. T., *Acta Cryst.*, **18**, 212-21 (1965).
- ¹⁵ Spiegel, M. R., *Theory and Problems of statistics*, (McGraw-Hill, New York, 1972).
- ¹⁶ Cullis, A. F., Muirhead, H., Perutz, M. J., Rossmann, M. G., and North, A. C. T., *Proc. R. Soc.*, **A65**, 15-38 (1962).
- ¹⁷ Dickerson, R. E., Weinzierl, J. E., and Palmer, R. A., *Acta Cryst.*, **B24**, 997-1003 (1968).
- ¹⁸ Wilson, A. J. C., *Acta Cryst.*, **2**, 318-31 (1949).
- ¹⁹ Blow, D. M., and Crick, F. H. C., *Acta Cryst.*, **12**, 794-802 (1959).
- ²⁰ Chothia, C., *J. molec. Biol.*, **75**, 295-32 (1973).
- ²¹ Ramachandran, G. N., and Sasisekharan, V., *Adv. Prot. Chem.*, **23**, 283-437 (1968).
- ²² Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **5** (National Biomedical Research Foundation, Washington D.C., 1972).
- ²³ Lewis, P. N., Momany, F. A., and Schraga, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2293-2297 (1971).
- ²⁴ Price, N. C., Reed, G. H., and Cohn, M., *Biochemistry*, **12**, 3322-3327 (1973).
- ²⁵ McLaughlin, A. C., Cohn, M., and Kenyon, G. L., *J. biol. Chem.*, **247**, 4382-4388 (1973).
- ²⁶ Cohn, M., Leigh, jun., J. S., and Reed, G. H., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 53-540 (1971).
- ²⁷ Watts, D. C., and Rabin, B. R., *Biochem. J.*, **85**, 507-516 (1962).
- ²⁸ Atkinson, A., thesis, University of Birmingham (1972).
- ²⁹ Birktoft, J. J., and Blow, D. M., *J. molec. Biol.*, **68**, 187-240 (1972).
- ³⁰ Schulz, G. E., and Schirmer, R. H., *Nature*, **250**, 142-144 (1974).

LETTERS TO NATURE

PHYSICAL SCIENCES

Large flare on the red dwarf star UV Ceti

ATTEMPTS to measure radio emission occurring simultaneously with the sporadic optical flares on the red dwarf dMe type stars have been hindered by the rarity of large flares. Apart from one case of a flare with an unusually long duration and a peak magnitude of 1.7 (ref. 1) the correlation so far established has been limited to flares of about 1 mag or less^{2,3}. On October 11, 1972 we were able to obtain simultaneous radio and optical recordings of an unusually large flare on the star UV Ceti (L726-8AB) during which the apparent magnitude of the star increased by more than 4.55 mag.

The event occurred during the period of cooperative observations of this star⁴ (October 1–15, 1972). The observations described here were made simultaneously by the Mk 1A radio telescope at Jodrell Bank, and the 30-inch Cassegrain reflector (f/13.5) at the Stephanion Astronomical Station in Peloponnese. The radio telescope was working on a frequency of 408 MHz (± 2 MHz, ± 3 dB). The aerial received linear polarisation and the output was obtained by the digital sampling process¹. A radio source of known strength was used for calibration. A Johnson dual channel photoelectric photometer⁵ was used on the 30-inch reflector.

The raw data in the optical case consisted of the photoelectric intensity I_t (minus the sky background) during the flare, compared with I_0 , the photoelectric intensity (minus the sky background) for the quiet star. The light variation is plotted in Fig 1 as the variation in the b colour magnitude $\Delta m(b) = 2.5 \lg I_t/I_0$. During the peak of the flare between 21 h 18 min 5.2 s and 21 h 18 min 53.4 s UT the recording pen was off scale. The other interruptions in this record are caused by measurements of the sky background or by checks of the position of the star in the field of view of the telescope. In the radio case the data are the digital printout (logger units) from the sampling made five times a second and printed at intervals of 30 s. The scale of flux units at 408 MHz is derived directly from the similar digital printout on the calibration source (3C33). The calibration was made between 20 h 27 min and 20 h 43 min UT.

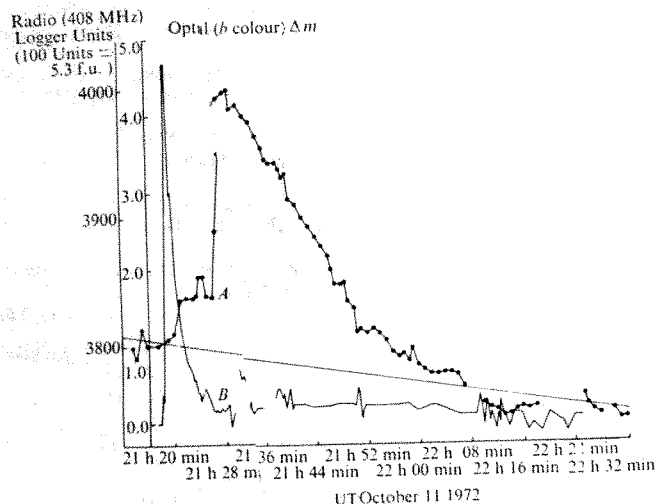


Fig. 1 The radio (A, 408 MHz) and photoelectric (B, b colour) records of the flare on UV Ceti on October 11, 1972. The ordinates for the radio record are digital logger units with 100 units = 5.3 flux units (10^{-26} W m⁻² Hz⁻¹).

No corrections have been made to these 408-MHz data plotted in Fig. 1 but because the flare occurred when the star was at an elevation of about 9° from Jodrell Bank there is a slight baseline slope caused either by ground effects or temperature drifts in the parametric amplifiers. The inclined abscissa indicates the estimated true baseline derived from recordings over the same azimuth-elevation range on preceding and following nights, when no flare occurred. In the case of the photoelectric records, measurements of a standard light source at 20 h 55 min and 23 h 06 min, differed by only 0.004 mag. Therefore, no corrections have been applied, and there are no corrections to $\Delta m(b)$ for atmospheric extinction. (The relative air-mass factors—secant of zenith distance—varied from 2.078 at 20 h 56 min, 1.777 at 22 h 44 min—upper culmination—to 1.787 at 23 h 06 min UT.) Measurements in the b colour of the companion star d (Fig. 1 of ref. 6) were made according to the recommendation of IAU Commission 27 (ref. 7). These measurements, at 20 h 56 min and 23 h 05 min UT were compared with the b colour measurements of UV Ceti at 21 h 06 min (quiescent state) and at 22 h 27 min UT. Flare star minus d were +0.54 mag and +0.45 mag respectively. The second order extinction coefficient K''_{b-v} at Stephanion is usually within 0.00 mag and -0.08 mag (ref. 8), and because $\Delta(b-v) \approx 1$ mag, the calculated values of these differences outside the Earth's atmosphere are approximately 0.62 mag and 0.52 mag respectively.

The rates of energy production during the flare are approximately 3.2×10^{25} erg s⁻¹ over a bandwidth of 400 MHz in the radio spectrum, and 2.5×10^{30} erg s⁻¹ over a bandwidth of 6.7×10^8 MHz in the optical spectrum. This ratio of 10^5 is similar to that for large solar flares but, as found in the previous measurements, the total energy output in the flare of 10^{31} to 10^{32} erg during the peak phase is at least equal to that of the normal photospheric continuum. In contrast, the total energy output of a large solar flare is equivalent to only about a millionth of the normal quiescent emission.

In many earlier radio-optical correlations there has been evidence that the peak of the radio emission follows the maximum phase of the optical flare by several minutes^{1,2}. The main feature of the correlation described here is the decisive evidence that the sharp front of the optical flare precedes that of the radio flare by approximately 8 min (the maxima are separated by 11 ± 1 min). The interpretation in terms of a solar flare type event in the atmosphere of UV Ceti is given in an accompanying paper⁹.

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¹ Lovell, B., *Nature*, **222**, 1126 (1969).

² Lovell, B., *Q. J. R. astr. Soc.*, **12**, 98 (1971).

³ Higgins, C. S., Solomon, L. H., and Bateson, F. M., *Aust. J. Phys.*, **21**, 725 (1968).

- ⁴ Chugainov, P. F., *IAU Inf. Bull. on Variable Stars* No. 605 (1971).
⁵ Johnson, H. L., *Annls Astrophys.* **18**, 292 (1955).
⁶ Luyten, W. J., *Astrophys. J.* **109**, 534 (1949).
⁷ *IAU Inf. Bull.* No. 326 (1969).
⁸ Asteriadis, G., Mavridis, L. N., and Tsoumis, A., in *Stars and the Milky Way System* (edit. by Mavridis, L. N.) (Springer-Verlag, Berlin, 1973).
⁹ Kahn, F., *Nature*, **250**, 125–126 (1974).

Model for a flare on the star UV Ceti

ON October 11, 1972 a large flare occurred on the star UV Ceti. It was observed simultaneously at photoelectric frequencies and at the radio frequency of 408 MHz (ref. 1). These observations provide enough information to describe a reasonably good model of the outer layers of the star, and of the overall mechanics of the flare.

The radio flare must have occurred in a region with low gas density, otherwise the radiation could not have propagated away. According to the observations there was a delay of about 500 s between the onset of the flare at optical and at radio frequencies. During this interval the disturbance from the optical flare must have spread through the corona of the star. The inevitable conclusion is that UV Ceti has a corona and a stellar wind. The energy required to heat the corona and to drive the wind must, presumably, come from the mechanical energy of the convective motions in and near the photospheric layers.

The general properties of flare stars have been described². For the star UV Ceti the data are as follows: mass = 10^{32} g, radius = 4.5×10^9 cm, and therefore surface gravity $g = 3.3 \times 10^5$ cm s⁻². When conditions are quiescent the surface temperature is $T_* = 3,000$ K, and the flux of radiation is 4×10^9 erg cm⁻² s⁻¹. The star has a surface area of 2.5×10^{20} cm² and its luminosity is 1.0×10^{30} erg s⁻¹.

Opacity tables³ can be used to construct a rough model for the atmosphere of the star. The table for an "Iben Mixture XII", with $X=0.8$ and $Z=0.01$, seems most appropriate. It becomes immediately apparent that the atmosphere must be convective even considerably above the level at which optical depth $\tau = 2/3$ is reached. The outer layers of the star are therefore fully convective; the pressure P is found to be 3.6×10^7 dyne cm⁻² and the density is 1.7×10^{-4} g cm⁻³ at $\tau=2/3$. The table given by Cox and Stewart³ has to be extrapolated to reach these values of pressure and density; the formula $\kappa = 9 \times 10^{-39} \rho^{0.45} T^{11}$ fits the opacity data reasonably well in the region concerned.

To generate a radiant energy flux of 4×10^9 erg cm⁻² s⁻¹ requires that the photospheric layer which can effectively radiate should have a surface density of 16 g cm⁻². As $\rho = 1.7 \times 10^{-4}$ g cm⁻³ this layer is only $\approx 10^5$ cm thick. Outside this layer the gas can hardly radiate at all.

The scale height, H , in the photosphere is about 6.5×10^5 cm and I assume that the mixing length for the convective motion is the same. To produce a convective flux of 4×10^9 erg cm⁻² s⁻¹ the rising and falling currents need an average speed $U = 2.0 \times 10^4$ cm s⁻¹, and a rising (or falling) element must have a temperature excess (or defect) of $\Delta T = 7$ K with respect to the mean temperature.

It seems probable that the convective motions will twist up any magnetic lines of force above the star. The turbulent pressure in the photosphere is $\rho U^2 = 6.8 \times 10^4$ dyne cm⁻². If this is equated to the magnetic pressure $H^2/4\pi$, then an estimate $H = 900$ gauss is obtained for the likely magnetic field strength at the surface of the star.

The photoelectric magnitude of UV Ceti increased by more than $\Delta m = 4.5$ during the flare¹. The peak increase lasted about 10 s. At 4,500 Å (a frequency of 6.7×10^{14} Hz) the normal energy output of the star is

$$\pi R^2 (8\pi h \nu^3 / c^2) \exp(-h\nu/kT) = 1.6 \times 10^{14} \text{ erg Hz}^{-1} \text{ s}^{-1}$$

An increase of 4.5 mag raises the energy flux by a factor of 63, to about 1.0×10^{16} erg Hz⁻¹ s⁻¹. Over a bandwidth of, for instance, 6.7×10^{14} Hz this corresponds to an energy output of 6.7×10^{30} erg s⁻¹ during the flare. But the optical flare is presumably a localised phenomenon above the surface of the star, not equally visible from all sides. I shall assume that the flare of October 11 was on the side facing the Earth. The energy received from it at the Earth would then be above average. I allow a factor 2.5 or 3 to take account of this. The estimate for the rate of energy production by the flare then becomes 2.5×10^{30} erg s⁻¹. Over a period of 10 s the energy output is 2.5×10^{31} erg.

The flare must occur in the lower corona. I shall take the ambient gas density there to be ρ_0 and the magnetic field strength in the region of the flare at the time of its occurrence to be H_0 . In accordance with the usual interpretation of solar phenomena I shall assume that the energy of the flare is released during the reconnection of the lines of force in a magnetic field. The Alfvén speed in the region will be $V_A = H_0 / (4\pi\rho_0)^{1/2}$; if L is the typical linear dimension of the flare region, the duration of the optical flare at its maximum can be estimated to be

$$\tau_0 = L/V_A = L\sqrt{4\pi\rho_0}/H_0 = 10 \text{ s} \quad (1)$$

The total magnetic energy in the flare region is

$$\mathcal{E}_0 \approx H_0^2 L^3 / 8\pi \quad (2)$$

If the light produced by the flare is a result of synchrotron radiation by fast electrons, then for the critical frequency $\omega_0/2\pi$ to be about right requires that, at the time of the optical flare,

$$\omega_{c,0} \approx 4.2 \times 10^{15} \approx \gamma_0^2 e H / mc \quad (3)$$

where $\gamma_0 mc^2$ is the typical energy of the electrons. If there are \mathcal{N}_0 electrons altogether, their total energy is $\mathcal{N}_0 \gamma_0 mc^2$ and their rate of production of radiant energy is

$$Q_0 = \mathcal{N}_0 \gamma_0^2 e^4 H_0^2 / m^2 c^3 = \mathcal{N}_0 \omega_{c,0} (e^3 / mc^2) H_0 \quad (4)$$

The estimate for Q_0 is 2.5×10^{30} erg s⁻¹. It follows from equations (3) and (4) that

$$\mathcal{N}_0 H_0 = 6 \times 10^{36} \text{ gauss} \quad (5)$$

But from relation (3) $\gamma_0 = 1.6 \times 10^4 H_0^{-1/2}$ and so

$$\mathcal{N}_0 \gamma_0 mc^2 = 10^{35} H_0^{-3/2} \quad (6)$$

is the total energy of the relativistic electrons present at any one time during the optical flare.

Further information can be deduced from the events following the visual flare. I postulate that after the reconnection of the lines of force a bubble of plasma with a magnetic field expands into the stellar corona, preceded by a shock wave. About 500 s later the bubble reaches the level at which radiation at 408 MHz can just propagate through the corona. I shall take this level to be at a height h_1 above the base of the corona. The local density of the plasma can be found from the condition that its refractive index is 0 at 408 MHz and this leads to $\rho_1 = 5 \times 10^{-15}$ g cm⁻³.

The expansion of the bubble can be described if something is known about the variation of density with height in the stellar wind. I assume the wind to be isothermal, with a pressure-density relationship $P/\rho = a^2 = \text{constant}$. Rough estimates indicate that the sonic point in the stellar wind will lie one or two stellar radii above the photosphere. In such a wind the variation of speed, u , with height is given by

$$(u - a^2/u) du = (2a^2/r - GM/r^2) dr \quad (7)$$

(see for example Kahn⁴). At the sonic point

$$u = a \text{ and } r = r_s = GM/2a^2 \quad (8)$$

To find how u varies with r near the sonic point put $u = a(1 + \epsilon)$ and $r = r_s(1 + \eta)$. With these substitutions equation (7) may be solved, correct to the second order in ϵ and η , in the form

$$\epsilon = \pm \eta \text{ or } u - a = \pm (a/r_s) (r - r_s) \quad (9)$$

The upper sign here applies for the case of a stellar wind, and the solution then simplifies to

$$u = ar/r_s \quad (10)$$

I shall apply this approximation all the way from the base of the corona to the level at which the 408 MHz radiation

can propagate. Then conservation of mass in the wind gives

$$\text{constant} = \rho v r^2 = \rho a^3 / r_s \quad (11)$$

and the density at height h above the photosphere is

$$\rho = \rho_0 R^3 / (R+h)^3 \quad (12)$$

Magnetic energy \mathcal{E}_0 is released in the lower corona during the visual flare. After time t the bubble has grown to linear dimensions h from its initial linear size L . Conservation of magnetic flux demands that the field strength should vary as h^{-2} , so that at the later time the energy content is $\mathcal{E} = \mathcal{E}_0 L/h$, the energy density is $\mathcal{E}_0 L/h^4$ and the magnetic pressure $P = \mathcal{E}_0 L/3h^4$. To expand, the bubble must push back the ambient coronal gas. The condition of momentum balance at the boundary of the bubble means that

$$P = \rho V^2 = \rho \dot{h}^2 \quad (13)$$

where $V = \dot{h}$ is the speed of expansion. It follows from equations (12) and (13) that

$$\rho_0 h^2 / (1+h/R)^3 = \mathcal{E}_0 L / 3h^4$$

and so the time τ_d required to reach height h_1 is given by

$$(\mathcal{E}_0 L / 3\rho_0)^{1/2} \tau_d = \int_0^{h_1} h^2 (1+h/R)^{-3/2} dh \quad (14)$$

The major contribution to the integral in this equation comes from the upper end of the range of integration, so that

$$\int_0^{h_1} h^2 (1+h/R)^{-3/2} dh \approx (2/3) h_1^{3/2} R^{3/2} \quad (15)$$

From equations (1) and (2) it follows that

$$(\mathcal{E}_0 / 3\rho_0)^{1/2} L^{1/2} = 6^{-1/2} (H_0^2 / 4\pi\rho_0)^{1/2} L^2 = 6^{-1/2} V_A L^2 \quad (16)$$

and so

$$6^{-1/2} L^3 \tau_d / \tau_0 = (2/3) h_1^{3/2} R^{3/2}$$

or

$$h_1 / R = (L^2 / R^2) (\tau_d \sqrt{3} / 2\tau_0 \sqrt{2})^{2/3} \quad (17)$$

For the flare of October 11, $\tau_d / \tau_0 = 50$, and the relationship becomes

$$h_1 / R = 9.8 L^2 / R^2 \quad (18)$$

Equations (1), (2), (12) and (18) together lead to a relationship between the energy \mathcal{E}_0 and linear dimensions L of the flare, in the form

$$\begin{aligned} \mathcal{E}_0 &= (1/2) \rho_0 V_A^2 L^3 = \rho_0 L^5 / 2\tau_0^2 = (\rho_1 L^5 / 2\tau_0^2) [1 + (h_1/R)]^3 \\ &= (\rho_1 L^5 / 2\tau_0^2) [1 + (9.8 L^2 / R^2)]^3 \end{aligned} \quad (19)$$

Inserting values for $\rho_1 \tau_0$ and R leads to the estimate in Table 1.

Table 1 Typical values for the parameters

L	10^9	2×10^9	3×10^9	cm
\mathcal{E}_0	8.3×10^{28}	2.2×10^{31}	1.0×10^{33}	erg
H_0	45	270	1,000	gauss

The results (Table 1) can be approximately represented by the formulae

$$H_0 = 2 \times 10^{-21} L^{5/2}, \mathcal{E}_0 = 3 \times 10^{-48} L^{8.5} \quad (20)$$

Comparison of the results in equation (20) with that in equation (6) shows that the total energy of the relativistic electrons present in the flare at any one time is

$$\mathcal{E}_{\text{rel}} = \mathcal{N}_0 \gamma_0 mc^2 = 10^{66} L^{-15/4} \quad (21)$$

It is obvious that $\mathcal{E}_{\text{rel}} < \mathcal{E}_0$, because the electrons derive their energy from the magnetic field. A lower limit to the linear dimensions of the flare is therefore given by

$$L \geq 1.7 \times 10^9 \text{ cm} \quad (22)$$

When there is equality in equation (22), \mathcal{E}_0 equals 1.6×10^{31} erg, barely enough to account for the visual energy output of the flare. If \mathcal{E}_0 exceeds \mathcal{E}_{rel} , for instance $\mathcal{E}_0 = \alpha \mathcal{E}_{\text{rel}}$ with $\alpha > 1$, then

$$L = 1.7 \times 10^9 \alpha^{0.08} \text{ cm} \quad (23)$$

so that even with $\alpha = 100$, say, the estimate for L is raised by only a factor 1.5. Furthermore, because \mathcal{E}_{rel} decreases as L increases, it seems most probable that \mathcal{E}_{rel} can never be as large as the total visual energy output of the flare, and that the synchrotron lifetime of the electrons is shorter than the duration of the flare. It must therefore be possible for electrons to pick up energy from the magnetic field as the flare evolves.

In the light of all these arguments it seems reasonable to conclude that $L \sim 2.5 \times 10^9$ cm. This implies that $\mathcal{E}_0 = 3.0 \times 10^{32}$ erg, $H_0 = 600$ gauss, $\mathcal{E}_{\text{rel}} = 6.0 \times 10^{30}$ erg, $\gamma_0 = 640$ and

$\mathcal{N}_0 = 1.0 \times 10^{34}$. The height h_1 equals 1.4×10^{10} cm and the estimate for the density in the lower corona becomes $\rho_0 = 3.8 \times 10^{-13} \text{ g cm}^{-3}$.

By the time the magnetised bubble reaches height h_1 it has expanded by a factor 5.6 in linear dimensions, and the magnetic field strength has dropped, by a factor of 1/31, to 20 gauss. If the radiation at 408 MHz also results from synchrotron emission, the energy $\gamma_1 mc^2$ of the electrons is then given by

$$\omega_{c,1} \approx 2.5 \times 10^9 = \gamma_1^2 e H / mc = 3.4 \times 10^8 \gamma_1^2$$

$$\text{or } \gamma_1 \sim 3$$

The observed output of the radio flare at its peak is 10 flux units (f.u.) $= 10^{-22} \text{ erg cm}^{-2} \text{ Hz}^{-1} \text{ s}^{-1}$. The distance to UV Ceti is 2.7 pc or 8×10^{18} cm and therefore the rate of output of energy by the star, per unit frequency interval, is $8 \times 10^{16} \text{ erg Hz}^{-1} \text{ s}^{-1}$, or about $3.2 \times 10^{25} \text{ erg s}^{-1}$, assuming a bandwidth of about 400 MHz. This means that there must be \mathcal{N}_1 electrons radiating, where

$$\mathcal{N}_1 \gamma_1^2 (e^4 H^2 / m^2 c^3) = (e^3 / mc^2) \mathcal{N}_1 H_1 \omega_{c,1} = 3.2 \times 10^{25}$$

There seem to be more relativistic electrons radiating during the radio phase of the flare than there were during the earlier, visual phase. But their individual energies are smaller, and their total energy at any one time is 4.0×10^{31} erg, about seven times that during the optical flare. For comparison, the magnetic energy in the bubble is now $\mathcal{E}_0 L/h_1 = 5.4 \times 10^{31}$ erg, still considerably larger than the particle energy. The acceleration process evidently still feeds energy to the electrons in the later stages of the flare.

The model of the flare has implications for the stellar wind. Take $r_s = 3R$ or $4R$ as possible values for the sonic radius. They correspond to

$$a = (GM/2r_s)^{1/2} = 1.7 \times 10^7 \text{ cm s}^{-1} \text{ or } 1.5 \times 10^7 \text{ cm s}^{-1}$$

and to temperatures $T = \bar{m} a^2 / k = 2.2 \times 10^8 \text{ K}$ or $1.6 \times 10^8 \text{ K}$. With the approximate form for the density variation in the stellar wind, the density at $r = r_s$ becomes $\rho_s = 1.4 \times 10^{-14} \text{ g cm}^{-3}$ or $6 \times 10^{-15} \text{ g cm}^{-3}$, and the predicted rate of mass loss

$$4\pi r_s^2 \rho_s a = 5 \times 10^{14} \text{ g s}^{-1} \text{ or } 3.4 \times 10^{14} \text{ g s}^{-1}$$

These values are not sensitive to the assumed value of r_s . They are of the same order as the mass loss rate inferred for another flare star, YZ CMi (ref. 4).

It is not obvious, with an isothermal model, how the amount of energy carried off by the stellar wind should be estimated, for in principle the wind speed keeps increasing indefinitely with distance. In practice, though, the isothermal condition is bound to fail at some reasonable distance. An acceptable first estimate is that each gram of stellar wind carries away energy of about $GM/R = 1.8 \times 10^{15} \text{ erg g}^{-1}$, and the total predicted energy loss into the wind is of the order of $8 \times 10^{29} \text{ erg s}^{-1}$. For comparison the luminosity of the quiescent star is $1.0 \times 10^{30} \text{ erg s}^{-1}$. The present model thus requires that a large fraction, at least 40%, of the energy brought by convection to the photosphere should later become available as mechanical energy for heating the stellar corona.

Finally, the magnetic energy stored in the region of the flare amounts to $\mathcal{E}_0 = 3.0 \times 10^{32}$ erg, localised above an area $L^2 \sim 6 \times 10^{18} \text{ cm}^2$ of the star. A supply of about $4 \times 10^9 \text{ erg cm}^{-2} \text{ s}^{-1}$ seems to be available as mechanical energy to drive the stellar wind. With an equal supply available to twist up the lines of force it would only take about $1.3 \times 10^4 \text{ s}$ (about 3.5 h) to charge up the magnetic field, ready for the flare.

I thank Sir Bernard Lovell for telling me about the observations of UV Ceti, and for discussion about its properties.

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¹ Lovell, A. C. B., and Mavridis, L. N., *Nature*, **250**, 124-125 (1974).

² Lovell, A. C. B., *Q. Jl R. astr. Soc.*, **12**, 98 (1971).

³ Cox, A. N., and Stewart, J. N., *Astrophys. J. Suppl. Ser.*, **19**, 243 (1970).

⁴ Kahn, F. D., *Nature*, **222**, 1130 (1969).

Redshifts of 1548 + 115a and 1548 + 115b in the theory of the generalised gravitational potential

In a recent communication Wampler *et al.*¹ reported the redshifts of the QSO pair 1548 + 115a, b. For the QSO 1548 + 115a the redshift is given as $z = 0.4359$ and for 1548 + 115b as $z = 1.901$. Since these QSOs are separated by approximately 5 arc s these authors concluded, primarily on the basis of a statistical argument, that these two objects are at the same cosmological distance and that the redshifts are therefore "discordant". In addition these authors noted that the wavelength ratio $(1 + z)_b / (1 + z)_a = 2.02$, which is sufficiently close to a factor of two for them to speculate that for such optically associated objects this "... is either an unfortunate coincidence or a profound mystery".

Stellar objects that are apparently connected but have considerably different redshifts have been reported previously. Arp², for example, has reported on pairs of galaxies which are joined by luminous bridges but which have different galaxy redshifts, and Burbidge *et al.* (BBSS)³ have published a statistical analysis of the proximities of 47 QSOs to bright galaxies, concluding that four of these QSOs were probably associated with their respective galaxies. Again in the Burbidge work the redshifts for the pairs are "discordant".

The work of Arp and BBSS has been discussed previously^{4,5} within the context of the generalised gravitational potential which is inferred from my general relativistic scalar field theory in the complex Weyl space⁶. In this theory the macroscopic theories of Einstein gravitational and Maxwell electromagnetism are united together with the microscopic scalar field in Ψ . When this theory is applied to the "Schwarzschild" problem for the nucleus, where the Newton-Einstein constant is negligible, the integration constant in the component g_{00} of the metric tensor is identified with a nuclear property, namely the nuclear core radius. Therefore for a stellar object with a density comparable with that of nuclear matter, where both constants are significant

$$g_{00} = 1 - ([2GM/c^2] + r_0 A^{1/3})/r \quad (1)$$

where G is the Newtonian gravitational constant, M the mass of the body, c the speed of light, r_0 the nucleon core radius and A the stellar mass number. The generalised gravitational redshift now becomes

$$z_G = [(2R_0 R/r_0 \rho^{1/3} (1 + R)) - 1]^{-1} \quad (2)$$

where

$$R = (r_0 c^2 / 2G m^{1/3}) M^{-2/3} \quad (2a)$$

and m is the nucleon mass, $R_0 = 1.21$ fm the nucleon radius and $\rho = D/D_0$ is the ratio of the density D of the stellar body to that of the density D_0 of the nucleon. Because of the "Schwarzschild" singularity we must also have in this theory that $z_G < 1$. And for a total redshift of z

$$z_v = (z - z_G / (1 + z_G)) \quad (3)$$

where z_v is the recessional redshift.

If we now apply the theory of the generalised gravitational potential to the pair 1548 + 115a, b and assume a negligible gravitational factor for 1158 + 115a (that is an object with both a negligible contribution from Einstein gravitation and a density much less than that of nuclear matter), we have $z_v = z = 0.4359$. If in addition we assume the maximum possible gravitational contribution from this theory ($z_G \approx 1$) we obtain from equation (3) $z_v \approx 0.451$ for 1548 + 115b; thus there is no need, in the context of this theory, to seek a further explanation of the wavelength factor of ~ 2 commented on by Wampler *et al.*

Stated in an alternative way, we assume that the total redshift given by Wampler *et al.* for 1548 + 115a represents the recessional redshift for both objects. In addition, however, we apply equation (3), utilising the maximum possible gravitational redshift from this theory ($z_G \approx 1$) for 1548 + 115b and obtain $z \approx 1.8718$ for 1548 + 115b, which is close to the result obtained by Wampler *et al.*

If the work⁷ of Cameron on neutron stars, which does not make use of equation (1), is nonetheless taken as a model for all stellar objects with neutron densities then the masses of these objects are of the order of one solar mass. It should be noted from equation (2) that gravitational redshifts in this theory can be appreciable for small masses. For example, for a solar mass with nuclear density ($\rho = 1$), $z_G = 0.776$. To account, therefore, for the energetics implied by the data on 1548 + 115b it has been suggested to me by Dr Howard Poss of Temple University that perhaps 1548 + 115b is actually an assemblage of such stars. This suggestion is, however, not to be restricted to the pair 1548 + 115a, b but must be generalised to any stellar system whose energetics cannot be sustained by a constraint of one solar mass, which is indeed how this general concept is applied in this theory for any stellar system with a "discordant" redshift. In addition, if cognisance is to be taken of the precision in the redshift calculations by Wampler *et al.* then one could postulate a small relative velocity between 1548 + 115a and 1548 + 115b with a resultant velocity component in the direction of the Earth for 1548 + 115a. This is a more definitive statement than that offered by these authors when discussing the precision of their measurements. Furthermore, if these authors mean by "spectral peculiarities" that there could exist associated QSO pairs with different redshifts not explainable on the basis of Einstein's theory and that therefore "... no pre-existing theory suggested them ...", then this statement is incorrect^{4,6}.

Both Bahcall *et al.* (BMB)⁸ and Hazard and Sanitt (HS)⁹ have pursued the work of BBSS by choosing different sample spaces. In the former case these authors considered a larger group of quasars in addition to the 3C quasars chosen by BBSS. Although they found additional quasars that are closer to bright galaxies than expected if these two types of objects were uncorrelated, the increase was not at all proportional to the BBSS finding. In this connection it should be noted that, from the point of view of the generalised gravitational potential, only three (3C232, 3C275.1, 3C309.1) of the original four in the BBSS list were consistent with a connection hypothesis, whereas the fourth (3C268.4) was not; that is to say that this theory predicts that observational data may be found to support the connection hypothesis for the first three quasars but no observational data will be found to establish a physical connection between 3C268.4 and NGC4138. If this theory were further taken into account then the original BBSS number would change from four to three probable associations. This in turn would modify somewhat the expectations of the work of BMB.

The latter work of HS also failed to confirm the BBSS results (restricted to 3CR types) when source and galaxy lists, other than those chosen by BBSS, were used. It should be noted that the elimination of 3C232 from the BBSS list would modify still further the expectations of BMB.

In an additional discussion Bahcall and Woltjer¹⁰ considered the pairs Ton 155, 156 and 1548 + 115a, b. They conclude that, when taken as a class, the close proximity of QSOs in each pair "may be comfortably explained as random coincidences ...".

The problem with all four papers quoted here^{3,6-10} that adopt a statistical approach to the redshift problem is that they each select (*a priori*) a subset of objects. The central physical problem, however, is whether or not there is an additional component in the redshift. The theory of the generalised gravitational potential claims there is, and to this end galaxy-galaxy pairs, QSO-galaxy pairs and QSO-QSO pairs should be examined with continually improved observational techniques, where the statistical analyses are of value only with respect to the choice of areas for concentrated observational effort. The question of whether or not QSOs are local or cosmological or consist of

both types of object will in any event obviously be influenced strongly by observational results obtained on the redshift problem.

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¹Wampler, E. J., Baldwin, J. A., Burke, W. L., Robinson, L. B., and Hazard, C., *Nature*, **246**, 203 (1973).

²Arp, H., *Astrophys. Lett.*, **7**, 221 (1971).

³Burbidge, E. M., Burbidge, G. R., Solomon, P. M., and Strittmatter, P. A., *Astrophys. J.*, **170**, 233 (1971).

⁴Cherry, N. H., *Nuovo Cim. Lett.*, **2**, 619 (1971).

⁵Cherry, N. H., *Int. J. theor. Phys.*, **8**, 47 (1973).

⁶Cherry, N. H., *Nuovo Cim.*, **4B**, 144 (1971).

⁷Cameron, A. G. W., *Astrophys. J.*, **130**, 884 (1959).

⁸Bahcall, J. N., McKee, C. F., and Bahcall, N. A., *Astrophys. Lett.*, **10**, 147 (1972).

⁹Hazard, C., and Sanitt, N., *Astrophys. Lett.*, **11**, 77 (1972).

¹⁰Bahcall, J. N., and Woltjer, L., *Nature*, **247**, 22 (1974).

Infrared heterodyne spectroscopy of CO₂ on Mars

THERE has been considerable interest in applying infrared heterodyne techniques to astronomical problems¹⁻³. We have used an infrared heterodyne spectrometer operating near 11 μm with a resolving power of 1.5×10^6 to obtain spectral line profiles of carbon dioxide absorption in the Martian atmosphere. The multichannel system determined shapes of three lines in the $10^0\ 0-00^0\ 1$ vibration-rotation band of $^{13}\text{C}^{16}\text{O}_2$, and found equivalent widths about 50 MHz ($0.0017\ \text{cm}^{-1}$). A lower limit of 670 MHz ($0.022\ \text{cm}^{-1}$) on the equivalent width of the P(20) line of $^{13}\text{C}^{16}\text{O}_2$ was also obtained.

Figure 1 shows a block diagram of the equipment. Two concave mirrors not shown in Fig. 1 matched the diameter and wavefront curvature of the laser and telescope beams at the combining beam splitter. The 9% of the laser beam which was reflected by the beam splitter and the 91% of the telescopic beam which was transmitted were focused on to a copper-doped germanium photomixer cooled to 4 K. The photomixer signals were amplified over a frequency band from 100 to 1,500 MHz and fed into a radiofrequency mixer with a variable local oscillator frequency. The output of the mixer was fed into a filter bank which simultaneously measured the power in eight channels, each 18 MHz wide, equally spaced from d.c. to 150 MHz. Each channel had a synchronous detector locked to the chopping frequency and followed by a low pass filter. The eight outputs of the filter bank were sampled and digitised by computer. To cancel offsets between the two parts of the sky seen by the dual beam chopper, the planet was periodically alternated from one beam to the other.

As a periodic calibration of the system sensitivity, a black body at 500 K was placed in front of the chopper. By placing the heliostat in autocollimation, the telescope transmission was found to be 0.85. Atmospheric transmission was determined by measuring heterodyne signals from the Moon and Mars as a function of zenith angle. Averaged over the band of 100 to 1,000 MHz, the zenith transmission on the P(20) line of $^{13}\text{CO}_2$ (wavelength $10.57\ \mu\text{m}$) was 0.45, lower than may be expected from previous measurements⁴

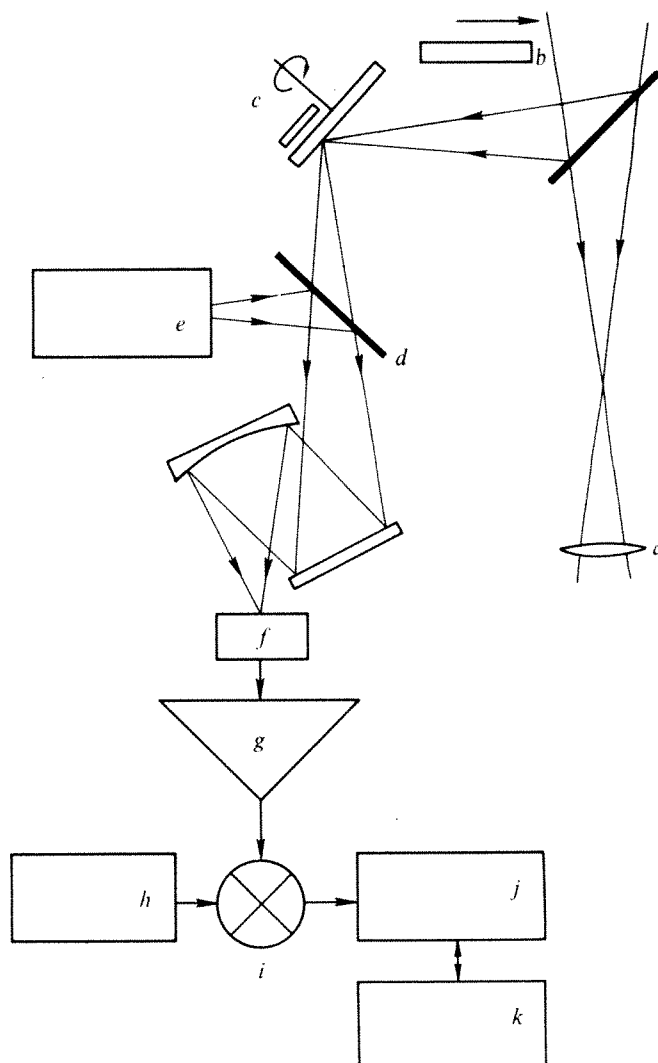


Fig. 1 Block diagram of the equipment located at the auxiliary solar telescope, located near the focal plane. *a*, Guiding eyepiece; *b*, black body; *c*, chopper; *d*, beamsplitter; *e*, CO₂ laser; *f*, germanium: copper photomixer; *g*, amplifier; *h*, radiofrequency local oscillator; *i*, radiofrequency mixer; *j*, eight channel filter bank; *k*, computer. The telescope consists of a steerable flat heliostat with a collecting area of about 0.4 m², followed by a fixed off-axis focusing mirror and three flat mirrors which direct the beam to the equipment. A rotating mirror in the focal plane of the telescope chopped the source against a sky background at 150 Hz. A linearly polarised laser used as an infrared local oscillator was operated in the TEM₀₀ mode, and stabilised on various rotational transitions of the $10^0\ 0-00^0\ 1$ band of CO₂.

and transmission of the $^{13}\text{CO}_2$ lines near $11.2\ \mu\text{m}$ was 0.90. The approximate standard deviation in each case was 0.05. The contribution of water vapour to the absorption was estimated to be 0.05 to 0.10 (ref. 5). The system sensitivity for each 18 MHz channel was thus calculated to be $5 \times 10^{-22}\ \text{Wm}^{-2}\ \text{Hz}^{-1}$ at the top of the Earth's atmosphere, assuming operation on a $^{13}\text{CO}_2$ line, radiation of one polarisation, and a signal to noise ratio of unity for one second of integration. Because of the antenna properties of the heterodyne receiver⁶ and the shape of the heliostat mirror, the system was only sensitive to radiation in an elliptical beam measuring 4 arc s in declination (dec) and 3 arc s in right ascension (RA). The angular diameter of Mars was about 14 arc s at the time of observation.

In order to understand how the spectral information was obtained it is necessary to consider how the spectrum was folded on itself in the two mixing operations (Fig. 2). The continuum infrared radiation is folded over into the absorption profile and decreases the fractional depth by a factor

of two. We assumed that the absorption lines were symmetric and tuned our radio frequency local oscillator to the expected centre of the absorption line. This frequency was calculated from the relative velocities and rotation rates of Earth and Mars, and typically had a value near 1,100 MHz. The output of the radio frequency mixer thus represents the amplifier output power folded about the frequency at line centre of the radio frequency local oscillator. This is quite accurate for the $^{13}\text{CO}_2$ lines because their widths are considerably less than both the velocity shift and the width of the entire filter bank. The $^{12}\text{CO}_2$ line, however, is very broad and its interpretation is more difficult.

In a computer simulation of the Martian atmosphere we assumed an atmosphere of 100% carbon dioxide consisting of plane-parallel layers of constant temperature and pressure (Fig. 3). The rate of change of temperature with altitude is conventionally defined as the lapse rate, and the height at which the pressure drops by a factor of e is similarly defined as the scale height. We assumed a constant lapse rate and a scale height, at the surface, of 11 km. Parameters used in the simulation were:

T_s = surface temperature (given separately for each line in Fig. 3);

$\Delta T_{AS} = T_s$ minus the temperature of the atmosphere at the surface;

Γ = temperature lapse rate (degrees km^{-1});

P_s = surface pressure (mbar).

In addition we defined the parameter

$$z = [P_s/6 \text{ mbar}] \cdot R(13/12) \cdot A(13/12).$$

This is proportional to the number of $^{13}\text{CO}_2$ molecules in the atmosphere and to the transition rate, where

$R(13/12)$ = ratio of isotopic species by number and

$A(13/12)$ = ratio of Einstein A coefficients for equivalent lines in the two isotopic species.

The best values of Γ , ΔT_{AS} , and z each depend to a large extent on which values are taken for the other two parameters (Table 1). This is because the number of absorbing molecules and their average temperature must remain nearly fixed in order to maintain the same absorption profile for each model. Four sets of these parameters which give equally good fits to the data are given in Table 1. Model 1 (Table 1) uses the adiabatic lapse rate for Mars. Higher lapse rates would be quickly reduced to the adiabatic case by convection. Model 4, with zero lapse rate, is physically unrealistic but is included to show that z does not continue to decrease as the lapse rate further decreases. The results themselves do not put upper bounds on ΔT_{AS} and z ; values for z which fit the data are, however, in all cases larger than expected and values of ΔT_{AS} were therefore chosen so as to minimise z .

Statistical errors in the data produce uncertainties in the model. The standard deviation of T_s is about 1.5 K for the P(16) line and about 3.0 K for P(22) and P(26); systematic errors in the calibration cannot, however, be completely ruled out. The value of T_s is obtained from the black body calibration. Based on this calibration our measurement of the lunar flux is about 15% higher than the published value and has a standard deviation of 3% but the discrepancy is, at least in part, because of imprecise knowledge of the Sun angle at the point observed. In any case the discrepancy is in the direction which, if corrected for, would reduce our measured flux from Mars and lower the surface temperature. The correlations between Γ , ΔT_{AS} , and z make uncertainties in these quantities difficult to estimate, but the errors are considerably smaller than the range allowed from model to model. For example, the standard deviation of z in Model 3 for the P(16) line is about 0.004 if Γ and ΔT_{AS} are kept fixed.

Previous data⁷ can be compared with the parameters we have fitted to our data. The effective continuum temperature near the $^{13}\text{CO}_2$ lines is about 250 K (Fig. 3). Mariner 9 (ref. 7), looking at the sub-solar point as we did, obtained

a temperature near 11 μm of about 260 K. Mariner 9 also measured a temperature lapse rate of 2.5 K km^{-1} and an atmospheric temperature at the surface about 25 K cooler than the surface itself. These values are close to those used in Model 2 (Table 1). The parameter z is of particular interest because our data indicate a higher value than expected in all models. Some limits can be placed on the ranges of the three factors comprising z . Mariner 9 reported a surface pressure of 6 mbar with a range from 4 to 8 mbar because of terrain changes. Our results place an upper limit of 12 mbar because at this pressure the effects of collisional line broadening would have become apparent. A value of 0.24 s^{-1} was taken for the Einstein A coefficient of $^{13}\text{CO}_2$ (refs 8,9). The correct value for $^{13}\text{CO}_2$ may, however, be somewhat different because this coefficient is strongly influenced by the Fermi resonance between the lower level of the absorbing transition and the 020 vibrational level. Even a small change in the mass of the carbon atom affects the strength of this interaction. Measurements of the relative output power of carbon dioxide lasers operating on the two isotopic species suggest that the ratio $A(13/12)$ is about 2/3 (C. Freed personal communication). Taking a value for surface pressure of

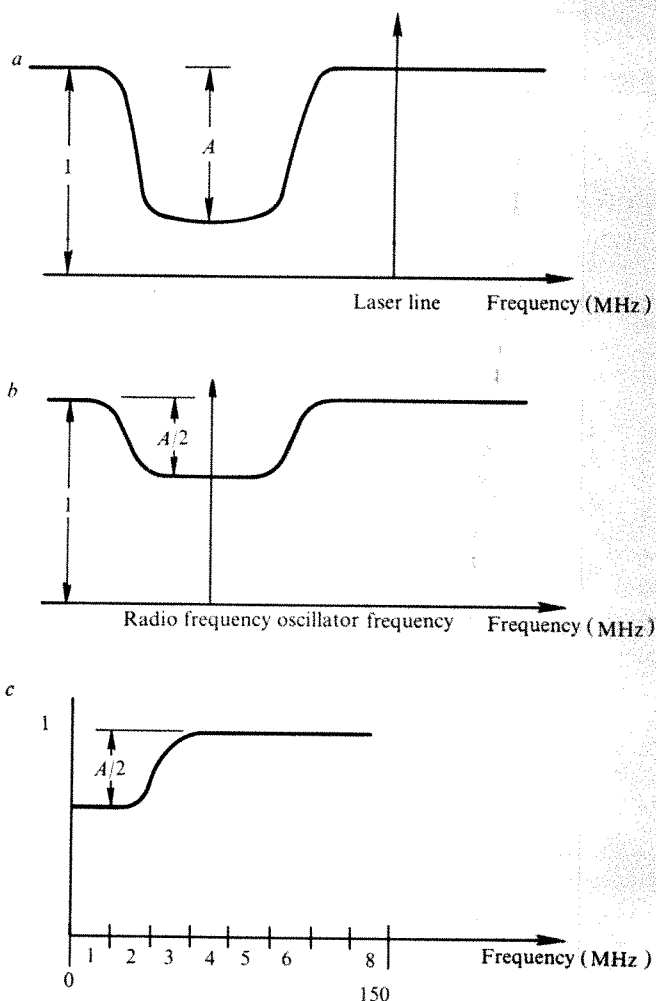


Fig. 2 Folding of the absorption line because of mixing: a, schematic representation of a Martian infrared absorption line shifted in frequency from a laser line of the same molecular transition by the relative motion of Earth and Mars. A is the fractional depth of the absorption. Infrared frequencies equally spaced on either side of the laser line appear at the same place in the radiofrequency spectrum of the amplifier output; b, the absorption line as it appears at the amplifier; c, the absorption line as it appears at the mixer output. The numbers along the abscissa represent the eight channels of the filter bank into which the mixer output is fed.

6 mbar, the terrestrial value of 0.011 for $R(13/12)$, and assuming a value of 2/3 for $A(13/12)$, gives a value of 0.007 for z , a number about three times smaller than those in Table 1 which fit our present measurements. The cause of this discrepancy is not clear.

Because $^{12}\text{CO}_2$ was expected to be 90 times more abundant than $^{13}\text{CO}_2$, we made the $^{12}\text{CO}_2$ measurements first. The results, however, were not as significant as those for $^{13}\text{CO}_2$. Because of the large width of the $^{12}\text{CO}_2$ line, measurements were made in bands 150 MHz wide distributed from 550 MHz below to 350 MHz above the expected frequency of the line centre. The bands could not be measured simultaneously because a suitably wide filter bank was not available. The measured flux was independent of frequency to within a standard deviation of 20%, indicating a line broader than the frequency range examined. The data are consistent with a continuum of 250 K folded into an absorption line with a flux of less than $2 \times 10^{-23} \text{ W Hz}^{-1}$ (200 K) and an equivalent width greater than 670 MHz (0.022 cm^{-1}). A computer simulation using a lapse rate of 2.5 K km^{-1} , a value for ΔT_{AS} of 30 K, and a surface pressure of 6 mbar predicts an equivalent width of 400 MHz for the $^{12}\text{CO}_2$ line. The difficulties of accurate cali-

Table 1 Parameters for computer simulation			
Model	Γ	ΔT_{AS}	z
1	5 K km^{-1}	25 K	0.027
2	2.5 K km^{-1}	30 K	0.02
3	1.3 K km^{-1}	35 K	0.017
4	0 K km^{-1}	45 K	0.02

bration mean that the discrepancy is probably not significant, although its direction is similar to that found for $^{13}\text{CO}_2$, corresponding to excess absorption.

The results demonstrate that infrared heterodyne spectroscopy can be used in astronomy for spectral resolution exceeding by several orders of magnitude that obtained with conventional techniques, although the copper-doped germanium photomixer used had a sensitivity about 25 times less than the theoretical limit of $2 \times 10^{-20} \text{ W Hz}^{-1}$ for heterodyne detectors at 11 μm . Continuously tunable infrared lasers would allow high resolution of lines anywhere in the infrared band and perhaps the detection of absorption lines of interstellar gas against bright sources.

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- Johnson, M. A., *J. opt. Soc. Am.*, **62**, 1350 (1972).
- de Batz, B., Granes, P. Gay, J., and Journet, A., *Nature*, **245**, 89-90 (1973).
- de Graauw, Th., and Van de Staadt, H., *Nature*, **246**, 73-75 (1973).
- King, S. R., Hodges, D. T., Hartwick, T. S., and Barker, D. H., *Appl. Opt.*, **12**, 1106-1107 (1973).
- McCoy, J. H., Rensch, D. B., Long, R. K., *Appl. Opt.*, **8**, 1471-1478 (1969).
- Siegman, A. E., *Proc. IEEE*, **54**, 1350-1356 (1966).
- Hanel, R., et al., *Icarus*, **17**, 423-442 (1972).
- Statz, H., Tang, C. L., and Koster, G. F., *J. appl. Phys.*, **37**, 4278-4284 (1966).
- Devir, A. D., and Oppenheim, U. P., *Appl. Opt.*, **8**, 2121-2123 (1969).
- Semiconductors and Semimetals*, vol. 5 (edit. by Willardson, R. K., and Beer, A. C.), (Academic Press, New York, 1970).

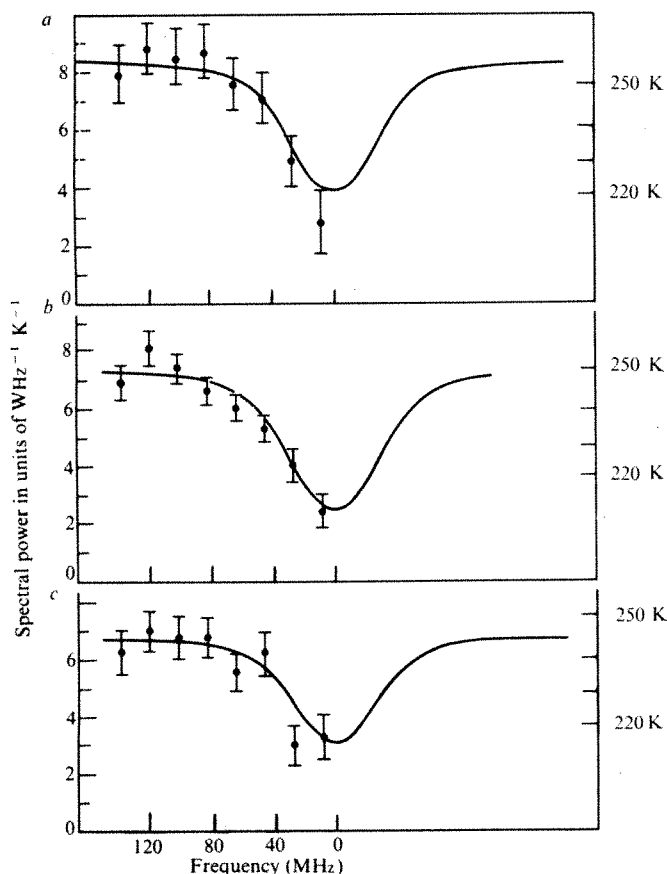


Fig. 3 Data obtained for the P(26) a, P(16) b, and P(22) c, rotational lines of $^{13}\text{CO}_2$. In a, $I_s=256 \text{ K}$, and equivalent width=50 MHz; b, $I_s=250 \text{ K}$, and equivalent width=66 MHz; c, $I_s=246 \text{ K}$, and equivalent width=49 MHz. The original data has been unfolded and plotted in the format of Fig. 2a as half of an infrared line. The ordinate is received spectral power in W Hz^{-1} divided by Boltzman's constant k and referenced to the top of the Earth's atmosphere. Absolute temperatures for black bodies of equal spectral radiance are shown at the right of the figure. Error bars represent plus and minus one standard deviation and correspond closely to predictions of the theory of heterodyne mixing¹⁰. The abscissa MHz is measured from the expected position of line centre. The P(26) and P(22) lines each represent about 1 h of observation, the P(16) line about 2 h. The solid curves represent absorption line profiles calculated in a computer simulation of the Martian atmosphere (see text).

Petrogenetic implications of argon isotopic evolution in the upper mantle

THE construction of models for the isotopic evolution of terrestrial argon (unlike those for strontium and lead¹⁻³), has been severely hampered by the fact that there are to our knowledge, no reported studies of the initial argon isotopic ratios in rocks of various ages. It has been estimated³ that the cosmic abundances of the stable argon isotopes are ^{40}Ar , 0.4%; ^{38}Ar , 14.1%; and ^{36}Ar , 85.6%. This gives a cosmic $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 0.005, which may reasonably be assumed to approximate closely the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the solid Earth at the time of formation.

The reported abundances of the argon isotopes in the terrestrial atmosphere⁴ at present are ^{40}Ar , 99.600%; ^{38}Ar , 0.063%; and ^{36}Ar , 0.337%, giving an atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 295.5. This anomalous situation is clearly related to the production of radiogenic ^{40}Ar derived from the natural decay of ^{40}K in the Earth's crust and mantle. An increase of

Table 1 Measured variation in ($^{40}\text{Ar}/^{36}\text{Ar}$)_i ratios

Source	($\times 10^6$ yr) Age	($^{40}\text{Ar}/^{36}\text{Ar}$) _i
1	Historic	282.9 → 294.8
2	Historic	292.1 → 320.4
3	0.075 ± 0.061	295.2 ± 11.1
4	0.120 ± 0.011	289.8 ± 3.8
5	0.194 ± 0.056	293.2 ± 6.0
6	0.259 ± 0.035	289.5 ± 7.3
7	0.534 ± 0.145	282.6 ± 14.6
8	12.4 ± 0.3	302 ± 6
9	15.3 ± 0.2	295 ± 3
10	15.8 ± 1.3	293 ± 19
11	53.1 ± 2.5	289 ± 21
12	64.8 ± 3.1	263 ± 21
13	55.4 ± 1.2	287 ± 9
14	119 ± 4	275 ± 8
15	410 ± 4	183 ± 70
16	415 ± 4	224 ± 37
17	411 ± 3	196 ± 61
18	770 ± 14	131 ± 88

(1) Basalts⁸; (2) single basalt flow²⁶; (3) – (7) New Zealand volcanics²⁷; (8) East Iceland volcanics²⁸; (9) Continental volcanics²⁸; (10) North-west Iceland volcanics²⁸; (11) – (13) Faeroes basalts²⁹; (14) Plutonic minerals⁷; (15) Continental margin, volcanics³⁰; (16) Plutonic biotites³⁰; (17) Plutonic biotites^{30,31}; (18) Continental dolerite dykes³².

more than four orders of magnitude over the initial terrestrial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio is indicated.

To demonstrate the isotopic evolution of argon in the solid Earth, we calculated the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios retained in analysed suites of mantle-derived igneous rocks, both volcanic and plutonic, of different ages (Table 1). The initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios were calculated in a manner exactly analogous to the determination of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, the intercept of an argon isochron following the linear equation:

$$(^{40}\text{Ar}/^{36}\text{Ar})_{\text{total}} = [\lambda_e/(\lambda_e + \lambda_B)](^{40}\text{K}/^{36}\text{Ar})(\exp(\lambda t) - 1) + (^{40}\text{Ar}/^{36}\text{Ar})_{\text{initial}}$$

A plot of the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios obtained from eighteen suites of mantle-derived samples against their time of emplacement or extrusion (Fig. 1) strongly suggests that there has been an increase in the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio with time. This we interpret as evidence for a significant increase in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the mantle over the past 800 Myr.

Implicit in this interpretation is the assumption that a significant proportion of the ^{36}Ar observed in the mass spectrum of argon extracted from terrestrial rocks and minerals is derived from the source region of the analysed samples and does not represent an atmospheric contaminant. We are supported in this contention by reported analyses of lava flows⁵⁻⁸ which have shown that most of the so-called ^{36}Ar 'contamination' comes not from the extraction line but is contained within the rocks themselves and is released along with ^{40}Ar during fusion. The initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios preserved in igneous rocks seem therefore to be representative of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in the source region of the parent magmas. The $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in any source region will increase with time at a rate dependent on the concentrations of potassium and ^{36}Ar , and the rate of argon release to the atmosphere.

Analyses of historically erupted lava flows^{5,6,8}, indicate initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios ranging from 282.9 to 350.0. Measured ratios greater than the present-day atmospheric ratio have been explained by the inclusion of xenoliths and xenocrysts in the analysed whole-rock samples but a satisfactory explanation has not been given for the lower ratios. The possibility of diffusion and fractionation of atmospheric argon into the cooling lava⁸ does not seem realistic to us. This variation of the observed $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in modern volcanic rocks may be due to inhomogeneities in the upper mantle or to remelting of older gabbroic rocks in layer 3 as suggested for some Icelandic rocks⁹. We therefore conclude, from the reported analyses of modern basalts, that the average $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the upper mantle is at present not greater than the atmospheric ratio (295.5) and possibly a little lower.

A similarity between the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the upper mantle and the terrestrial atmosphere leads us to the conclusion that it is the Earth's mantle, not the crust, that is the major source of atmospheric argon. Despite the obvious generation of radiogenic ^{40}Ar in the potassium-rich crust of the Earth, there is little evidence that significant outgassing of crustal argon is occurring today. It has been variously suggested (see Rankama¹⁰, for a summary) that the ^{40}Ar present in the Earth's atmosphere has been gradually released from crustal rocks throughout geological time as a result of weathering processes¹¹ or volcanism¹². Alternative models involving major degassing of the crust and mantle during the first 10^9 years of the Earth's history have been suggested by Nicolet¹³ and Damon and Kulp¹⁴, while Turekian^{15,16} and Schwartzman¹⁷ advocated continuous transfer of argon from both the crust and mantle. Modern plate tectonic theory provides a viable mechanism for the continual release of argon from the mantle, by way of

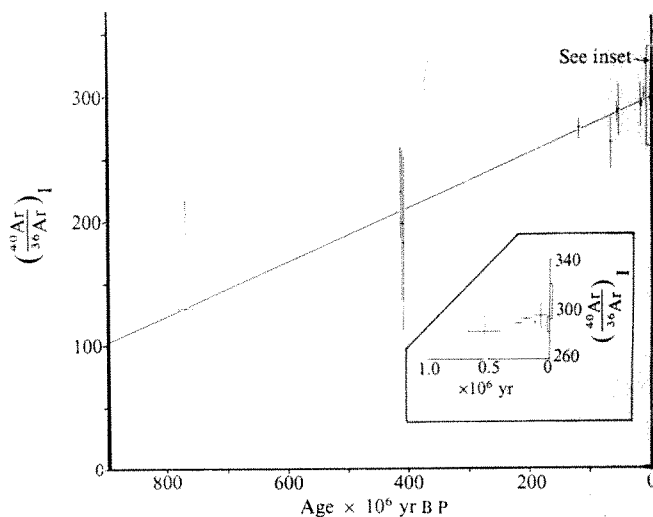


Fig. 1 Variation of the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the upper mantle-derived igneous rocks over the past 800 Myr. This variation is considered to reflect the variation in argon isotopic ratio of the upper mantle region with time.

volcanism on active plate boundaries, but there is no simple mechanism available for the release of argon accumulating in the Earth's crust. Furthermore, a large component of crustal argon in the atmosphere would result in a present-day atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio much greater than 295.5.

It is generally assumed that minerals melting and recrystallising in the Earth's crust under igneous and metamorphic conditions will lose argon to the atmosphere as a result of temperature-controlled diffusion processes. In reality, what is more likely to occur under these conditions is lattice diffusion and isotopic homogenisation of argon, with effective retention in the whole-rock system, a situation analogous to strontium isotopic homogenisation under similar conditions. Very little argon would escape from the crust directly to the atmosphere, except perhaps along major fracture zones. Homogenisation of the argon isotopes would have the effect of equalising the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the whole-rock system, so that initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios measured in crustal rocks should be greater than the present-day atmospheric ratio. Excessively high initial ratios have been recorded in metamorphic rocks, expressed in terms of an 'excess' ^{40}Ar concentration¹⁸⁻²¹.

To test our conclusions we have made a semiquantitative analysis of the available data. Any relevant calculations suffer, of course, from uncertainties in the estimation of potassium abundances^{22,23} in the Earth (Table 2). The calculated mass of radiogenic ^{40}Ar produced in the Earth from the decay of ^{40}K consequently has a large error which is propagated through the calculations.

The mass of ^{40}Ar produced in the Earth is calculated assum-

Table 2 Estimate of potassium and radiogenic argon in the Earth

	Mass of shell ²⁴ ($\times 10^{21}$ tons)	Potassium ^{22,23} K % ($\times 10^{15}$ tons)	Radiogenic ⁴⁰ Ar ($\times 10^{15}$ tons*)
Crust	0.024	320 \pm 55	1.350
Mantle and core	5.941	500 \pm 200	0.008
Total	5.965	820 \pm 207	0.014
Pyrolite	0.441	500 \pm 200	0.108
		(ref. 25)	0.0668 \pm 0.0267
Atmosphere			0.0653 (ref. 11)

* ⁴⁰Ar calculated from the potassium abundances with an age of 4,500 Myr for the Earth.

ing the Earth to be 4,500 Myr old. The observed mass of ⁴⁰Ar in the atmosphere (0.065×10^{15} tons), compared to the calculated mass of radiogenic ⁴⁰Ar produced in the Earth (0.11×10^{15} tons), suggests that only 60% of all the ⁴⁰Ar produced in the Earth has been released to the atmosphere.

In calculating the mass of ⁴⁰Ar in the mantle we make the following assumptions: (1) All ⁴⁰Ar in the atmosphere is derived from the solid Earth. (2) The upper mantle and crust are separate systems with respect to argon isotopic evolution. (3) Sediments make up approximately 5% of all crustal material²⁴, therefore not more than 5% of crustal argon has been released to the atmosphere and perhaps as little as 1% (ref. 10).

From assumption (3), taking 5% of crustal ⁴⁰Ar as being the maximum released to the atmosphere, the balance of the ⁴⁰Ar in the atmosphere is assumed to have come from the mantle, so (in units of 10^{15} tons):

$$\begin{aligned} \text{⁴⁰Ar in atmosphere} &= (0.0653 \pm 0.0033) \\ \text{⁴⁰Ar released from crust (5\%)} &= (0.0021 \pm 0.0004) \\ \therefore \text{⁴⁰Ar released from mantle} &= (0.0632 \pm 0.0034) \\ \therefore \text{⁴⁰Ar remaining in mantle} &= (0.0036 \pm 0.0269) \end{aligned}$$

The large error in the estimation of ⁴⁰Ar remaining in the mantle arises because the abundance is the difference between two nearly equal and poorly known numbers.

For purposes of calculation, we now assume that: (4) All the potassium, and thus radiogenic ⁴⁰Ar, is concentrated in an upper mantle region with a pyrolite²⁵ composition. (5) This pyrolite layer is approximately 200 km thick and has a mass of 0.441×10^{21} tons (Table 1). (6) The argon isotopic composition is homogeneous throughout the pyrolite layer.

The maximum concentration of ⁴⁰Ar at present in this pyrolite layer can now be calculated knowing the mass of the layer and the mass of ⁴⁰Ar remaining in the upper mantle. Thus the maximum ⁴⁰Ar concentration in the total pyrolite layer is $(8.2 \pm 61.0) \times 10^{-3}$ p.p.m. ⁴⁰Ar.

The present-day ³⁶Ar concentration in the upper mantle can be estimated from the observed ³⁶Ar concentrations in modern volcanic rocks derived from the mantle. The concentration of ³⁶Ar in young volcanic rocks, measured in our laboratory, after correcting for line blank, is estimated to be $(2.4 \pm 1.1) \times 10^{-3}$ p.p.m. ³⁶Ar. Because these rocks have been at least partially outgassed on extrusion, this value serves at least as a minimum concentration for the ³⁶Ar in the upper mantle source region.

Assuming that the present-day ⁴⁰Ar/³⁶Ar atomic ratio in the upper mantle source region is 295 ± 10 (or 327 weight ratio), then the present-day ⁴⁰Ar concentration in the upper mantle is at least $(7.8 \pm 3.6) \times 10^{-3}$ p.p.m. ⁴⁰Ar. This value is close to that estimated in the pyrolite layer by the difference method. This near coincidence could be due to the cancelling out of the different assumptions (1) to (6), or it might indicate that Hurley's potassium abundances^{22,23} are more precise than indicated in Table 2 and that most of the ⁴⁰Ar at present in the atmosphere has been released from the mantle.

According to our model, approximately 95% of all the ⁴⁰Ar produced in the mantle has been lost to the atmosphere. At least that percentage of ³⁶Ar must also have been lost, assuming no isotopic fractionation. From assumptions (5) and (6), using the observed concentration of ³⁶Ar in mantle-derived volcanic rocks, the mass of ³⁶Ar in the pyrolite shell (200 km thick) is estimated to be $(1.05 \pm 0.50) \times 10^{10}$ tons at present.

This must represent at most 5% of the ³⁶Ar originally present in the mantle. Therefore, 4,500 Myr ago, the mass of ³⁶Ar in the mantle was at least $(21.0 \pm 9.0) \times 10^{10}$ tons. There is at present 20.0×10^{10} tons of ³⁶Ar in the atmosphere, so it is quite reasonable to postulate that the majority of ³⁶Ar in the atmosphere has also come from the mantle.

Protocrustal material, with an average potassium concentration of 1.350%^{22,23}, would yield in 4,500 Myr a concentration of ⁴⁰Ar ten times greater than that in the upper mantle. The net result of this would be to give ⁴⁰Ar/³⁶Ar ratios in the deep crust that are an order of magnitude greater than the ⁴⁰Ar/³⁶Ar ratio in the upper mantle.

Initial ⁴⁰Ar/³⁶Ar ratios on igneous rocks which have not undergone isotopic homogenisation since crystallisation should yield information about the source region. It is thus possible to distinguish clearly between partial melting in a low potassium environment (such as the upper mantle) and partial melting of old sialic material. Initial ⁴⁰Ar/³⁶Ar ratios should also be very sensitive to any geological contamination (assimilation) of a basaltic magma by older potassium-rich material.

In summary: (1) Initial ⁴⁰Ar/³⁶Ar ratios of mantle-derived igneous rocks have increased steadily over the past 800 Myr. (2) The present-day ⁴⁰Ar/³⁶Ar ratios in the upper mantle and atmosphere are very similar. (3) The majority of argon at present in the atmosphere has been derived from the mantle rather than the crust. (4) Initial ⁴⁰Ar/³⁶Ar ratios on igneous rocks are potentially more sensitive petrogenetic indicators than strontium or lead isotopes.

Calculation of argon isochron parameters from the literature suffers from several omissions in the presentation of K-Ar age data for publication and we suggest the following minimum list of items that need to be reported: (1) The measured argon ratio in air. (2) The system line blank and isotopic ratio. (3) The weight of sample fused. (4) The concentration of ³⁶Ar measured or the percentage of 'atmospheric' to 3 significant figures.

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- 1 Armstrong, R. L., *Rev. Geophys.*, **6**, 175 (1968).
- 2 Sinha, A. K., and Tilton, G. R., *Geochim. cosmochim. Acta*, **37**, 1823 (1973).
- 3 Suess, H. E., *J. Geol.*, **57**, 600 (1949).
- 4 Neir, A. O., *Phys. Rev.*, **77**, 789 (1950).
- 5 Mussett, A. E., and Dalrymple, G. B., *Earth planet. Sci. Lett.*, **4**, 422 (1968).
- 6 Dalrymple, G. B., *Earth planet. Sci. Lett.*, **6**, 47 (1969).
- 7 Hayatsu, A., and Carmichael, C. M., *Earth planet. Sci. Lett.*, **8**, 71 (1970).
- 8 Krumenacher, D., *Earth planet. Sci. Lett.*, **8**, 109 (1970).
- 9 O'Nions, R. K., and Gronvold, K., *Earth planet. Sci. Lett.*, **19**, 397 (1973).
- 10 Rankama, K., *Progress in Isotope Geology*, (Interscience, New York, 1963).
- 11 Rankama, K., *Isotope Geology* (Pergamon, Oxford, 1954).
- 12 Shillibeer, H. A., Russell, R. D., *Geochim. cosmochim. Acta*, **8**, 16 (1955).
- 13 Nicolet, M., *Bull. Acad. r. Belg. Cl. Sci.*, 482 (1956).
- 14 Damon, P. E., and Kulp, J. L., *Geochim. cosmochim. Acta*, **13**, 280 (1958).
- 15 Turekian, K. T., *Geochim. cosmochim. Acta*, **17**, 37 (1959).

- ¹⁶ Turekian, K. T., in *The origin and evolution of atmospheres and oceans* (edit. by Cameron and Brancazio), (Wiley, New York, 1964).
- ¹⁷ Schwartzman, D. W., *Geochim. cosmochim. Acta*, **37**, 2479 (1973).
- ¹⁸ Brewer, M. S., *Earth planet. Sci. Lett.*, **6**, 321 (1969).
- ¹⁹ Wanless, R. K., Stevens, R. D., and Loveridge, W. D., *Eclog. geol. Helv.*, **63**, 345 (1970).
- ²⁰ Giletti, B. J., *Earth planet. Sci. Lett.*, **10**, 157 (1971).
- ²¹ Harper, C. T., and Schamel, S., *Earth planet. Sci. Lett.*, **12**, 129 (1971).
- ²² Hurley, P. M., *Geochim. cosmochim. Acta*, **32**, 273 (1968).
- ²³ Hurley, P. M., *Geochim. cosmochim. Acta*, **32**, 1025 (1968).
- ²⁴ Mason, B., *Principles of Geochemistry*, third ed. (Wiley, New York, 1966).
- ²⁵ Ringwood, A. E., *J. geophys. Res.*, **67**, 857 and 4473 (1962).
- ²⁶ Baksi, A. K., thesis, Univ. Toronto (1970).
- ²⁷ McDougall, I., Polach, H. A., and Stipp, J. J., *Geochim. cosmochim. Acta*, **33**, 1485 (1969).
- ²⁸ Moorbath, S., Sigurdsson, H., and Goodwin, R., *Earth planet. Sci. Lett.*, **4**, 197 (1968).
- ²⁹ Tarling, D. H., and Gale, N. H., *Nature*, **218**, 1043 (1968).
- ³⁰ Brown, J. Flett, thesis, Univ. Oxford (1973).
- ³¹ Westoll, N. D. S., and Miller, J. A., *Scott. J. Geol.*, **5**, 11 (1969).
- ³² Baadsgaard, H., and Mueller, P. A., *Geol. Soc. Am. Bull.*, **84** (1973).

Plate tectonics, volcanism and the lithosphere in British Columbia

THE most important feature of the present tectonic situation off the west coast of Canada is the ridge-trench-fault type triple junction between the Juan de Fuca, Pacific and American lithospheric plates near the continental margin, at approximately 51°N (Fig. 1). The half spreading rate for the Juan de Fuca Ridge, based on the magnetic lineation pattern, is 2.9 cm yr⁻¹, and the calculated rate of motion along the Queen Charlotte transform fault between the American and Pacific Plates is 6 cm yr⁻¹ (ref. 1). The main part of the Juan de Fuca Ridge is probably spreading parallel to the north-east trending Blanco fracture zone which marks the southern edge of the plate at approximately 43.5°N. The vector diagram for the relative motion of the three plates during the late Cainozoic suggests

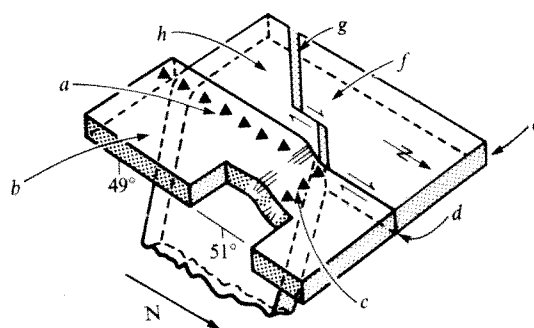


Fig. 2 The Canadian Cordillera and the adjacent north-east Pacific Ocean viewed from the north-east. a, 'arc' volcanics over the subducted part of the Juan de Fuca Plate; b, American Plate; c, lithosphere; d, Queen Charlotte Transform Fault; e, Pacific Plate; f, east-west volcanic lineaments; g, Juan de Fuca Ridge; h, Juan de Fuca Plate. Note the flexing of the American plate and the associated east-west topographic and volcanic lineaments (f) at approximately 51°N; the oblique underthrusting of the Pacific Plate along the Queen Charlotte Fault (d), and the associated north-south volcanic belt of British Columbia north of 53°N.

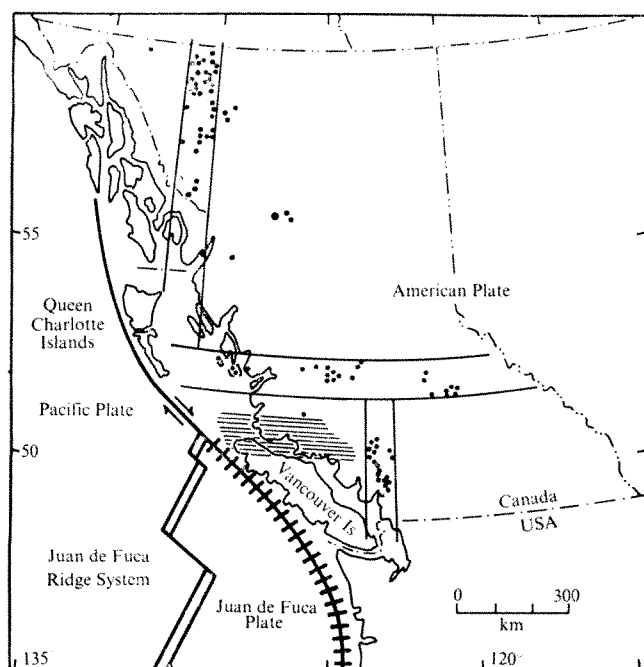


Fig. 1 Plate boundaries (after Atwater¹) and the volcanic (after Souther⁶) and topographic lineaments of the Canadian Cordillera. ●, Volcanic centres; double heavy lines, ridges; heavy, crossed, lines, trench; heavy line with reversed arrows, transform fault; thin close-set lines, east-west topographic lineaments.

that the Juan de Fuca Plate is being thrust obliquely below the American Plate at 2.5 cm yr⁻¹ in a direction of N 35° E (ref. 1).

Travel-time anomalies for the Seattle earthquake which occurred at a depth of 60 km, indicate the existence of a high velocity slab dipping eastwards at 50° beneath south-western Canada and the north-western United States². The magnetic anomalies of the Juan de Fuca Plate continue across the continental slope, and there is evidence of steep reverse faulting on the slope parallel to the continental margin³. Both these lines of evidence indicate recent subduction of the Juan de Fuca Plate below the American plate, as does the presence, further inland, of the late Cainozoic andesitic volcanic belt of the Cascades.

In the absence of a Benioff seismic zone, however, it is difficult to say how far the subducted Juan de Fuca Plate may extend to the east below the Cordillera. I here suggest that a number of phenomena within the Canadian part of the Cordillera, in the vicinity of the triple junction between the Juan de Fuca, Pacific and American Plates, can be explained if the influence of the subducted plate extends at least 500 km eastwards from the continental margin.

The most obvious physiographic feature of the Canadian Cordillera is that the area south of 51°N, the latitude of the triple junction, is approximately 500 m higher than that to the north. Geologically, there is more granitic 'basement' exposed to the south than there is to the north⁴, and in the eastern metamorphic belt of the Cordillera, the highest grades of metamorphism are again south of 51°N and decrease rapidly northwards⁵. These features all suggest that there has been a greater degree of uplift and erosion south of 51°N.

More than 90% of the approximately 150 Pleistocene and Recent volcanic centres which have been identified in the Canadian Cordillera⁶, fall within the two north-south belts and one east-west belt (Fig. 1). The volcanic centres are generally short-lived cinder cones with alkali olivine basalt flows, but at least 20 are composite volcanoes with flows ranging from picrite basalt, through alkaline andesite and dacite, to rhyolite. The north-south line of volcanic centres in southern British Columbia could be the northern continuation of the Cascade andesitic volcanic centres, and part of a volcanic arc over the subducted portion of the Juan de Fuca Plate. The greater arc-trench distance in Canada may indicate that the plate dips eastwards at a shallower angle north of 49°N. The east-west volcanic belt is at approximately the same latitude as the triple

junction on the continental margin, and the belt may be related to an upward bending of the American Plate over the northern edge of the Juan de Fuca Plate⁷, with magma generated by partial melting in the zone of tension at the base of the lithosphere (Fig. 2). South of the east-west volcanic belt, the reverse bend causes tension in the upper part of the lithosphere. The consequent east-west fracturing has been emphasised by subsequent erosion, producing the prominent east-west lineations in the Coast Mountains (Fig. 2). Preliminary interpretation of gravity data over Queen Charlotte Sound suggests that the east-west topography of the Coast Mountains continues below the sedimentary cover of the continental shelf north of Vancouver Island. The oldest identified sediments overlying this topography are late Miocene⁸, which indicates that the triple junction to the west has remained at approximately the same latitude for the last 15 Myr.

The model in Fig. 2 also relates the north-south volcanic belt of north-western British Columbia to a lithospheric flexure, in this case caused by the oblique convergence of the Pacific and American Plates. Data from this northern region are scarce, but one fault-plane solution for the 1949 Queen Charlotte earthquake (54.1°N, 132.6°W) requires right lateral slip, with some underthrusting of the Pacific Plate below the American Plate⁹. Seismic profiling across the southern end of the Queen Charlotte Fault (52°N) has shown a raised block of oceanic crust at the foot of the continental slope¹⁰. It is possible that this block was raised by reverse faulting similar to that found along the continental slope further south, where the faulting is believed to be related to subduction of the Juan de Fuca Plate below the American Plate³. In the St Elias mountains near the junction of the borders of Alaska, Yukon and British Columbia, there are marine Pliocene sediments which have been raised at least 6,400 m since deposition⁶. Thus, there is evidence that the Pacific Plate has been thrust obliquely under the American Plate, with the western margin of the latter raised to the north of the triple junction (Fig. 2). Tension at the base of the bent plate could have caused the observed volcanic lineament. With this interpretation the Queen Charlotte Fault cannot be regarded as a true transform fault (compare with Fig. 1) because of the element of convergence between the Pacific and American Plates.

The half wavelength of the flexure in the American Plate between the east-west volcanic belt and the parallel zone of topographic lineaments to the south, is 200 km and, assuming that the density between the lithosphere and the supporting asthenosphere is 3.3 g cm^{-3} and $g = 980 \text{ cm s}^{-2}$ the apparent flexural rigidity of the plate¹¹ will be $1.3 \times 10^{22} \text{ Nm}$. The thickness of the lithosphere¹¹, using a Poisson's Ratio of 0.25 and a Young's Modulus of $7 \times 10^{10} \text{ N m}^{-2}$ will then be 28 km. A similar value for the thickness of the lithosphere has been obtained from a study of the deformation of glacial lake shorelines by Fulton and Walcott¹² who estimated the lithosphere in the Cordillera south of 51°N to be 20–50 km thick. Even within these wide limits, the lithosphere is considerably thinner than the 110 km found below stable areas of the crust.

The concept of an abnormally thin lithosphere below the southern part of the Canadian Cordillera supports earlier ideas. For instance, Caner¹³ invoked a layer of possible partial melting in the lower crust to account for anomalous variations in the Earth's magnetic field in the south-eastern part of the Cordillera. Wickens¹⁴ has shown that the low velocity layer equivalent to the asthenosphere, approaches the base of the crust below the southern part of the Cordillera. The thickness of the lithosphere is approximately equal to the depth of the M discontinuity derived from refraction seismic data, suggesting that the upper surface of the asthenosphere approaches the base of the crust in this part of the Cordillera. The P_n velocity over the subducted part of the Juan de Fuca Plate is low (approximately 7.8 km s^{-1} , Berry, personal communication, 1973) and I have concluded⁷ that the density of the upper mantle below the central part of the Cordillera has also to be lower than normal in order to reconcile the gravity and refrac-

tion seismic results. As may be expected from the model in Fig. 2, P_n for the central part of the Cordillera increases to a more normal value of 8.1 km s^{-1} north of the latitude of the triple junction and the area overlying the Juan de Fuca plate.

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- ¹ Atwater, T., *Bull. geol. Soc. Am.*, **81**, 3513–3535 (1970).
- ² McKenzie, D. P., and Julian, B., *Bull. geol. Soc. Am.*, **82**, 3519–3524 (1971).
- ³ Barr, S. M., thesis, Univ. British Columbia (1972).
- ⁴ *Geol. Surv. Can., Map 1250A*.
- ⁵ Monger, J. W. H., and Hutchison, W. W., *Geol. Surv. Pap. Can.*, **70–33** (1971).
- ⁶ Souther, J. G., *Can. J. Earth Sci.*, **7**, 553–568 (1970).
- ⁷ Stacey, R. A., *Can. J. Earth Sci.*, **10**, 615–628 (1973).
- ⁸ Shouldice, D. H., *Bull. Can. Petrol. Geol.*, **19**, 405–436 (1971).
- ⁹ Hodgson, J. G., and Milne, W. G., *Bull. seism. Soc. Am.*, **41**, 221–242 (1951).
- ¹⁰ Srivastava, S. P., *et al.*, *Can. J. Earth Sci.*, **8**, 1265–1281 (1971).
- ¹¹ Walcott, R. I., *J. geophys. Res.*, **75**, 3941–3954 (1970).
- ¹² Fulton, R. J., and Walcott, R. I., *Mem. geol. Soc. Am.* (in the press).
- ¹³ Caner, B., *J. Geomagn. Geoelectr.*, **Kyoto**, **22**, 113–129 (1970).
- ¹⁴ Wickens, A. J., *Can. J. Earth Sci.*, **8**, 1154–1162 (1971).

Isotopic analysis of the deep structure in the Tyrrhenian Sea

THE monotonic distribution of temperature and salinity with depth, observed in most oceans of the world is replaced, in certain areas, by a peculiar 'staircase' structure in which sheets of water of constant temperature and salinity are separated by comparatively thin layers, across which there is a sharp gradient. Although its origin is speculative, the structure is often associated with the intrusion of warm saline water into a water mass that is colder and fresher. The structure is present in the eastern Atlantic, west of Gibraltar, below the characteristic Mediterranean outflow^{1,2}, and in the Tyrrhenian Sea below the inflow of the Levantine Intermediate Water through the Strait of Sicily^{3,4}.

We have attempted to distinguish the layers in the Tyrrhenian Sea by isotopic analysis of water samples collected from the different layers.

The Mediterranean is divided into two main basins by the Strait of Sicily. The eastern part is referred to as a 'concentration basin': the amount of water lost by evaporation exceeds the amount gained by precipitation and river discharge. In order to maintain the water and salt balance, two opposite flows connect the eastern and western basins through the Strait of Sicily. The upper flow, towards the east, carries in more 'Atlantic' water of lower salinity than the opposite flow at the bottom^{5,6}.

The deeper flow, the Levantine Intermediate water, is characterised by a salinity of about 38.7‰ and a temperature of about 14°C, and, after passing the sill, descends to a level of stability at a depth of about 600 m in the Tyrrhenian Sea. As it extends northwards and eastwards, it can be identified by the observed maxima in temperature and salinity profiles. It is the water mass below this maximum which, at some locations, exhibits the 'staircase' structure, although at other locations the more normal monotonic decrease to the bottom is observed.

In May 1972, samples were taken from 24 stations in the Tyrrhenian Sea, using a salinity-temperature-density (STD) probe. In addition, Nansen casts immediately followed the STD casts in five of the locations. At the station which exhibited the most pronounced structure, samples were taken at six depths below the salinity maximum (Fig. 1).

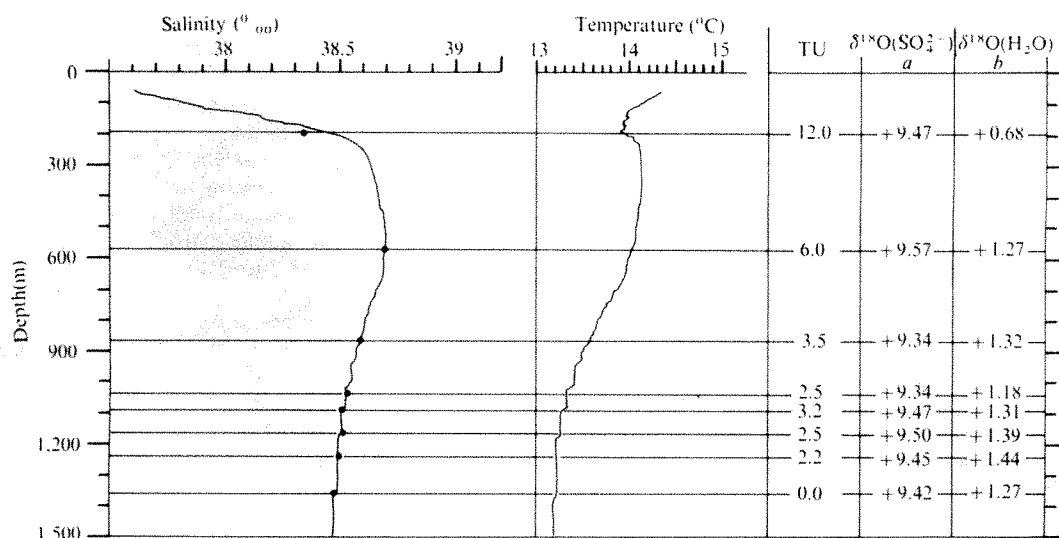


Fig. 1 Salinity, temperature and isotopic profiles at hydrographic station 19B1 in the low Tyrrhenian sea (39° 59.0'N, 12° 42.5'E). a, b, results relative to SMOW.

The isotopic analysis concerned the concentration of the stable ^{18}O content of water and of the dissolved sulphate, and the tritium concentration in superficial and deep seawater samples.

The procedure used in the preparation of samples for measuring the $^{18}\text{O}/^{16}\text{O}$ ratios in sulphate ions has been described⁷. The oxygen isotope analyses were carried out using the graphite-reduction technique⁸.

Oxygen isotope ratios are given in δ notation:

$$\delta^{18}\text{O} = \left\{ \left[\frac{(^{18}\text{O}/^{16}\text{O})_{\text{sample}}}{(^{18}\text{O}/^{16}\text{O})_{\text{standard}}} \right] - 1 \right\} \times 10^3$$

The results are reported relative to Standard Mean Ocean Water (SMOW)⁹. Isotopic analyses of the CO_2 were made using an ATLAS M86 mass spectrometer. The expected error of the $\delta^{18}\text{O}(\text{SO}_4^{2-})$ measurements was estimated at $\pm 0.07\text{‰}$.

Water oxygen isotopic samples were prepared using the isotopic equilibration technique¹⁰. The expected error of the $\delta^{18}\text{O}(\text{H}_2\text{O})$ measurements is $\pm 0.1\text{‰}$.

Tritium is formed in the atmosphere by, essentially, three mechanisms: the interaction of the nucleonic components of the cosmic radiation with the constituents of the atmosphere; direct origin from the Sun; and thermonuclear weapon tests. Most of the atmospheric tritium will form water and enters the oceans as tritiated water by molecular exchange across the air-water interface.

The concentrations are reported as TU (tritium unit); that is, the number of tritium atoms relative to 10^{18} hydrogen atoms¹¹. The accuracy of the tritium concentration measurement is approximately ± 1 TU.

Table 1 presents the results of the isotope analysis together with the observed temperature and salinity of the samples. All

Table 1 Hydrological stations, STD and isotopic results

	Depth (m)	Temperature (°C)	Salinity (‰)	TU	$\delta^{18}\text{O}(\text{SO}_4^{2-})^*$	$\delta^{18}\text{O}(\text{H}_2\text{O})^*$
	0		37.685(B)†			
Station 15 (38° 27.8'N, 11° 16.2'E)	46	15.61		8.0	+9.65	
	267	13.93		7.7	+9.49	
	746	13.29		1.5	+9.31	
	975	13.09	38.466			
	0		37.512(B)		+9.64	
Station 17 (39° 24.4'N, 11° 43.0'E)	150	13.67	38.298	9.5	+9.38	
	450	14.06	38.705	6.0	+9.42	
	998	13.28	38.515	0.0	+9.45	
	0		37.494(B)	12.5	+9.38	+0.69
	191	13.86	38.334	12.0	+9.47	+0.68
	571	14.03	38.689	6.0	+9.57	+1.27
Station 19B1 (39° 59.0'N, 12° 42.5'E)	864	13.60	38.589	3.5	+9.34	+1.32
	1,039	13.36	38.530(STD)	2.5	+9.34	+1.18
	1,092	13.30	38.511	3.2	+9.47	+1.31
	1,165	13.31	38.510	2.5	+9.50	+1.39
	1,236	13.25	38.492	2.2	+9.45	+1.44
	1,355	13.21	38.476	0.0	+9.42	+1.27
	0		37.412(B)			
Station 21 (40° 27.8'N, 12° 37.6'E)	49	14.57	37.552	8.0	+9.41	
	521	14.03	38.699	5.0	+9.50	
	1,175	13.25	38.497	1.0	+9.38	
	1,430	13.19	39.477	0.0	+9.39	
	0		37.554(B)			
Station 24 (41° 17.7'N, 11° 03.6'E)	179	13.86	38.440	9.5	+9.45	
	525	13.92	38.665	4.0	+9.41	
	846	13.53	38.572	2.0	+9.50	
	992	13.29	38.510	0.0	+9.47	

* Results relative to SMOW.

† (B), Bucket samples from the surface.

STD casts were made with a Nansen bottle attached above the probe for calibration purposes. Corrected depths were calculated using unprotected thermometers.

The $\delta^{18}\text{O}(\text{SO}_4^{2-})$ values range from 9.31‰ to 9.65‰, with an average value of 9.44‰. This agrees well with a previously reported value of 9.47 ± 0.19 ‰ from the Tyrrhenian Sea¹². In the region of pronounced step structure (Station 19B1) the $\delta^{18}\text{O}(\text{SO}_4^{2-})$ values lie between 9.57‰ at 547 m depth and 9.34‰ at 900–1,000 m.

The $\delta^{18}\text{O}(\text{H}_2\text{O})$ analysis, made only at station 19B1, showed an increase from a value of about 0.7‰ in the surface waters to a value of about 1.3‰ in water below 600 m.

A net decrease of tritium concentration with depth can be seen at all stations. The tritium concentration in the thermocline area (Station 19B1) is very uniform (about 12 TU) and considerably higher than in subjacent waters. In the intermediate Levantine water (about 600 m) the tritium concentration is 6 TU.

In the region of the stepped structure the tritium content decreases with depth (2.8 ± 0.6 TU), but the disparity is a little lower than the expected error. The deepest water sample is 'dead'.

It must be admitted that the results of this first attempt to detect significant differences in the isotope concentration of the separate layers are inconclusive. There is no doubt that both in the layered structure and at adjacent stations where the layering is not marked, the tritium concentration decreases with depth, and the tritium contents in the stepped portion of the TDS profile at station 19B1 may be taken as an indication that the observed steps concern a water mass showing a uniform apparent age, intermediate to those of the overlying Levantine and underlying deeper waters. If, as is probable, the stepped water mass results from mixing between the Levantine Intermediate water and the deep, 'dead', water, then at different times correspondingly different degrees of mixing and, consequently, different tritium concentrations will occur. Thus, because they have the same tritium contents, the observed layers may be formed at the same time or at very close times. There is also some indication that the tritium concentration of the Levantine Intermediate water (300–600 m) decreases to the north, though again, the differences are roughly comparable to the accuracy of the measurements.

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- ¹ Tait, R. I., and Howe, M. R., *Deep Sea Res.*, **15**, 275 (1968).
- ² Howe, M. R., and Tait, R. I., *Deep Sea Res.*, **17**, 963 (1970).
- ³ Johannessen, O. M., and Lee, O. S., *Saclant ASW Research Centre, Special Report*, M-74 (1972).
- ⁴ Johannessen, O. M., and Mellberg, L. E., *Saclant ASW Research Centre, Technical Report No. 206* (1972).
- ⁵ Molcard, R., *Proceedings Saclantcen Conference*, **7** (1972).
- ⁶ Molcard, R., *Proceedings Saclantcen Conference*, **7** (1972).
- ⁷ Cortecci, G., and Longinelli, A., *Earth planet. Sci. Lett.*, **4**, 325 (1968).
- ⁸ Longinelli, A., and Cortecci, G., *Rc. Soc. miner. ital.*, **XXVI**, 733 (1970).
- ⁹ Craig, H., *Science*, **133**, 1833 (1961).
- ¹⁰ Epstein, S., and Mayeda, T., *Geochim. cosmochim. Acta*, **4**, 213 (1953).
- ¹¹ Cameron, J. F., *Radioactive dating and methods of low-level counting* (International Atomic Energy Agency, Vienna, 1967).
- ¹² Longinelli, A., and Craig, H., *Science*, **156**, 56 (1967).

Ophiolites and oceanic crust

OPHIOLITES consist of a pseudostratiform sequence, of harzburgite, tectonite, ultramafic and mafic cumulates sometimes including gabbro and quartz diorite (plagiogranite) intrusions, dolerite dyke swarms, pillow lava¹, and deep-sea sediments^{2–4}. This assemblage occurs in all Phanerozoic mountain systems and is interpreted as fossil oceanic crust and uppermost mantle^{5–10}. Outstanding problems include differences between the chemical properties of ophiolites and rocks thought to represent present-day oceanic crust^{11,12}, the lack in some complexes of recognised dyke swarms or cumulates, and the relative thinness of ophiolite mafic rocks compared with standard oceanic crustal sections^{5,8,13}.

We here attempt to resolve some of these difficulties by comparing data on oceanic crustal structure, with data on selected, relatively complete, ophiolite complexes. We also discuss possible relationships between age, spreading rate, and crustal structure, and suggest a more precise correlation between oceanic crustal and ophiolitic layers.

Table 1 presents data on both the internal structure of ophiolite complexes used in this comparison, and our inferred thicknesses of oceanic layers 2 and 3 (see refs 13 and 14).

Several workers have discussed the variation of oceanic crustal structure with age and rate of spreading^{15–17}. Sutton *et al.*¹⁸, reported a series of combined wide-angle reflection-refraction measurements from the Pacific Ocean, which present a somewhat different structural picture from the normal oceanic model. In particular they found abundant evidence for a basal layer of $V_p = 7.1\text{--}7.6 \text{ km s}^{-1}$ between the main oceanic layer (layer 3) and the mantle. They suggested that this layer is widespread, but seldom recognised. We here compare their results with the ocean crustal ages¹⁹ and spreading rates²⁰.

The thicknesses reported by Sutton *et al.*¹⁸ show little relationship to age of crust (Fig. 1). This contrasts with observed increases in the thickness of layer 3 (ref. 17) but it may be because of inadequate data.

Figure 2 shows that the thicknesses of the oceanic and the basal layer, respectively decrease and increase as the spreading rate increases. Comparison of seismic velocity (V_p) with spreading rate (Fig. 3) reveals little change in the oceanic layer but an apparent decrease with increasing rate in the basal layer.

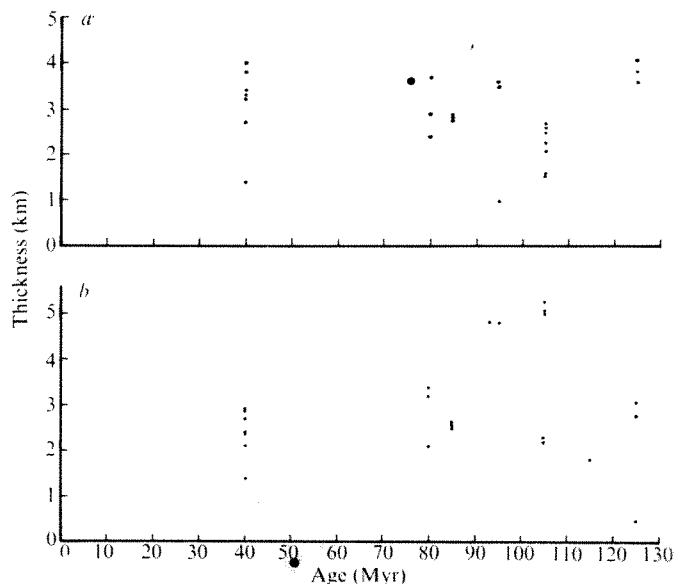


Fig. 1 Relationship between the thicknesses of the oceanic (a, $V_p = 6.2\text{--}7.0 \text{ km s}^{-1}$) and basal (b, $V_p = 7.1\text{--}7.8 \text{ km s}^{-1}$) layers¹⁸ and the age of the crust.

Table 1 Age, thickness, and structure of selected ophiolite complexes

Complex	Thickness (km)				Oceanic correlation		
	Transition	Gabbro	Diabase	Lava	Layer 3	Layer 2	Total
Vourinos ⁴ (V)	1	1.6	~0.5	~0.5	1.6	1	2.6
Troodos ^{13,26} (T)	0.5	0.5	2.3	0.9	0.5	3.2	3.7
Kizil Dag ²⁷ (KD)	2.0	2		0.4	0-2	0.4-2.4	2.4
Semail ^{6,7} (S)		3	2	1	3	3	6
Othris ²⁸ (O)		0.25		0.6	0-0.25	0.6-0.85	0.85
Pindos ^{25,27} (Pi)		~1	~1	0.3	1	1.3	2.3
Papua ³⁰ (Pa)	0.5	4		4-6	4	5±1	9
Elba ^{31,32} (E)		0.1	1.1		0.1	1.1	1.2
Betts Cove ³³ (BC)		0.2	1	1	0.2	2	2.2
Canyon Mountain ⁹ (CM)		3.1		0.4	3.1	0.4	3.5
Bay of Islands ⁸ (BI)	0.8	2.6	0.6	1.2	2.6	1.8	4.4

The thicknesses of the units are only approximate. They are either taken from the references indicated, or measured from published cross sections.

The sections of Sutton *et al.*¹⁸ closely resemble those of the selected ophiolite complexes (Fig. 4). The range of thickness of the oceanic layer (approximately 1-5 km), is surprisingly similar to the inferred range of thickness of layer 3 (gabbro) in ophiolites (0.25-4 km).

Furthermore, the presence of a basal layer intermediate in velocity between the oceanic layer and the mantle, also has a geological counterpart in ophiolite complexes, that is, the mixed mafic-ultramafic or 'transition' zones of many

there is slow cooling, with a lot of diffusion and secondary enlargement, resulting in a thinner, higher velocity, basal layer, and a thicker, lower velocity, oceanic layer. Data from fast spreading ridges suggest that cooling is faster, with less diffusion, and a high degree of space filling, giving rise to a thicker, lower velocity, basal layer, and a thinner, higher velocity, oceanic layer (see Figs 3 and 4). It must be emphasised, however, that the seismic data are sparse and highly variable and that the mixed mafic-ultramafic zones of many ophiolites are complicated by pyroxenite and gabbro dykes, and above all, are commonly partially serpentinised. It is possibly these geological features which give rise to the scatter in Figs 1-3.

Some olivine cumulates in the Pindos complex²⁵ contain as much as 25% plagioclase and 2% pyroxene, whereas olivine cumulates in the Vourinos complex²¹ are essentially monomineralic (dunites). These relationships imply that primary cumulus processes are first order determinants of oceanic and basal layer thicknesses and velocities. Pindos may have formed from a faster spreading ridge than Vourinos.

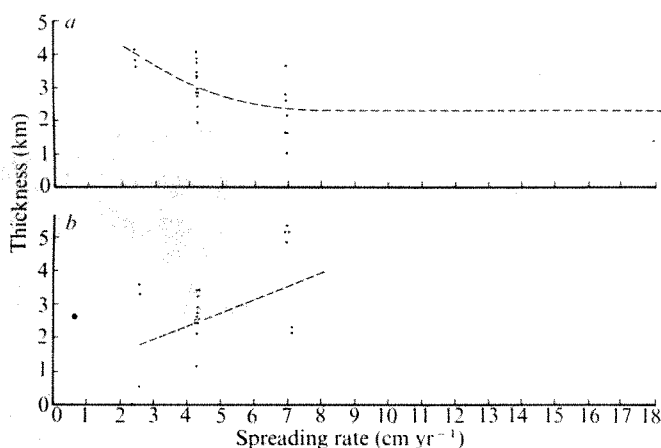


Fig. 2 Relationship between the thicknesses of the oceanic (a) and basal (b) layers¹⁸ and the spreading rate.

ophiolites. These zones are composed principally of layered cumulates, which in at least one case (Vourinos) are arranged in cyclic units²¹. The interstices between cumulus crystals (for example, olivine) may be filled by material of the same composition as the crystal itself²² (thereby forming, for example, a dunite); or they may be filled by other minerals (for example, plagioclase and pyroxene)²² thereby forming, for example, a plagioclase hertzolite or wehrlite. As the postcumulus material comprises as much as 50% of a cumulate, fairly large differences of seismic velocity can be expected, depending on the relative amounts of 'secondary enlargement space filling' material.

These differences are of fundamental importance in any consideration of magmatic processes at a ridge, because 'secondary enlargement' apparently is controlled by the rate of accumulation of cumulus crystals in layered cumulus complexes²³. When the rate of accumulation is slow, diffusion operates to produce enlargement. When the rate is rapid, the original magma effectively is trapped and 'space filling material' is formed. This relationship is evident in the Stillwater complex where there is an inverse relationship between the thickness of section of olivine cumulate and the amount of enlargement²⁴.

Thus, the data suggest that in slow spreading ridges,

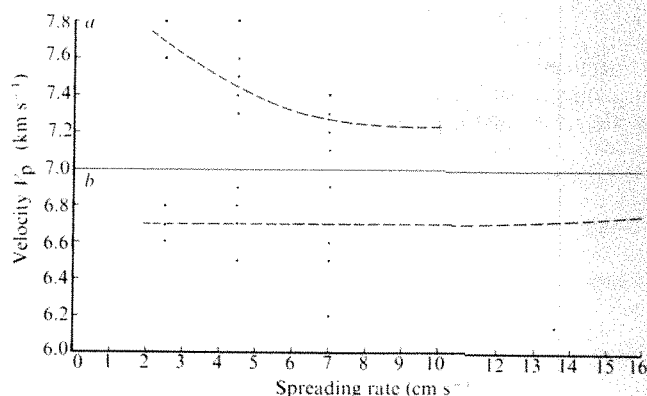


Fig. 3 Relationship between velocity (V_p) in basal (a) and oceanic (b) layers and spreading rate.

Completely unserpentinised ultramafics are rare in ophiolite complexes, but occur in a few, such as Vourinos or Papua. Possibly the serpentinised-fresh ultramafic contact in these complexes represents the seismic Moho rather than the mafic-ultramafic contact. Whether serpentinised or not, it is apparent that the basal layer-mantle interface may not correspond to the magmatic-metamorphic contact of ophiolites^{1,21}.

It is not yet possible to infer a spreading rate for a given ophiolite complex by comparing the internal structure and petrology with seismic data of oceanic crust, but some trends are emerging. It seems reasonable to assume, however, an oceanic crustal mode as follows: layer 2=pillow lava, and dolerite, or zeole-greenschist

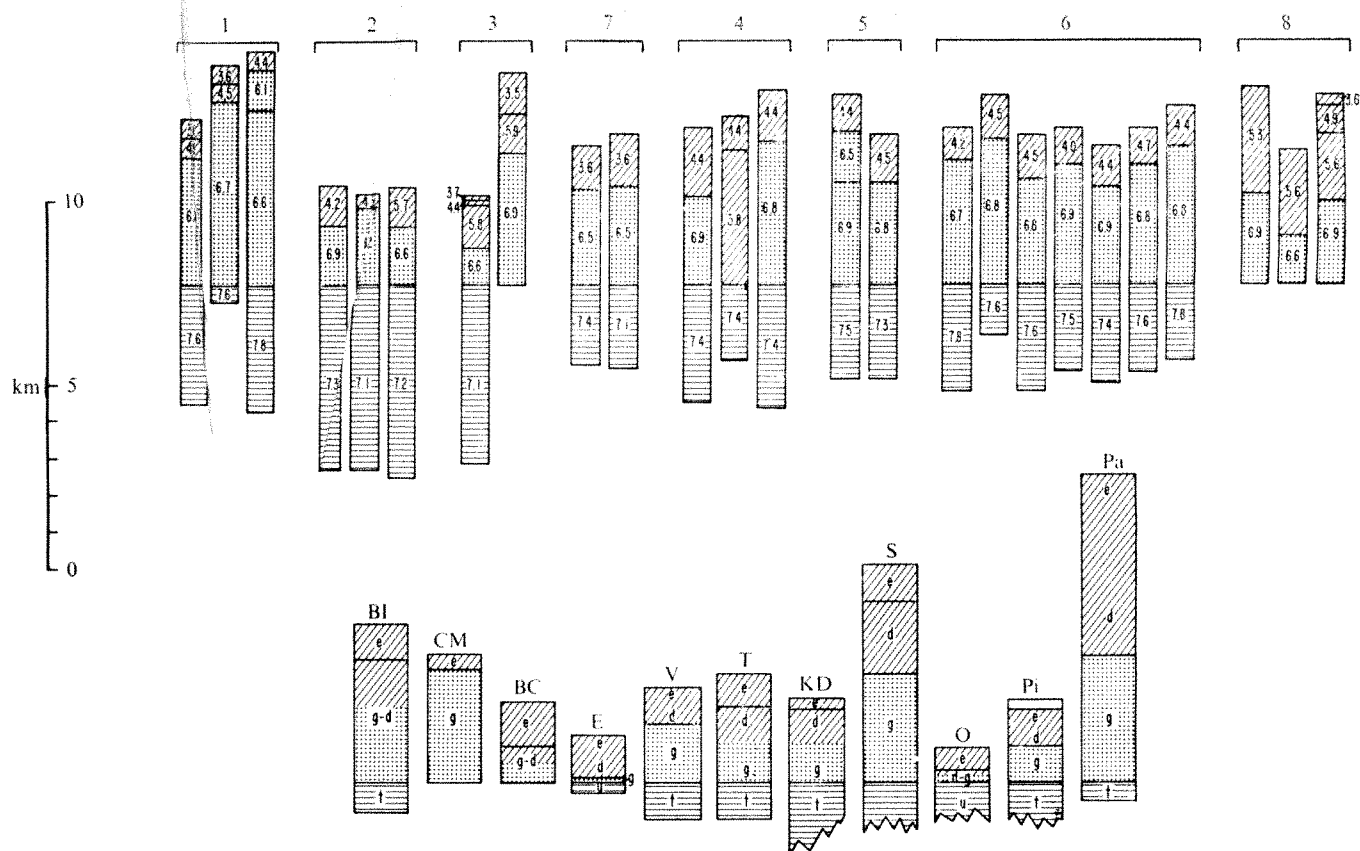


Fig. 4 Upper row, crustal sections of the Pacific basin¹⁸. Numbers over sections are sites of Sutton *et al.*¹⁸. Numbers within sections are seismic velocities (km s⁻¹). Lower row, selected ophiolite complexes. e, Extrusives, generally mafic pillow lava; d, dolerite; g, gabbro +, mixed mafic-ultramafic or 'transition' zone. Symbols for ophiolite complexes are from Table 1.

facies equivalents^{15,16}, oceanic layer (layer 3)=gabbro, and quartz diorite (plagionite) and/or amphibolite; basal layer=a mixed ultramafic cumulate zone together with partly serpentinised tectonite.

Refinement of the model awaits more data on ophiolite, oceanic crustal lithologies, and thicknesses and elastic properties, obtained either seismically or by drill sampling. The elastic properties of ocean ridge dredge samples confirm that layer 2 is basaltic and the oceanic layer (layer 3) is gabbroic, but identification of basal layer rocks is unlikely using this approach, and coherent stratigraphy is difficult to establish in dredge hauls. The ultimate aim is the calibration of ophiolite spreading rates, and thus those of now vanished Phanerozoic oceans.

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¹ Nicolas, A. and Jackson, E. D., *Schweiz. miner. petrogr. Mitt.*, **53**, 385 (1972).

² Anon., *Geotimes*, **17**(12), 24 (1972).

³ Maxwell, J. C., in *The Megatectonics of Continents and Oceans*, (edit. by Johnson, H., and Smith, B. L.), 167 (Rutgers University Press, New Brunswick, 1969).

⁴ Moores, E. M., *Spec. Pap. geol. Soc. Am.*, **118** (1969).

⁵ Coleman, R. G., *J. geophys. Res.*, **76**, 1212 (1971).

⁶ Reinhardt, B. M., *Schweiz. miner. petrogr. Mitt.*, **49** (1969).

⁷ Glennie, K. W., *et al.*, *Bull. Am. Ass. Petrol. Geol.*, **57**, 5 (1973).

⁸ Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **76**, 3179 (1970).

⁹ Moores, E. M., *Nature*, **228**, 837 (1970).

¹⁰ Temple, P. G., and Zimmerman, J., *Geol. Soc. Am., Abstr.*, **7**, 221 (1969).

¹¹ Abbate, E., Bortolotti, V., and Passerini, P., *Boll. Soc. geol. Ital.*, **91**, 239 (1972).

¹² Miyashiro, A., *Earth planet. Sci. Lett.*, **19**, 218 (1973).

¹³ Vine, F. J., and Moores, E. M., *Mem. geol. Soc. Am.*, **132**, 195 (1972).

¹⁴ Poster, C. K., *Nature phys. Sci.*, **243**, 2 (1973).

¹⁵ Menard, H. W., *Science*, **157**, 923 (1967).

¹⁶ Shor, G. G., jun., Menard, H. W., and Raitt, R. W., in *The Sea* (edit. by Maxwell, A. E.), **4**, Part 2 (Interscience, New York, 1971).

¹⁷ Goslin, J., Beuzart, P., Francheteau, J., and LePichon, X., *Mar. geophys. Res.*, **1**, 418 (1972).

¹⁸ Sutton, G. H., Maynard, G. L., and Hussong, D. M., *Monogr. Am. geophys. Un.*, **14**, 193 (1971).

¹⁹ Larson, R. L., and Chase, C. G., *Bull. geol. Soc. Am.*, **83**, 3627 (1972).

²⁰ Larson, R. L., and Pitman, W. C., III, *Bull. geol. Soc. Am.*, **83**, 3645 (1972).

²¹ Jackson, E. D., Moores, E. M., and Green, H. W. II, *Bull. geol. Soc. Am.* (in the press).

²² Jackson, E. D., in *Ultramafic and Related Rocks* (edit. by Wyllie, P. J.), 20 (Wiley, New York, 1967).

²³ Hess, H. H., *Mem. geol. Soc. Am.*, **80** (1960).

²⁴ Jackson, E. D., *Prof. Pap., US geol. Surv.*, 358 (1961).

²⁵ Parrot, J. F., *Cah. ORSTOM, ser. Geologie*, **1**, 35 (1969).

²⁶ Fox, P. J., Schreiber, E., and Peterson, J. J., *J. geophys. Res.*, **78**, 5155 (1973).

²⁷ Moores, E. M., and Vine, F. J., *Phil. Trans. R. Soc.*, **A268**, 443 (1971).

²⁸ Dubertret, L., *Notes et Memoires sur le Moyen-Orient*, **VI** (1955).

- ²⁸ Heyns, A. J., Nisbet, E. G., Smith, A. G., Welland, M. J. P., and Rex, D. C., *Zeitschr. der Deutschen Geol. Gessell* (in the press).
- ²⁹ Terry, J., *C.r. somm. Séanc. Soc. geol. Fr.*, **1**, 12 (1972).
- ³⁰ Davies, H. L., *Bull. Aust. Bur. Miner. Res., Geol. Geophys.*, **128** (1971).
- ³¹ *Carta Geologica d'Italia, Foglio 126, Isola d'Elba*, **1**, 100.
- ³² Bignazzi, G., Bonodonna, F. P., Ferrara, G., and Innocenti, F., *Fortschr. de Mineralogie*, **50**, 51 (1973).
- ³³ Upadhyay, H. D., Dewey, J. F., and Neale, E. R. W., *Proc. geol. Ass. Can.*, **24**, 27 (1971).

Optical absorption phenomena at electrode surfaces

THERE has been a considerable expansion recently, in the application of optical techniques, such as internal reflection and specular reflection spectroscopy, ellipsometry, interferometry, holography and optical microscopy, to the study of adsorption phenomena in electrochemical reactions¹⁻³.

In general, these methods have been used to study the optical properties of the electrode material and of thin films adsorbed on to their surfaces. Internal reflection spectroscopy and specular reflection spectroscopy have both been used to monitor the surface concentration of an electroactive species⁴⁻⁶.

We describe here some preliminary observations of the optical absorption phenomena which occur above a platinum cathode surface when dilute aqueous solutions of metal cations containing a preponderant concentration of an indifferent electrolyte, such as an alkali metal halide, are electrolysed at low current densities and controlled potentials in a silica cuvette.

Well defined transient absorption spectra are obtained both 'on' the cathode surface and as much as 10^6 – 10^7 Å into the solution phase. The wavelengths of the absorption maxima of the transient absorption bands bear a close relationship to the shorter wavelength absorption lines of the same atomic species in a gas phase. Furthermore, the peak intensities of these transient signals are proportional, in each case, to the concentration of the free electroactive cations in solution. No corresponding signals are observed at the anode or in the absence of the electroactive species, or at potentials below that required to discharge the particular cation. The signals are most intense at grazing incidence on the cathode, but they are also found in the solution phase when the cathode is below the analysing light beam.

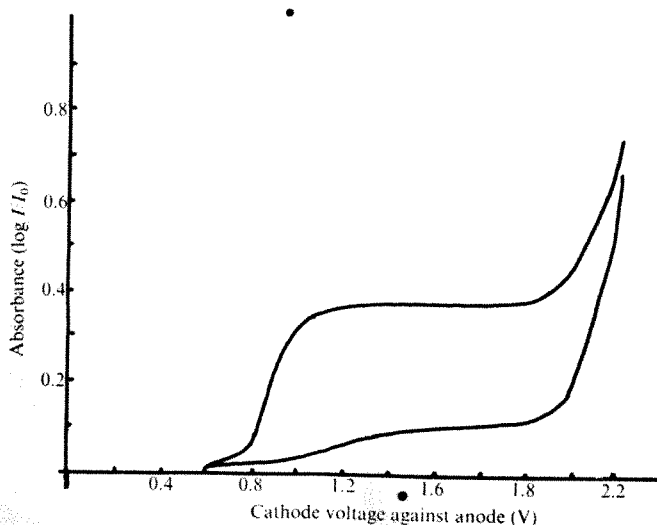


Fig. 1 Variation of absorbance (207 nm) with voltage. Upper curve, 5 p.p.m. Zn^{2+} in 0.03 M K_2SO_4 ; lower curve, 0.03 M K_2SO_4 only.

These observations suggest the transient existence of hydrated free atoms in the solution, close to the cathode surface. It is probable that these are formed by a process of electron tunneling from the cathode with the formation of hydrated electrons which subsequently reduce the hydrated, or otherwise liganded, cations.

These experiments suggest to us the basis of a potentially useful analytical trace technique of hydrated (solvated) atomic absorption spectroscopy (T. S. West, Fourth International Conference on Atomic Spectroscopy, Toronto, unpublished). The technique may also prove useful for the examination of surface mechanisms in electrodeposition and electrochemistry in general.

A simple ultraviolet/visible spectrophotometer was built from a hydrogen lamp and power supply, a tungsten filament lamp, a monochromator, and a photomultiplier tube with a Branden-

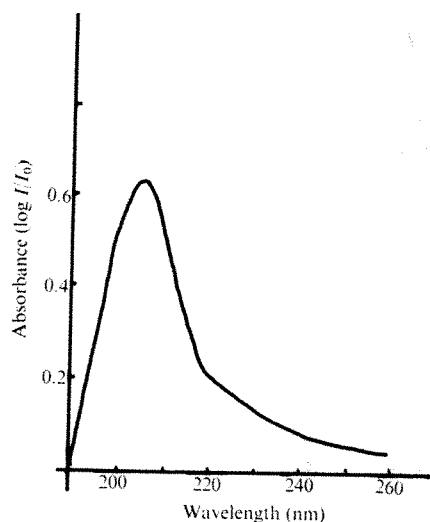


Fig. 2 Variation of absorbance with wavelength; 10 p.p.m. Zn^{2+} in 0.03 M K_2SO_4 at -1.4 V.

burg type 475R e.h.t. power supply. The output was monitored by a chart recorder. A 20-mm silica cell was used, with two platinum plate electrodes inserted. A potentiostatic waveform source was used as a signal generator. The alignment was such that a narrow collimated beam of light passed over the surface of the cathode, with the anode kept well clear of the light path. The cathode could be racked upwards or downwards in the light beam.

Each element was studied by using the highest possible potential difference in order to find a wavelength at which a maximum transient increase in absorption was obtained. At that wavelength, the optimum electrode pretreatment conditions and potential difference were found, and, using these, the variations of absorbance with wavelength and with concentration of metal cation were obtained.

The stock solutions were diluted to give solutions of (typically) 10 parts per million (p.p.m.) in 0.03 M K_2SO_4 or 0.1 M KCl . A 5 cm³ aliquot was placed in the cell and de-aerated with oxygen-free nitrogen. The gain of the system was adjusted so that zero absorbance was registered, and a potential step (square wave) was imposed on the electrodes so that the electrode in the light path became the cathode. After the change in absorbance had been recorded, the cell was emptied (by suction) and the working electrode was washed with dilute base electrolyte while a high positive potential (up to 5.0 V) was applied—that is, it was cleaned by anodic stripping. If necessary, any surface oxide was reduced by reversing the polarity of the system.

Using this technique we studied the following metal ions: Cu^{2+} , Cd^{2+} , Fe^{2+} , Fe^{3+} , Pb^{2+} , Zn^{2+} , Co^{2+} and Ni^{2+} . On applying a potential difference between the electrodes a transient increase in absorbance was observed. This change in absorbance

has been interpreted as absorption of light by reduced species generated on or near the cathode surface.

A variation of absorbance with voltage was observed, in most cases, after a simple step function had been applied, that is, the potential difference was stepped from 0.0 V to the preset required value. Pulsed square wave functions were also used (both between zero and the required voltage and between positive and negative values) but, in general, these did not increase the magnitude of the absorbance obtained. The magnitude of the potential difference is limited by cathodic evolution of hydrogen (it was limited to about -2.2 V in 0.03 M K_2SO_4). At the absorption maximum, plots of absorbance against potential difference were obtained. These have the appearance of d.c. polarograms; as the potential difference is increased, the absorbance increases until a plateau region occurs. This persists as the applied potential difference is progressively increased until the background electrolyte begins to be reduced, or hydrogen is evolved, at which point a rapid increase in absorbance, resulting from scattering, is observed. Zinc typically has a plateau region of -1.1 to -2.0 V (Fig. 1).

With an oxidised cathode an increased signal was obtained at lower potential differences. At -2.0 V the nature of the pre-treatment had no effect on the magnitude of the absorbance signals observed for any of the cations.

With the background electrolyte alone, no absorbance was observed at wavelengths greater than 210 nm. Below this, a band with a maximum absorbance of about 0.25 (half bandwidth 10 nm) was observed at 197 nm.

Plots of absorbance against wavelength were made at the optimum observed conditions. Generally, these are, not unexpectedly, much broader than gas phase absorption lines and have a single maximum occurring in the ultraviolet. For example, zinc has a maximum at 207 nm and a half bandwidth of 16 nm (Fig. 2). The spectra for the other metals so far examined are broader.

Absorbance against concentration was plotted at the absorption maximum for each metal. Generally, the plots were linear, over a variety of concentration ranges. The zinc calibration was linear between 1 and 12 p.p.m.

The absorption typically reached its maximum value after approximately 30 s and then decayed to the initial value over a period of up to 2 min if the applied potential was maintained. The decay was more rapid if the potential difference reverted to zero at, or just after, the maximum absorbance had been reached.

The maximum absorbance was only maintained if an oscillating voltage function (both positive and negative going) was used at a frequency greater than 0.1 Hz.

Although some metals give rise to more intensely absorbing species, there is a concentration range for each metal so far studied, over which the absorbance is a linear function of concentration. This, together with the possibility of achieving selectivity with a suitable choice of wavelength and voltage, or with selective complexing and masking (as in the preconcentration step in voltammetric stripping analysis), points the way to analytical applications.

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¹ Hansen, W. N., *Spectrochemical Observation of an Electrochemical Reaction by means of Internal Reflection Spectroscopy, in Modern Aspects of Reflectance Spectroscopy* (edited by Wendlandt, W. N., and Hecht, H. G.), (Plenum Press, New York, 1966).

² *Faraday Society Symposium on Optical Studies of Adsorbed Layers at Interfaces*, 4 (Faraday Society, London, 1970).

³ *Advances in Electrochemistry and Electrochemical Engineering* (edit. by Delahay, P., and Tobias, C. W.), 9 (Wiley, New York, 1973).

⁴ Hansen, W. N., Osteryoung, R. A., and Kuwana, T., *J. Am. chem. Soc.*, **88**, 1062 (1966).
⁵ Kuwana, T., and Strojek, J. N., *Trans. Faraday Soc.*, **45**, 134 (1968).
⁶ Kissinger, P. T., and Reilly, C. N., *Analyt. Chem.*, **42**, 12 (1970).

BIOLOGICAL SCIENCES

Comparison of predicted and experimentally determined secondary structure of adenyl kinase

It is generally accepted that the action of a protein cannot be understood until its three-dimensional structure is known. At present, X-ray analysis of protein crystals is the only method of obtaining such structural information. It is to be feared, however, that many important proteins will never give suitable crystals so that one is obliged to consider other approaches to structure elucidation. Renaturation experiments indicated¹⁻⁴ that the three-dimensional structure of many if not all proteins is a unique function of their amino acid sequence. Therefore, in principle one should be able to determine these structures by using only the information contained in the sequence. A first step in this direction is the prediction of secondary structures (α helices, β pleated sheets, β bends) in globular proteins from amino acid sequences. Several prediction schemes have been devised to this end⁵⁻²³. It is the aim of this paper to demonstrate directly the current standing of such methods.

The enzyme adenyl kinase (adenylate kinase, EC 2.7.4.3, molecular weight 21,600) (ref. 24) was taken as a test object. Its amino acid sequence²⁵ was distributed to several groups known to work on prediction schemes. None of these groups knew the three-dimensional structure²⁶ of the protein when giving their prediction; and they were not informed about structural relationships²⁷ between adenyl kinase and other proteins. Therefore, none of the predictions can be biased by previous structural information.

The predicted and the experimentally determined secondary structures in adenyl kinase are compiled in Fig. 1. The experimental data are derived from a 3 Å electron density map. Although such a map is not detailed enough to show hydrogen bonds directly, most of the secondary structures are clearly visible. There are considerable differences between the prediction methods that have been applied. Detailed descriptions of the individual schemes can be found in the references given in the legend of Fig. 1. As shown in the lowest part of Fig. 1 the α helix predictions correspond very well with the experimental data. Two groups found nine, two groups found eight, and one group found seven out of the 10 helices. Only one of these five groups predicted a wrong piece of chain as being helical. The results in Fig. 1 indicate, however, that it is still difficult to find the correct starting and termination point of a helix.

A conventional way of estimating the success of a particular prediction scheme is to compare the prediction with the experiment in every single residue²⁸. Therefore, scores have been made which are listed on the right side of Fig. 1. One group gave an inverse prediction, that is, helix breakers instead of helical residues. To avoid confusion this kind of prediction has not been scored. A 'joint prediction' of α helices was produced by adding predictions A to J at each residue and displaying the result as a histogram. This histogram is in good agreement with the experimental data except for the C-terminal region.

Four groups predicted those residues that are involved in pleated sheet structures and one group assigned sheet-breaking residues. Again, scores have been taken and a joint prediction histogram has been produced. The results show that the three central strands (around residues 12, 91, and 116) of the five-

Temperature and Life

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Professor Dr. **Jes Christophersen**, Universität Hohenheim, Fachgruppe Lebensmitteltechnologie, Stuttgart;
Professor Dr. **Herbert Hensel**, Institut für Physiologie der Universität, Marburg, and
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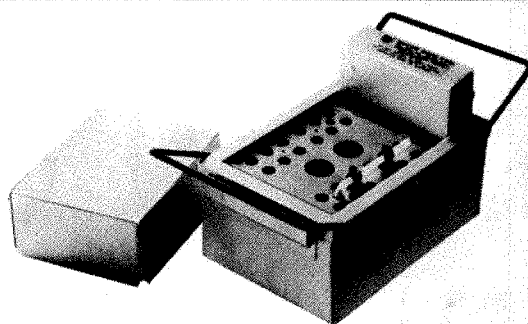
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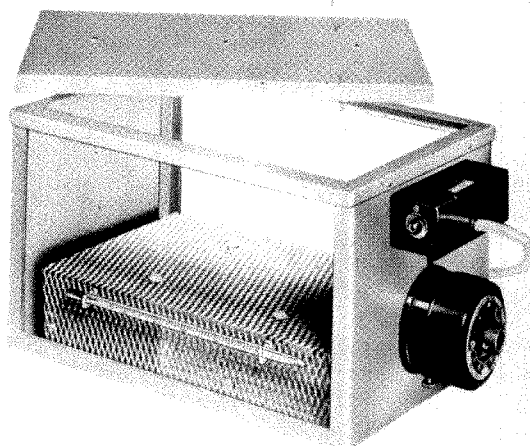
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Molecular hybridization with ³H-labeled Polyuridylic acid (Poly U) has been used ¹⁻² to detect Poly A sequences in viral RNA.

1. D. Gillespie, S. Marshall, and R.C. Gallo, Nature **236**, 227 (1972).

2. J. Schlom, D. Colcher, S. Speigelman, S. Gillespie, and D. Gillespie, Science **179**, 697 (1973).



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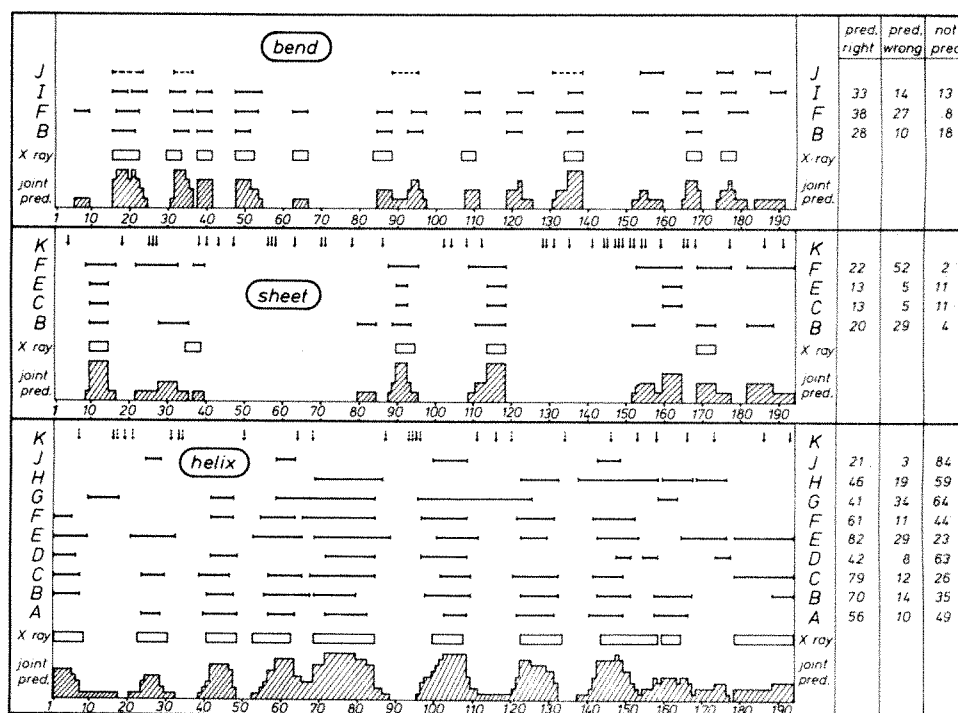


Fig. 1 Comparison of predicted and experimentally determined α helices, strands of β -pleated sheets, and bends in adenylyl kinase. The experimental data (X-ray) have been derived from a 3 Å electron density map. At this resolution the exact geometry of bends cannot be evaluated with certainty. Therefore, the experimentally determined bends in Fig. 1 are defined as changes of more than 120° in the overall direction of the polypeptide chain and without reference to any hydrogen bonding scheme. None of the predictions is biased by previous structural information about the enzyme. The predictions A to K have been supplied by A, Barry and Friedman; B, Chou and Fasman^{21,22}; C, Finkelstein and Pitsyn^{10,11}; D, Levitt and Robson^{14,32}; E, Lim²³; F, Nagano²⁰; G, Burgess and Scheraga⁸; H, Burgess and Scheraga⁸; I, Burgess and Scheraga¹³; J, Burgess and Scheraga¹⁹; K, Kabat and Wu¹⁸. They are based upon prediction schemes that have been described in the corresponding references. (The method applied by Barry and Friedman has not been published yet. It uses an analysis of the distribution of amino acids occurring at both ends of helices to define potential starting and termination points of helices in a sequence. In this method the residues three before and three after, as well as the residue defined as the beginning (or end) of an helix, are considered to be structurally significant. The predictions are based on data for 90 helices from 23 distinct three-dimensional protein structures.) Although predictions are usually made as probability profiles, all groups converted their profiles beforehand to yes or no decisions for each residue by comparing the probabilities with a given threshold value. Thus, every prediction depends on the threshold value applied³². This procedure simplified the comparison appreciably. But concomitantly, it reduced the amount of information contained in any of the predictions. Kabat and Wu predicted helix and sheet breaking residues, which are indicated by vertical arrows. Dashed arrows point to residues that are helix breaking but with a lower probability. Dashed lines in the bend prediction J indicate 'multiple bend regions'. A bend is predicted to be anywhere within such a region. Three joint prediction histograms have been produced by adding predictions A to J for helix, predictions B, C, E, and F for sheet and predictions B, F, I, and J for bends, respectively. Scores of correctly and incorrectly predicted residues as well as residues not predicted are listed on the right side of the figure.

stranded parallel pleated sheet in adenylyl kinase have been predicted correctly by all groups. This is not true, however, for the two strands at the edges of this sheet. Scores and histograms indicate that the accuracy of the β -sheet prediction is lower than the accuracy of helix or bend prediction. This might be related to the fact that the sheets are predominantly formed by interactions between residues that are far apart along the polypeptide chain (= nonlocal interactions) whereas helices and bends are caused by interactions between neighbours along the chain (= local interactions). Obviously, local interactions are more easily recognised in an amino acid sequence than nonlocal interactions, because the latter require assumptions about the three-dimensional structure.

The results in the upper part of Fig. 1 as well as the corresponding scores indicate that bends of the polypeptide chain are often successfully predicted. The joint prediction histogram shows that essentially only the bend around residue 64 has been missed. The bends predicted at residues 95 and 122 turned out to be directional changes in the polypeptide chain of only 60° and 90° , which is less than the 120° required in the definition for an experimentally determined bend (see legend of Fig. 1).

As shown in Fig. 1 some of the secondary structures have been predicted consistently whereas others were less readily recognised. The α helices in the middle of the chain and the strands in the centre of the sheet and the bends near to the N terminus were predicted consistently. There is less agreement in the C-terminal region. It is conceivable that consistent predictions are obtained in chain segments where local inter-

actions dominate the formation of secondary structures and that the predictions become more inaccurate whenever non-local interactions predominate. Thus, the prediction problems in the C-terminal region would be understood if during the folding process the C-terminal end is wrapped around a pre-existing molecular core. Nonlocal interactions with this core might force the terminal chain into its final secondary structure.

Adenylyl kinase contains a structurally rather independent domain consisting of the three helices between residues 41 and 84. The consistent prediction of these helices indicates that their formation might be dominated by local interactions. Moreover, if local interactions dominate, the helices can form spontaneously so that they may be nucleation points and the domain may be a nucleus^{21,29-31} in the folding process.

In conclusion it seems fair to state that the joint prediction histograms allow the assignment of most of the helices and bends and more than half of the sheet strands. It must be remembered, however, that adenylyl kinase is a protein with abundant secondary structures and that joint prediction histograms of other proteins might be less informative. For the same reason the merit or demerit of any prediction scheme should not be judged from its success with this particular protein alone.

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- ¹ Jardetzky, O., Thielmann, H., Arata, Y., Markley, J. L., and Williams, M. N., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 257-261 (1971).
- ² Anfinsen C. B., Haber, E., Sela, M., and White, jun., F. H., *Proc. natn. Acad. Sci., U.S.A.*, **47**, 1309-1314 (1961).
- ³ Anfinsen, C. B., *Biochem. J.*, **128**, 737-749 (1972).
- ⁴ Ikai, A., Fish, W. W., and Tanford, C., *J. molec. Biol.*, **73**, 165-184 (1973).
- ⁵ Guzzo, A. V., *Biophys. J.*, **5**, 809-822 (1965).
- ⁶ Prothero, J. W., *Biophys. J.*, **6**, 367-370 (1966).
- ⁷ Schiffer, M., and Edmundson, A. B., *Biophys. J.*, **8**, 29-39 (1968).
- ⁸ Kotelchuck, D., and Scheraga, H. A., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 14-21 (1969).
- ⁹ Lewis, P. N., Go, N., Go, M., Kotelchuck, D., and Scheraga, H. A., *Proc. natn. Acad. Sci., U.S.A.*, **65**, 810-815 (1970).
- ¹⁰ Ptitsyn, O. B., and Finkelstein, A. V., *Dokl. Akad. Nauk SSSR*, **195**, 221-224 (1970).
- ¹¹ Ptitsyn, O. B., and Finkelstein, A. V., *Biofizika (USSR)*, **15**, 757-768 (1970).
- ¹² Leberman, R., *J. molec. Biol.*, **55**, 23-30 (1971).
- ¹³ Lewis, P. N., Momany, F. A., and Scheraga, H. A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2293-2297 (1971).
- ¹⁴ Robson, B., and Pain, R. H., *J. molec. Biol.*, **58**, 237-259 (1971).
- ¹⁵ Wu, T. T., and Kabat, E. A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 1501-1506 (1971).
- ¹⁶ Finkelstein, A. V., and Ptitsyn, O. B., *J. molec. Biol.*, **62**, 613-624 (1971).
- ¹⁷ Nagano, K., *J. molec. Biol.*, **75**, 401-421 (1973).
- ¹⁸ Kabat, E. A., and Wu, T. T., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1473-1477 (1973).
- ¹⁹ Burgess, A. W., Ponnuswamy, P. K., and Scheraga, H. A., *Israel J. Chem.* (in the press).
- ²⁰ Nagano, K., *J. molec. Biol.* (in the press).
- ²¹ Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 211-222 (1974).
- ²² Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 222-245 (1974).
- ²³ Lim, V. I., *J. molec. Biol.*, (in the press).
- ²⁴ Noda, L., *The Enzymes* (edit. by Boyer, P. D.), **8**, 279-305 (Academic Press, New York, 1973).
- ²⁵ Heil, A., Müller, G., Noda, L. H., Pinder, T., Schirmer, I., Schirmer, R. H., and v. Zubern, I., *Eur. J. Biochem.*, **43**, 131 (1974).
- ²⁶ Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H., *Nature*, **250**, 120-123 (1974).
- ²⁷ Schulz, G. E., and Schirmer, R. H., *Nature*, **250**, 142-144, (1974).
- ²⁸ Lewis, P. N., and Scheraga, H. A., *Archs. Biochem. Biophys.*, **144**, 576-583 (1971).
- ²⁹ Ptitsyn, O. B., Lim, V. I., and Finkelstein, A. V., *Proc. 8th FEBS Meeting*, Vol. 25 (edit. by Hess, B.), 421-429, (North-Holland, Amsterdam, 1972).
- ³⁰ Wetlauffer, D. B., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 697-701 (1973).
- ³¹ Ptitsyn, O. B., *Biophys. Chem.* (in the press).
- ³² Krigbaum, W. R., and Knutton, S. P., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2809-2813 (1973).

Topological comparison of adenyl kinase with other proteins

A COMBINATION of amino acid sequence analysis¹ and X-ray analysis has yielded the atomic structure of the enzyme adenyl kinase² (adenylate kinase, EC 2.7.4.3). No protein of which the structure is known is so closely related to adenyl kinase that an amino acid sequence comparison³ or an exact geometrical comparison of the structures⁴⁻⁶ can be expected to indicate a relationship. Similarities with other proteins become apparent, however, if one restricts the comparison to topologies, that is, to chain folds without reference to the exact geometry. Since chain folds are particularly well conserved during evolution⁷ such a procedure might reveal distant relationships.

The most important structural feature of adenyl kinase is its central five-stranded parallel pleated sheet²; therefore, we shall only discuss proteins containing a similar motif. Central parallel sheets with more than three strands are present in subtilisin⁸⁻¹⁰, flavodoxin^{11,12}, and various dehydrogenases¹³⁻¹⁶. The sheets of these proteins are sketched in Fig. 1 according to a proposal of Rossmann (personal communication). The drawings contain no geometrical detail. Only the sheet strands and the approximate path of the connections between these strands (through the upper and lower side of the sheet), that is, only the sheet topology, is given. In such a presentation the topology is fully described by the strand sequence in the sheet and the above-below pattern of the connections. Since there are finite, calculable numbers of possible strand sequences and possible connection patterns, the number of possible topologies (M) is also finite and calculable.

This number can be used to define a quantitative measure of relationship between two structures. Assuming, for instance, each of two proteins contains an n -stranded parallel sheet with identical topology; since such a sheet can exist in M different topologies, the probability that by chance both sheet topologies are identical is $1/M$. Or, in other words, the significance of their relationship, which one might call 'figure of topological relatedness', is $M:1$. We shall now derive such figures within the group of protein structures shown in Fig. 1.

First we have to evaluate the number of possible strand sequences in an n -stranded parallel sheet. This is equal to $n!/2$, the number of permutations of n items reduced by a factor of 2. The halving is necessary because symmetrically related strand sequences as $ABCD$ and $DCBA$ can be superimposed by rotation. Second, we take into account that the $n-1$ connections between the strands run either through the upper or the lower side of the sheet, giving rise to 2^{n-1} possible above-below connection patterns. Combining strand sequences and connection patterns we find that there are $M = 2^{n-1} \times n!/2 = 2^{n-2} \times n!$ possible topologies. For simplicity this counting scheme disregards further topological differences which arise whenever connections cross each other as, for example, connections $A-B$ and $B-C$ in subtilisin.

One can use this formula for a comparison within the dehydrogenase family, for example, for a comparison between

Table 1 'Figures of topological relatedness' between protein structures

Compared sheet topologies		No. of strands in common for both sheets	No. of connections in common for both sheets	Uncorrected figure of topological relatedness	Reduction factor	Corrected figure of topological relatedness	Figure of topological relatedness including binding region
Adenyl kinase	Subtilisin	5	3*	480 : 1	1 × 2	240 : 1	2400 : 1
Adenyl kinase	Flavodoxin	4	3	96 : 1	2 × 2	24 : 1	240 : 1
Adenyl kinase	Any dehydrogenase	4	3	96 : 1	2 × 3	16 : 1	160 : 1
Subtilisin	Flavodoxin	5	4	960 : 1	2 × 1	480 : 1	4800 : 1
Subtilisin	Any dehydrogenase	5	4	960 : 1	2 × 2	240 : 1	2400 : 1
Flavodoxin	Any dehydrogenase	5	4	960 : 1	1 × 2	480 : 1	4800 : 1
A dehydrogenase	Another dehydrogenase	6	5	11520 : 1	1 × 1	11520 : 1	115200 : 1

* Adenyl kinase and subtilisin have five strands but only three (and not four) connections in common because connections *B-C* are different. No reduction factor for this disagreement has been applied, however, since connection *B-C* has only slipped around a sheet edge.

lactate dehydrogenase¹³ and liver alcohol dehydrogenase¹⁵. Both structures contain six-stranded parallel sheets with identical topology (Fig. 1), although there exist $M=2^{6-2} \times 6! = 11,520$ possible topologies for such a sheet. Therefore, their "figure of topological relatedness" is 11,520 : 1.

The reasoning can be extended to topologies that are identical in the central strands of the sheet but that do show differences at the sheet edges. The procedure is best described by com-

paring adenyl kinase with flavodoxin. Both structures have a four-stranded sheet (*ACDE*) and three connections (*A-B=A-C*, *C-D*, *D-E*) in common. This yields an "uncorrected figure of topological relatedness" of $2^{4-2} \times 4! : 1$ or 96:1. For the comparison, however, one strand in each protein has been discarded. When selecting these strands an arbitrary choice of one out of two possibilities in each protein (left or right edge) has been made. Since such a choice reduces the significance, a reduction factor of $2 \times 2 = 4$ was applied to the "uncorrected figure of topological relatedness", yielding a "corrected figure of topological relatedness" of 24:1 (see Table 1). An analogous procedure has been followed when comparing other pairs of structures (Table 1).

The structural relationship within the dehydrogenase family is not only indicated by correspondence of sheet topologies but also by correspondence of nucleotide binding positions¹⁶. As shown in Fig. 1, the other proteins contain similar sites at similar positions relative to the sheet. Therefore, we shall include this kind of information about relationship into the proposed quantitative measure.

For sake of simplicity we do not distinguish between different kinds of sites but merely refer to them as being a "binding region". In the view given in Fig. 1, for all proteins this binding region is located in front of the carbonyl ends of the central sheet strands, that is, above the plane of the paper. In order to use the same reasoning as before, we estimate how many positions of such a binding region we would have accepted as being different. For instance, we would have been able to distinguish between left side, centre, and right side of the sheet as well as between above, in front, in the rear, and below the sheet, that is, we would have differentiated between about 10 positions.

Since all proteins show the same binding region position out of these roughly estimated 10 possibilities, there is an additional significance of their relationship of 10:1. Thus, the 'corrected figure of topological relatedness' (Table 1) may be multiplied by a factor of 10, yielding the overall 'figure of topological relatedness including binding region' between the structures (Table 1). If a closer correspondence of the binding regions could be established, there would be more than 10 distinguishable positions and therefore a larger additional factor.

The results in Table 1 show that adenyl kinase is rather closely related to subtilisin and less closely to flavodoxin and to the dehydrogenase family. This corresponds to the strand sequence reversal *BC* compared with *CB* between adenyl kinase and subtilisin on the one hand and the dehydrogenases on the other. A relationship between the dehydrogenases, flavodoxin and subtilisin is clearly indicated.

These relationships point to either divergent or convergent evolution. Convergent evolution would be expected if the resulting topology is energetically favoured or if the topology is favoured in the dynamical folding process¹⁸ of the polypeptide chain. But this does not seem very likely. In the case of divergent evolution, however, we have to postulate a common ancestor for enzymes as distant from each other as the

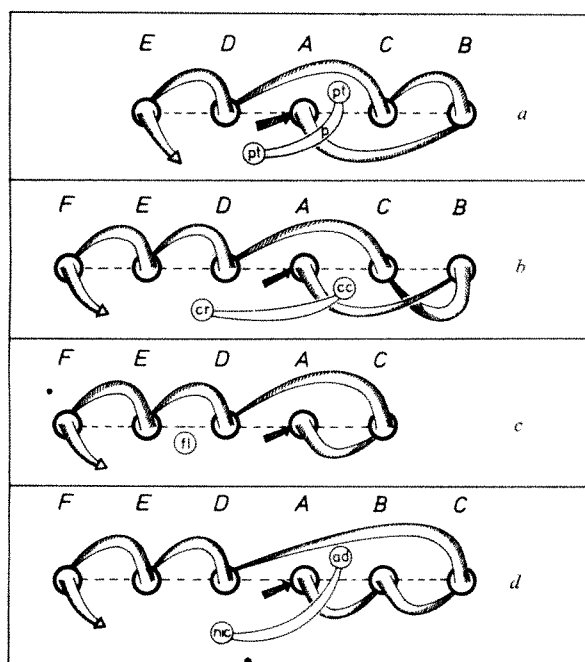


Fig. 1 Topologies of proteins containing a parallel sheet. *a*, Adenyl kinase²; *b*, subtilisin⁹⁻¹⁰; *c*, flavodoxin^{11,12}; *d*, NAD-binding globules (domains) of lactate dehydrogenase¹³, s-malate dehydrogenase¹⁴, liver alcohol dehydrogenase¹⁵, and D-glyceraldehyde-3-phosphate dehydrogenase¹⁶; *pt*, hydrophobic pockets, that is, presumed binding sites for adenine²; *p*, phosphate position in the catalytic centre of adenylate kinase; *cr*, hydrophobic crevice, that is, presumed binding site for aromatic or apolar side chain¹⁰; *cc*, catalytic centre; *fl*, FMN; *nic-ad*, nicotinamide and adenine moieties of the dinucleotide NAD. In the case of adenyl kinase the five heavy circles are the five strands in the sheet. (The drawing corresponds to the linear diagram given in Fig. 3 of the adenyl kinase structure description², if one views this figure from the top along the strands in the sheet, that is, along the plane of the paper, to the bottom.) The carbonyl ends of these strands point toward the viewer. The arrows indicate the direction of the polypeptide chain³, that is strands *ABCDE* in the sheet are ordered alphabetically along the course of the chain. The sheet topologies of the other proteins have been drawn correspondingly. The topologies of the NAD-binding globules of the four dehydrogenases are identical¹⁶. The same topology might be present in horse muscle phosphoglycerate kinase¹⁷. The location of substrates (*pt*, *cr*), cosubstrates (*fl*, *nic-ad*), and catalytic centres (*cc*, *p*) are all in front of the carbonyl ends of the central strands in the sheet, that is above the plane of the paper.

bacterial extracellular protease subtilisin and the mammalian intracellular adenylyl kinase.

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- ¹ Heil, A., Müller, G., Noda, L. H., Pinder, T., Schirmer, I., Schirmer, R. H., and v. Zabern, I., *Eur. J. Biochem.*, **43**, 131–144 (1974).
- ² Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H., *Nature*, **250**, 120–123 (1974).
- ³ Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **5**, (National Biochemical Research Foundation, Washington DC, 1972).
- ⁴ McLachlan, A. D., *Nature New Biol.*, **240**, 83–85 (1972).
- ⁵ Rao, S. T., and Rossmann, M. G., *J. molec. Biol.*, **76**, 241–256 (1973).
- ⁶ Rossmann, M. G., and Liljas, A., *J. molec. Biol.*, **85**, 177–181 (1974).
- ⁷ Timkovich, R., and Dickerson, R. E., *J. molec. Biol.*, **79**, 39–56 (1973).
- ⁸ Schubert Wright, C., Alden, R. A., Kraut, J., *Nature*, **221**, 235–242 (1969).
- ⁹ Drenth, J., Hol, W. G. J., Jansonius, J. N., and Koekoek, R., *Cold Spring Harbor Symp. quant. Biol.*, **36**, 107–116 (1971).
- ¹⁰ Kraut, J., Robertus, J. D., Birktoft, J. J., Alden, R. A., Wilcox, P. E., and Powers, J. C., *Cold Spring Harbor Symp. quant. Biol.*, **36**, 117–123 (1971).
- ¹¹ Watenpugh, K. D., Sieker, L. C., Jensen, L. H., Legall, J., and Dubourdieu, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3185–3188 (1972).
- ¹² Andersen, R. D., Apgar, P. A., Burnett, R. M., Darling, G. D., Lequesne, M. E., Mayhew, S. G., and Ludwig, M. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3189–3191 (1972).
- ¹³ Rossmann, M. G., Adams, M. J., Buehner, M., Ford, G. C., Hackert, M. L., Lentz, jun., P. J., McPherson, jun., A., Schevitz, R. W., and Smiley, I. E., *Cold Spring Harbor Symp. quant. Biol.*, **36**, 179–191 (1971).
- ¹⁴ Hill, E., Tsernoglou, D., Webb, L., and Banaszak, L. J., *J. molec. Biol.*, **72**, 577–591 (1972).
- ¹⁵ Brändén, C. I., Eklund, H., Nordström, B., Boiwe, T., Söderlund, G., Zeppezauer, E., Ohlsson, I., and Åkeson, Å., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2439–2442 (1973).
- ¹⁶ Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossmann, M. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3052–3054 (1973).
- ¹⁷ Blake, C. C. F., and Evans, P. R., *J. molec. Biol.*, **84**, 585–601 (1974).
- ¹⁸ Wetlaufer, D. B., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 697–701 (1973).

Stimulatory capacity of human T and B lymphocytes in the mixed leukocyte culture

LYMPHOCYTES can be separated into thymus-dependent (T) and thymus-independent (B) cells on the basis of ontogeny, surface membrane differentiation markers, and function. Thus, some functions can be attributed solely to T or B cells, whereas others require T/B cell cooperation^{1,2}. When two populations of allogeneic lymphoid cells are cultured together in the mixed leukocyte culture assay (MLC), cellular interaction leads to blastic transformation and subsequent proliferation of a portion of the cultured cells. This proliferation response originates in T lymphocytes^{3–5,30,31}. But secondary T cell-mediated B cell proliferation may in some species contribute to the overall proliferative response of T and B cells^{6–9}. The question then arises: which cell type acts as stimulator in MLC? Is the stimulus to proliferate equally transferred by T and B cells, or are the antigenic determinants responsible for MLC activation represented exclusively or preferentially on the surface of only T or B lymphocytes? Studies in mice^{8–11} indicated that T and B cells possess equal stimulatory capacities. However, in other reports the stimulatory capacity in mouse MLC was almost

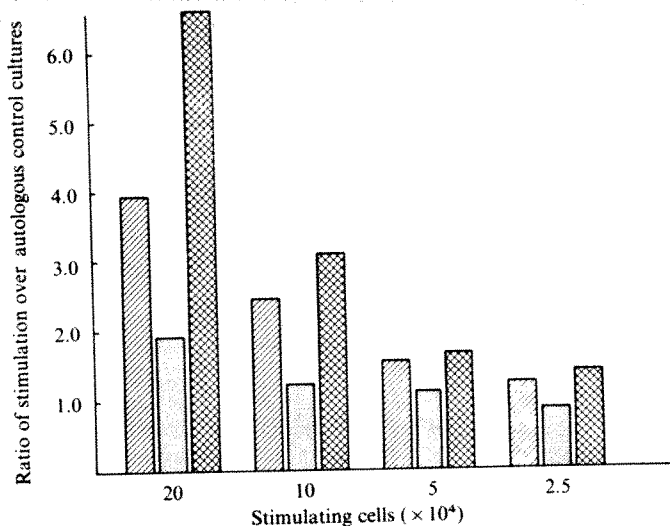


Fig. 1 Stimulatory capacity of unseparated T and B peripheral blood lymphocytes in human MLC. Lymphocytes (2×10^6) were stimulated with decreasing numbers of irradiated allogeneic cells. Ordinate: ratio of stimulation (^3H -TdR uptake in allogeneic cultures over ^3H -TdR uptake in corresponding autologous cultures), as calculated from the means of triplicate cultures. Abscissa: number of stimulating cells. Left bar, Unseparated lymphocytes; Centre bar, T lymphocytes; Right bar, B lymphocytes.

exclusively attributed to B lymphocytes (refs 3 and 12, and personal communication from D. H. Sachs).

We have attempted to answer this same question for human peripheral blood lymphocytes and report here that the proliferative response in human MLC is largely the result of stimulation by B cell-bound alloantigens.

Cells responding in MLC were prepared from heparinised venous blood by centrifugation over a Ficoll-Hypaque gradient¹². This preparation regularly contained more than 90% lymphocytes, the rest being monocytes and an occasional granulocyte.

Rosette formation of human T lymphocytes with unsensitised sheep erythrocytes (SRBC)¹⁴ was utilised to separate peripheral blood lymphocytes, used as stimulating cells in MLC, into rosetting (T) and nonrosetting (B) cells. To remove monocytes, which stimulate, although weakly, in MLC¹⁵, heparinised whole blood was incubated with sterile carbonyl iron powder for 60–90 min at 37° C with continuous agitation. Subsequent centrifugation over a Ficoll-Hypaque gradient yielded lymphocyte preparations containing less than 0.1% monocytes. Using a modification of the method of Wybran *et al.*¹⁶, these lymphocytes were rosetted with neuraminidase-pretreated SRBC (N-SRBC) which bind more firmly to human T lymphocytes than do untreated SRBC^{17,18}. Samples of 0.25 ml lymphocytes in Hanks balanced salt solution (HBSS) (10^7 ml^{-1}), 0.25 ml of foetal calf serum (absorbed with SRBC; Microbiological Associates) and 0.5 ml of N-SRBC (10^8 ml^{-1}) were added to plastic test tubes, centrifuged for 8 min at 200g, and further incubated at room temperature for 60 min. The gently resuspended pellets of all tubes (containing rosetted and nonrosetted lymphocytes) were pooled, and centrifuged over a Ficoll-Hypaque gradient (30 min at 400g); rosetting cells formed the pellet and nonrosetting cells remained at the interface between the layers. The pellet lymphocytes (termed T cells here) were washed twice in HBSS, and resuspended in culture medium (see below) to the concentration needed. No efforts were made to lyse or remove the N-SRBC so as to manipulate the lymphocytes as little as possible: orienting experiments showed that the capacity of lymphocytes to stimulate or respond in MLC was unaltered by addition of similar numbers of N-SRBC. The interface cells were washed, rosetted and again centrifuged over a Ficoll-Hypaque layer, to remove residual rosetting cells. The inter-

face cells thus obtained will be termed B cells here. This procedure effectively separated T and B cells: preparations of B cells contained less than 1% rosetting lymphocytes; and less than 2% of the T lymphocytes carried detectable amounts of surface immunoglobulins, as determined by direct immunofluorescence techniques¹⁷.

MLC (1 ml each) were set up in duplicate or triplicate with 400 ml Eagle's minimal essential medium (MEM) supplemented with 75 ml of decomplexed pooled human serum, 10 ml of L-glutamine (200 mM), 10 ml of a mixture of streptomycin and penicillin (5,000 U ml⁻¹ each), and 5 ml of nonessential amino acids (Flow Laboratories). To obtain one-way stimulation in MLC, stimulating allogeneic lymphocytes were irradiated (2,500 rad; 1,660 rad min⁻¹ from a caesium source) immediately before addition in various numbers to 2×10^5 responding cells. Responsiveness in these one-way MLCs was attributed to the nonirradiated cells, whereas stimulation was considered to be a function of the irradiated cell populations. Cultures were maintained in a humidified 5% CO₂-air atmosphere at 37° C for 7 d. Lymphocyte stimulation was assessed from the uptake of tritiated thymidine (³H-TdR) into DNA during the last 4 h in culture; cells were processed as described previously¹⁹.

Decreasing numbers of stimulating unseparated, T or B lymphocytes were added to 2×10^5 responding cells. Figure 1 demonstrates that B cells stimulated three times more vigorously than T lymphocytes using a ratio of responder:stimulator cells of 1:1. When compared with unseparated lymphocytes, T cells possessed significantly lower and B cells higher stimulatory capacity ($P < 0.001$). At lower responder:stimulator ratios, these differences were less pronounced.

Mixtures with different proportions of T and B cells were prepared, and used for stimulation of allogeneic lymphocytes. Figure 2 shows the proliferative response at three different concentrations of stimulating cells. Again, the stimulatory capacity of B cells was more than three times that of T cells at a responder:stimulator ratio of 1:1. Increasing percentages of T cells among stimulating cells led to decreasing stimulation. Similar, but less striking findings were obtained with lower numbers of stimulating cells. Could the stimulatory capacity of T cells be altered by formation of rosettes? Adherence of N-SRBC may expose previously masked T cell antigens for MLC stimulation; alternatively, T cell alloantigens may be removed or altered so that the T cells' stimulatory capacity is reduced or lost. The data presented here refute these objections. The stimulatory capacity of mixed T and B cells, using a T:B cell ratio of 2:1, was close to that of unseparated lymphocytes. Since there is a T:B cell ratio of approximately 2:1 in unseparated normal peripheral blood lymphocytes^{14,17}, it can be concluded that T cells possess their original MLC-stimulating capacity after rosette formation. Data in Fig. 2 further suggest that B and T cells contribute independently and additively to the stimulatory capacity of unseparated lymphocytes. To test whether T cells could suppress the stimulatory capacity of B cells, a constant number of stimulating B cells (2×10^5) and increasing numbers of stimulating T cells (0 to 2×10^5) were added to 2×10^5 responding lymphocytes. The proliferative lymphocyte response increased with increasing numbers of stimulating T cells; this observation excluded T cell suppression of the stimulatory capacity of B cells.

These results demonstrate that the major stimulus in human MLCs has to be attributed to B cell-bound alloantigens, and that human T cells possess only weak MLC-stimulatory capacity. The strength of the stimulatory capacity of the T cell preparations renders it unlikely that the few residual contaminating B cells were solely responsible for this stimulation; true T cell-bound stimulatory capacity seems to exist in man.

We propose the following hypothesis to explain the strong

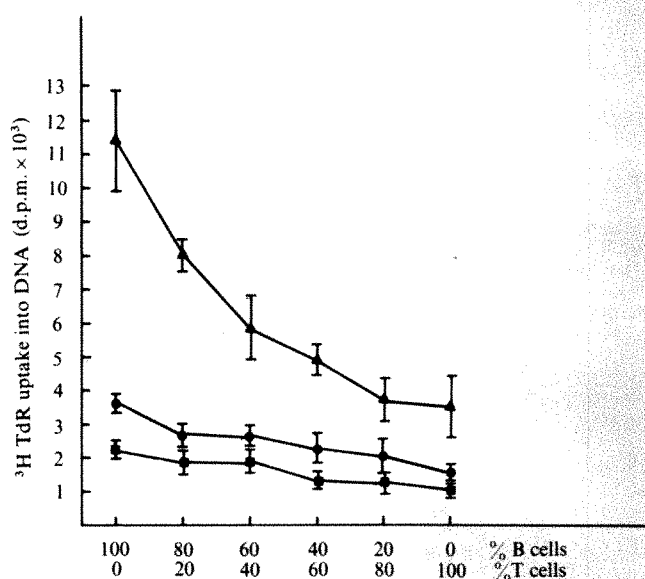


Fig. 2 Stimulatory capacity of human T and B lymphocytes in MLC. Responding cells (2×10^5) were stimulated with mixtures of allogeneic T and B cells at three different concentrations. Abscissa: percentages of T and B cells in the stimulating cell mixtures. Ordinate: ³H-TdR uptake into DNA (d.p.m.); indicated are the means \pm one s.d. of triplicate cultures. Δ , 2×10^5 stimulating cells; \bullet , 5×10^4 stimulating cells; \blacksquare , 2×10^4 stimulating cells.

B cell and weak T cell stimulatory capacity in human MLC. Serologically defined (SD) alloantigens (H-2 in the mouse, HL-A in man) were originally thought to be responsible for MLC activation²⁰. From studies in intra-H2 recombinant mouse strains²¹ and in siblings with a crossing over event within the major histocompatibility complex (MHC)^{20,22,23}, however, it is generally accepted that genes closely linked to, but genetically separable from, the two SD loci code for MLC activation (MLC loci, LD loci). Since genetic differences in the Ir region are required for strong MLC stimulation between congenic mouse strains^{21,24}, Ir-linked genes have been implicated as coding for MLC-activating gene products²⁵. A B-cell-specific alloantigen, coded by Ir-linked genes²⁶, has been suggested to be responsible for the strong one-way MLC activation observed between two recombinant mouse strains^{12,27}. But at least one other MLC locus exists within the MHC of mice, coding for a weakly stimulating alloantigenic system²⁴.

Two MLC loci have also been suggested in man, one coding for a strong and the other coding for a weak MLC-stimulating alloantigenic system^{28,29}. It is tempting to speculate that, in further analogy to the findings in the mouse, the differences in the MLC-stimulatory capacity between human T and B cells are due to different expression of MHC-determined alloantigenic gene products on T and B cells: the genetic locus responsible for strong MLC stimulation may code for B-cell-specific alloantigens, whereas the gene products of the second locus, coding for weak MLC-stimulating alloantigens, may be represented on T (and B?) cells. Further studies on the stimulatory capacity of T and B cells in siblings with a recombinational event in the MHC may substantiate this hypothesis.

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- ¹ Miller, J. F. A. P., *Int. Rev. Cytol.*, **33**, 77 (1972).
- ² Feldman, M., *Transplant Proc.*, **5**, 43 (1973).
- ³ Plate J. M. D., and McKenzie, I. F. C., *Nature new Biol.*, **245**, 247 (1973).
- ⁴ Andersson, L. C., Nordling, S., and Hayry, P., *J. exp. Med.*, **138**, 324 (1973).
- ⁵ Johnston, J. M., and Wilson, D. B., *Cell. Immun.*, **1**, 430 (1970).
- ⁶ Piquet, P.-F., and Vassalli, P., *J. exp. Med.*, **136**, 962 (1972).
- ⁷ Vischer, T. L., *Clin. exp. Immun.*, **11**, 523 (1972).
- ⁸ Wagner, H., *J. Immun.*, **109**, 630 (1972).
- ⁹ v. Boehmer H., *J. Immun.*, **112**, 70 (1974).
- ¹⁰ Harrison, M. R., *J. Immun.*, **111**, 1270 (1973).
- ¹¹ Cheers, C., and Sprent, J., *Transplantation*, **15**, 336 (1973).
- ¹² Fathman, C. G., Schwartz, R., Handwerker, B., and Sachs, D. H., *Fedn. Proc.*, **33**, 774 (1974).
- ¹³ Boyum, A., *Scand. J. clin. Invest.*, suppl. 21 (1968).
- ¹⁴ Jondal, M., Holm G., and Wigzell, H., *J. exp. Med.*, **136**, 207 (1972).
- ¹⁵ Blaese, R. M., Oppenheim J. J., Seeger, R. C., and Waldmann, T. A., *Cell. Immun.*, **4**, 228 (1972).
- ¹⁶ Wybran, J., Chantler, S., and Fudenberg, H. H., *Lancet*, **i**, 126 (1973).
- ¹⁷ Lohrmann, H.-P., and Novikovs, L., *Clin. Immun. Immunopath.* (in the press).
- ¹⁸ Weiner, M. S., Bianco, C., and Nussenzweig, V., *Blood*, **42**, 939 (1973).
- ¹⁹ Lohrmann, H.-P., Graw, C., and Graw, R. G., jun., *J. exp. Med.* (in the press).
- ²⁰ Amos, D. B., and Bach, F. H., *J. exp. Med.*, **128**, 623 (1968).
- ²¹ Bach, F. H., Widmer, M. B., Bach, M. L., and Klein, J., *J. exp. Med.*, **136**, 1430 (1972).
- ²² Yunis, E. J., and Amos, D. B., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 3031 (1971).
- ²³ Eijssvoegel, V. P., Koning, L., de Groot-Kooy, L. Huisman, S. L., van Rood, J. J., and Schellekens, P. Th. A., *Transplantn. Proc.*, **4**, 199 (1972).
- ²⁴ Meo, T., Vives, G., Rijnbeek, A. M., Miggiano, V. C., Nabholz, M., and Shreffler, D. C., *Transplantn. Proc.*, **5**, 1339 (1973).
- ²⁵ Meo, T., Vives, G., Rijnbeek, A. M., Miggiano, V. C., Nabholz, M., and Shreffler, D. C., *Transplantn. Proc.*, **5**, 1339 (1973).
- ²⁶ Sachs, D. H., and Cone, J. L., *J. exp. Med.*, **138**, 1289 (1973).
- ²⁷ Lozner, E. C., Sachs, D. H., Shearer, G. M., and Terry, W. D., *Science*, **183**, 757 (1974).
- ²⁸ Eijssvoegel, V. P., van Rood, J. J., du Toit, E. D., and Schellekens, P. T. A., *Eur. J. Immun.*, **2**, 413 (1972).
- ²⁹ Dupont, B., Good, R. A., Hansen, G. S., Jersild, C., Nielsen, L. S., Park, B. H., Svegaard, A., Thomsen, M., and Yunis, E. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 52 (1974).
- ³⁰ Lohrmann, H.-P., Novikovs, L., and Graw, R. G., jun., *J. exp. Med.* (in the press).
- ³¹ Lohrmann, H.-P., and Whang-Peng, J., *J. exp. Med.* (in the press).

Successful construction of chimaeric rabbit

CHIMAERIC mice can be made by aggregation of zona-free cleavage stage embryos, and these animals have proved useful in analysing various aspects of development and differentiation. Animals of this type are particularly suited to test models which propose that genetic information is transferred between cells, for example during antibody formation. It has been reported that RNA extracted from immunised rabbits can induce cells homozygous for one immunoglobulin allotype to synthesise antibody molecules carrying a foreign allotype characteristic of the animals from which the RNA had been extracted¹⁻³. Although chimaeric mice have been used to search for gene transfer during the normal *in vivo* response to antigen, these results have not proved conclusive⁴.

The concept of gene transfer in the immune response

Table 1 The immunoglobulin allotypic markers in the parental rabbits and their offspring

		Coat Colour	Heavy chain		Light chain	
			a1	a3	b4	b5
Parental colony	1	White	+		+	
	2	Black		+	+	+
Offspring	1	White	+		+	
	2	Chimaeric	+	+	+	+

The pattern of allotypic markers seen in the offspring has been consistently observed over an 8 month period. The threshold of detection of the 5b allotypic marker was less than 1/625 of the concentration in normal serum.

originated from work in rabbits and, in addition, the distribution of the allotypic markers in different parts of the immunoglobulin molecule⁵ make the rabbit the ideal species to answer the problem.

It is unlikely that chimaeras formed by aggregation of zona-free cleavage stage embryos will be successful in the rabbit as it has been shown that zona-free blastocysts fail to implant (C. E. Adams, unpublished). Chimaeric mice can be constructed, however, by the injection of isolated inner cell mass cells or the insertion of inner cell masses into the complete blastocyst still surrounded by a zona pellucida⁶. Here we report the successful application of this method to the rabbit.

Inner cell masses were dissected from 4-d-old blastocysts obtained from black rabbits bearing the heavy chain allotype a3 and the light chain allotypes b4 and b5, and individual inner cell masses were inserted into blastocysts, also 4 d old, obtained from white rabbits carrying the heavy chain allotype a1 and the light chain allotype b4. The blastocysts were allowed to repair during a brief incubation in culture (up to 2 h) and then returned to the uterus of pseudopregnant white rabbits which carried the a1 and b4 allotypes. Pseudopregnancy was induced 4 d before the transfer of the blastocysts.

Five blastocysts were transferred to each of two rabbits. Two offspring were obtained from one of these rabbits, and none from the other. One of the offspring was an albino rabbit, but the other was a chimaera with mixed coat colour (Fig. 1).



Fig. 1 Chimaeric rabbit, 37 d old.

Allotype analysis was carried out on the serum obtained from the two rabbits and the results are shown in Table 1. The albino rabbit contained only the a1 and b4 allotypes but the chimaeric rabbit contained the a1, a3, b4 and b5 allotypes. Further chimaeric animals with this gene combination are being produced and we are at present collecting reagents to look for gene transfer in the existing chimaera. Phenotypically the chimaeric animal is female and has produced a litter of nine albino rabbits following mating with a New Zealand white buck. Ten metaphases from circulating blood have proved to be XX. We hope animals of this type will provide an unambiguous answer to the problem of gene transfer in the immune response.

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Note added in proof: A further chimaeric rabbit has been obtained since this report was submitted for publication.

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¹ Adler, F. L., Fisher, M., and Dray, S., *J. Immun.*, **97**, 554 (1966).² Bell, C., and Dray, S., *J. Immun.*, **107**, 83 (1971).³ Bell, C., and Dray, S., *Cell. Immun.*, **6**, 375 (1973).⁴ Munro, A. J., Day, K., and Gardner, R. L., *Immunology* (in the press).⁵ Mage, R. G., *Prog. Immun.* (edit. by Amos, B.), **47** (Academic Press, 1971).⁶ Gardner, R. L., in *Advances in the Biosciences* (edit. by Raspe, G.), **6**, 279 (Pergamon, Oxford, 1971).

Is chiasma determination sequential?

Most cytological studies of chiasma distribution in higher plants and animals are limited to qualitative statements on the pattern of chiasma distribution along bivalents. A few quantitative studies exist, however, based on detailed measurements of chiasma positions along the extended diplotene bivalents of grasshopper and locust spermatocytes¹⁻³. These studies differ in certain respects but agree in concluding that chiasma formation or at least chiasma determination (assuming that the determination of sites for exchange is separable from exchange itself⁴) occurs sequentially along bivalents. To this extent these studies largely endorse the view originally proposed by Mather^{5,6} which was inferred from the property of chiasma interference and from observations on the frequencies of crossing over in different chromosome regions of *Drosophila melanogaster*.

The cumulative frequency distributions of chiasmata along meiotic bivalents of the locust, *Schistocerca gregaria*^{1,3} and of four species of British grasshopper², demonstrate clearly that chiasmata do not occur with equal frequencies in all chromosome regions. Certain regions, notably the telomeric, distal, ends of both metacentric and telocentric bivalents form chiasmata much more frequently than expected if chiasma distribution were uniform. This distinct peak of distally located chiasmata in all the species included in the studies quoted above has been widely interpreted as evidence that chiasma determination follows a sequential pattern starting at the telomere and extending to other bivalent regions. There is also a distinct tendency, especially in *Schistocerca gregaria*^{1,3}, for bivalents which form only one chiasma (monochiasmate bivalents) to have that chiasma localised distally. This tendency has also been held to demonstrate a sequence in chiasma determination since, for example, it is reasoned that the first-formed chiasma is the

least likely to be lost when chiasma frequency is reduced. There is also an implicit suggestion, based on this observation, of nonindependence between chiasmata formed at the telomere and those formed elsewhere, such that chiasma formation in nontelomeric regions is conditional upon previous telomeric chiasma formation—which again implies a sequence.

A critical examination of these claims shows that the case for a sequential mechanism of chiasma determination is not proved. The frequency peaks of chiasmata in certain chromosome regions, notably at the telomeres, merely denote that these regions enjoy a high probability of chiasma formation and this evidence does not necessarily imply that these chiasmata are the first formed. Likewise, the strict distal localisation of chiasmata in monochiasmate bivalents need not imply a sequence originating at the telomere. Indeed, the argument that nontelomeric chiasmata are conditional upon previous telomeric chiasma formation can be shown to contain a logical fallacy. The spurious impression of dependence is caused by the almost invariable occurrence of one chiasma in the telomeric region.

Observations on the frequency distribution of chiasmata along the bivalents of grasshoppers and locusts therefore make possible, but do not require, a sequential mechanism of chiasma determination. Other models of chiasma determination, including nonsequential models, are not excluded by this evidence and are equally valid if the evidence is taken at its face value.

An inherent feature of all sequential models of chiasma control is the necessary assumption that the control of the chiasma formed first differs fundamentally from the control of subsequent chiasmata. The principal controlling factor identified in these sequential models is chiasma interference but it is evident that this can only influence the position of the second and any subsequent chiasmata, and it follows that the position of the chiasma formed first must be determined by a quite separate control, although the actual mechanism of exchange initiation may be the same for the first chiasma as for subsequent chiasmata. The chiasma properties of a recently described meiotic mutant of rye raise some doubts concerning this distinction between various categories of chiasmata. This mutant genotype was isolated among the segregants from an interspecific cross^{7,8}, and is defective in the control of chiasma distribution⁹. Wild-type rye is characterised by pronounced distal chiasma localisation, so that bivalent arms forming a single chiasma normally have that chiasma localised in a distal, telomeric, position. Additional chiasmata, when they occur, necessarily form in more interstitial locations. According to sequential models of chiasma determination, the distal chiasmata should be regarded as the first formed and as such under a separate control from other, interstitial, chiasmata whose positions should be governed by chiasma interference.

In the distributional mutant of rye, all chiasmata are distributed in an apparently uncontrolled manner. Frequent instances of interstitial and even proximal chiasmata occur even in monochiasmate bivalents⁸, indicating a breakdown of localisation and a more random distribution pattern along bivalents than is found in wild-type rye. In addition, chiasma interference seems to be greatly reduced in intensity, or even absent, in this mutant⁸ (as judged from the Poissonian nature of the between-bivalent frequency distribution of chiasmata¹⁰). These findings indicate that a single control apparently governs the positions of all chiasmata and therefore determines both chiasma interference and chiasma localisation in rye, otherwise one might expect a differential response of these two factors to a change in the controlling mechanism. An extension of this comparison to include a large collection of diverse rye genotypes⁹ indicates that this correlated response is general and widespread (Fig. 1). Furthermore, there is evidence of similar correlated effects in certain meiotic mutants of *Drosophila melanogaster* which show both increased coincidence values (that is, reduced interference) and also nonuniform reductions in crossover frequencies along chromosomes (that is, reduced localisation)¹¹.

There is considerable evidence, therefore, for a single genetic control which governs the positions of all the chiasmata formed

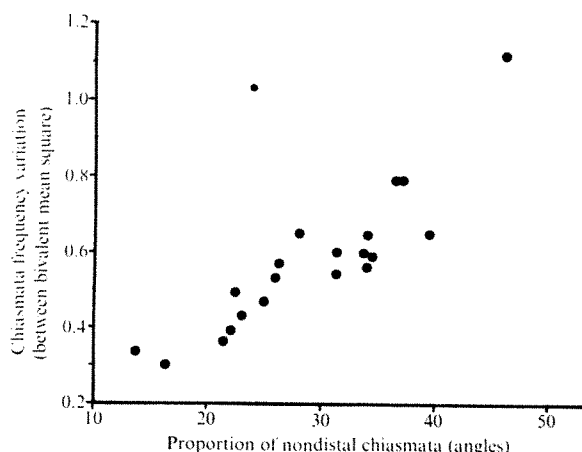


Fig. 1 The relationship of between-bivalent chiasma frequency variation (between-bivalent mean square) and chiasma positional distribution along bivalents (proportion of non-distal chiasmata) for a range of rye genotypes (from Jones⁹). The between-bivalent mean square can be regarded as a rough measure of the strength of chiasma interference¹⁰ and the proportion of nondistal chiasmata is a measure of the degree of chiasma localisation. These variables are clearly correlated ($r = 0.9$; $P < 0.001$) thus indicating that these diverse aspects of chiasma variation are controlled jointly.

in a bivalent, irrespective of their relative positions. The distinction between the chiasmata formed first and subsequent chiasmata, indicated by sequential models of chiasma determination, is difficult to reconcile with this conclusion. Proponents of sequential models could still argue that separate position-determining controls for the first and subsequent chiasmata have a factor in common, but as yet there is no evidence (for example, from the analysis of mutants) for the existence of unique factors associated with these hypothetical separate controls. Models based on nonsequential determination of all chiasmata are therefore at least as acceptable on present evidence and merely require that chiasma interference and other factors such as chiasma localisation be regarded as manifestations or consequences of control rather than control mechanisms in their own right.

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- ¹ Henderson, S. A., *Heredity*, **18**, 173–190 (1963).
- ² Southern, D. I., *Chromosoma*, **22**, 164–191 (1967).
- ³ Fox, D. P., *Chromosoma*, **43**, 289–328 (1973).
- ⁴ Sandler, L., Lindsley, D. L., Nicoletti, B., and Trippa, G., *Genetics*, **60**, 525–558 (1968).
- ⁵ Mather, K., *J. Genet.*, **33**, 207–235 (1936).
- ⁶ Mather, K., *Biol. Rev.*, **13**, 252–292 (1938).
- ⁷ Jones, G. H., and Rees, H., *Heredity*, **19**, 719–730 (1964).
- ⁸ Jones, G. H., *Chromosoma*, **22**, 69–90 (1967).
- ⁹ Jones, G. H., *Heredity* (in the press).
- ¹⁰ Haldane, J. B. S., *Cytologia*, **3**, 54–65 (1931).
- ¹¹ Carpenter, A. T. C., and Sandler, D. L., *Genetics* (in the press).

Decreased antibody formation in mice exposed to lead

SEVERAL environmental contaminants have been demonstrated to be synergistic to infectious agents. Polychlorinated biphenyls¹, arsenicals², cobalt sulphate³ and sulphur dioxide⁴ increased the mortality of animals infected with viral agents. Lead nitrate enhanced the mortality of mice to *Salmonella typhimurium*⁵. Some of these compounds are apparently immunosuppressive as circulating antibody titres to infectious agents from animals exposed to lead, cadmium, mercury⁶, DDT (ref. 7) and polychlorinated biphenyls⁸ were significantly lower than those from the control animals. This study was undertaken to determine if the decreased circulating antibody in animals that were exposed to lead was a result of a decrease in the number of cells producing antibody.

Swiss Webster mice 28 d old were given lead orally (1,375, 137.5 or 13.75 p.p.m.) as lead acetate in deionised water for 56 d. The controls were given deionised water. There were 120 mice in each group. The diet fed to all the mice was contaminated with 1.12 p.p.m. lead⁹.

After 56 d, all the mice were inoculated intraperitoneally with 0.2 ml. of a 2% suspension of sheep red blood cells (SRBC). Ten mice in each group were killed on days 3–7 to measure primary immune response (19S or IgM antibody) and on days 8–14 for the secondary response (7S or IgG antibody) after a second inoculation of SRBC on day 7. An additional group of mice were inoculated with SRBC while they remained on 137.5 p.p.m. lead. The spleen from each mouse was immediately removed, cut into pieces, and forced through a nylon mesh into sterile medium 199. The spleen cell suspension was counted in a haemocytometer by the trypan blue exclusion method and diluted to 1×10^7 spleen cells ml⁻¹. The number of plaque forming cells (PFC) was measured according to a modification of Cunningham and Szenberg¹⁰. Three pieces of double-sided tape $\frac{1}{2}$ inch wide were laid across

a clean microscope slide (75 × 25 mm) dividing it into two equal areas. Two clean coverslips (22 mm square) were placed on the tape such that two edges of each coverslip were firmly attached to the tape and formed two shallow chambers. A mixture containing 88% spleen cells (1×10^7), 2% SRBC, 5% complement and 5% medium 199 was delivered by a syringe into the two chambers. These were sealed with vaseline and incubated at room temperature for 2 h. The plaques were counted using a light microscope. The number of plaques per million spleen cells was calculated from the number of plaques observed per μ l of the initial mixture used.

The same procedure was used to assay the secondary response (animals killed on days 8–14). Medium 199 was replaced in the mixture by developing antiserum (Microbiological Associates, Bethesda, Maryland No. 52-122). The number of plaques per million spleen cells was corrected by determining the sensitivity of the developing antiserum and by determining the number of plaques that occurred in spleen suspensions from non-antigen (SRBC) stimulated mice.

The mice were bled every 2 weeks to observe the erythrocytes for basophilic stippling and to determine packed cell volume. Serum was collected before death for haemolysin titration. One kidney was collected at necropsy and stored at -70°C for lead analysis. The other kidney was fixed in 10% buffered formalin for microscopic examination.

There was a reduction in the number of spleen cells producing 19S antibody in each dose of lead exposed mice (Fig. 1) but the decrease was most remarkable in the mice that received 1,375 p.p.m. lead. The peak response was on day 5 for all groups of mice but those given 13.75 and 1,375 p.p.m. lead also had a lag in their response as only a few plaques developed on day 4. The secondary immune response was markedly affected in all of the lead exposed animals. Even those mice that received the lowest lead dose (13.75 p.p.m.) had a significant decrease in the number of 7S plaque-forming cells. The control mice had 500 plaque-forming spleen cells on day 2 and the mice with 13.75 p.p.m. lead had 158 plaques per million spleen cells (Fig. 2). Furthermore, the mice on 137.5 and 1,375 p.p.m. lead had a lower response and a delay of 1 d in their peak response. They also remained much lower throughout the 7 d test period than did the controls. The mice that remained on 137.5 p.p.m.

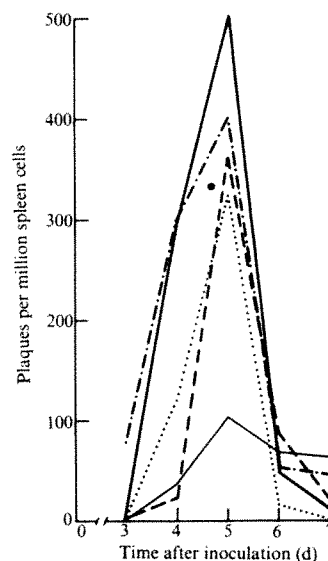


Fig. 1 Primary immune response. The largest lead dose (1,375 p.p.m.) administered to the mice resulted in a five-fold decrease in antibody forming cells as compared to the controls. A 1 d lag in the antibody response occurred in two lead groups (13.75 and 1,375 p.p.m.). There was a highly significant difference in the means at days 3, 4 and 5 as determined by the analysis of variance. —, Control; ---, 13.75 p.p.m. Pb; - · -, 137.5 p.p.m. Pb; · · · · ·, 137.5 p.p.m. Pb (continuous); ———, 1,375 p.p.m. Pb.

lead throughout the study had fewer plaques, particularly IgG, than did those in which lead was discontinued at the time of inoculation of antigen (Figs 1 and 2).

All of the mice that received 1,375 p.p.m. lead exhibited basophilic stippling of the erythrocytes at 2 weeks of exposure. Basophilic stippling occurred in some of the mice that received 137.5 and 13.75 p.p.m. lead at 4 weeks and in all of those mice at 8 weeks. A few of the control mice had basophilic stippling at 6 and 8 weeks as the diet contained 1.12 p.p.m. lead. The packed cell volume was not significantly different for the various groups throughout the experiment.

As the sera collected at the time of death were haemolysed from the lead the mice received, the haemolysin titrations were quite variable and were considered inaccurate for comparing the circulating antibody titres with the number of plaque-forming cells.

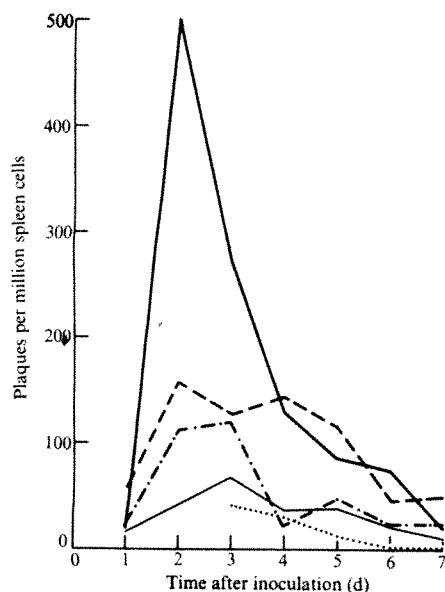


Fig. 2 Secondary immune response. Day 1 represents that day after the second inoculation of the antigen (SRBC), or actually day 8 of the experiment. All lead exposed mice had a highly significant decrease in antibody forming cells as compared to the controls. The two larger doses of lead (13.75 and 1,375 p.p.m.) produced a delay of 1 d in their peak response. The antibody production was the lowest in those mice that were on lead continuously until death. There was a significant difference in the means at day 3 and 4 and a highly significant difference at days 1, 2 and 7 as determined by the analysis of variance. —, Control; ---, 13.75 p.p.m. Pb; — · —, 137.5 p.p.m. Pb; · · · · ·, 1,375 p.p.m. Pb (continuous); —, 1,375 p.p.m. Pb.

Histopathology disclosed a marked necrosis of the tubular epithelial cells in the renal cortex, especially near the cortical medullary junction, of the mice that were exposed to 1,375 p.p.m. lead. Many intranuclear inclusion bodies (stained with haematoxylin and eosin (H & E)) were in the renal epithelial cells of these mice for the 14 d period. The renal necrosis was not as extensive and there were fewer intranuclear inclusions in the mice that received 137.5 p.p.m. lead. Also, at day 14, the renal necrosis was not as severe and only an occasional inclusion could be found. There was moderate necrosis of the renal epithelial cells and an occasional intranuclear inclusion until day 5 in the mice that had been given 13.75 p.p.m. lead. From days 6–9, a narrow zone of mild degeneration and necrosis of the epithelial cells was in the cortex adjacent to the cortical medullary junction. Intranuclear inclusions were not observed by H & E stain. From days 10–14, the kidneys appeared to be normal and did not differ from the control kidneys. The Ziehl-Neelsen stain was used to confirm the acid-fast inclusion body that contained lead.

Table 1 Renal concentration of lead from mice that were exposed to lead for 56 days in the drinking water. The kidneys were collected 3 and 14 days after lead was removed from the water

Drinking Water p.p.m. lead	Mean p.p.m. of renal lead*	
	Day 3	Day 14
1,375	16.4 ± 1.97	11.0 ± 0.96
137.5	9.3 ± 0.62	6.7 ± 0.92
13.75	1.0 ± 0.13	0.9 ± 0.09
No lead (control)	0.6 ± 0.09	0.4 ± 0.05

*Wet weight

The kidneys were analysed for lead content by atomic absorption spectrophotometry using a micro-carbon furnace. The renal concentrations of lead at 3 and 14 d after exposure are listed in Table 1.

It has been shown¹¹ that lead acetate impairs hepatic phagocytic activity in rats but does not alter the primary or secondary immune response. More recently, however, lead acetate markedly reduced the serum antibody titre of pseudorabies virus in rabbits⁶. In the present study, lead acetate markedly decreased the number of cells forming antibody to SRBC suggesting that the decrease in circulating antibody is due to a decrease in antibody formation. Other environmental contaminants such as polychlorinated biphenyls⁸, cadmium⁹, mercury⁶, DDT (ref. 7), and sulphur dioxide¹² have also resulted in reduction of circulating antibody in animals.

Mice given sulphur dioxide for 135 d had an enhancement of antibody production but at 192 d the response was a suppression of antibody¹³. It was suggested that high concentrations of sulphur dioxide served as an adjuvant. Lead, however, is immunosuppressive and the larger amount accumulated results in a smaller quantity of antibody synthesised. The group of animals that continued to receive lead until killed had a very slight IgG antibody response and would be most susceptible to infectious agents.

The smallest dose in this study was 13.75 p.p.m. lead in the drinking water, so the mice were ingesting about 0.069 mg of lead d⁻¹. As approximately 10% of ingested lead is absorbed¹³, each mouse was actually receiving about 0.0069 mg of lead into its system each day. A significant decrease in antibody forming cells, particularly 7S, occurred at this dose. The adult human normally ingests 0.3 mg of lead d⁻¹ and 2 mg d⁻¹ can produce toxicity¹⁴.

The intranuclear inclusion bodies in the kidneys are composed of lead and protein¹⁵. They are an acute manifestation of lead poisoning and can occur as early as 24 h after a single intraperitoneal injection¹⁶. In the present study, mice that received 13.75 p.p.m. lead had intranuclear inclusions up to 5 d after removal from lead, but they were not identified thereafter by H & E staining. Also, the cellular degeneration and necrosis in the renal cortex from this group of mice was not as severe at day 6 and could not be recognised by day 11; indicating that there was apparently rapid removal of lead from the kidney after discontinuing administration of lead as well as regeneration of the damaged tubular epithelium.

We have shown that chronic exposure to lead produces a significant decrease in antibody synthesis, particularly IgG, indicating that the memory cell is involved. These results also indicate that the reduced antibody synthesis is responsible for the increased mortality from bacterial and viral diseases in animals that are chronically exposed to lead.

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- ¹ Friend, M., and Trainer, D. O., *Science*, **170**, 1314 (1970).
- ² Gainer, J. H., and Pry, T. W., *Am. J. vet. Res.*, **33**, 2299 (1972).
- ³ Gainer, J. H., *Am. J. vet. Res.*, **33**, 2067 (1972).
- ⁴ Fairchild, G. A., Roan, J., and McCarroll, J., *Arch. environ. Hlth*, **25**, 174 (1972).
- ⁵ Hemphill, F. E., Kaeberle, M. L., and Buck, W. B., *Science*, **172**, 1031 (1971).
- ⁶ Koller, L. D., *Am. J. vet. Res.*, **34**, 1457 (1973).
- ⁷ Wassermann, M., Wassermann, D., Kedar, E., and Djauaherian, M., *Bull. environ. Contam. Tox.*, **6**, 426 (1971).
- ⁸ Koller, L. D., and Thigpen, J. E., *Am. J. vet. Res.*, **34**, 1605 (1973).
- ⁹ Schroeder, H. A., Balassa, J. J., and Vinton, W. H., *J. Nutrition*, **36**, 51 (1965).
- ¹⁰ Cunningham, A. J., and Szenberg, A., *Immunology*, **14**, 599 (1968).
- ¹¹ Trejo, R. A., DiLuzio, N. R., Loose, L. D., and Hoffman, E., *Exp. molec. Path.*, **17**, 145 (1972).
- ¹² Zarkower, A., *Arch. env. Hlth*, **25**, 45 (1972).
- ¹³ Chisolm, J. J., *Scient. Am.*, **224**, 15 (1971).
- ¹⁴ Goyer, R. A., *Am. J. Path.*, **64**, 167, (1971).
- ¹⁵ Goyer, R. A., *et al.*, *Lab. Invest.*, **22**, 245 (1970).
- ¹⁶ Choie, D. D., and Richter, G. W., *Science*, **177**, 1194 (1972).

Dissociation between effects of nerve growth factor on tyrosine hydrolase and tubulin synthesis in sympathetic ganglia

TREATMENT of neonatal animals with nerve growth factor (NGF) promotes general growth and differentiation of the peripheral sympathetic nervous system. NGF markedly increases the outgrowth of axons and at the same time causes the appearance of many microtubules in electron-microscopic pictures¹. Moreover, as a manifestation of enhanced differentiation NGF produces a selective induction of tyrosine hydroxylase (TH) and dopamine β -hydroxylase in sympathetic ganglia². These two enzymes catalyse key steps in the biosynthesis of noradrenaline and are located selectively in adrenergic neurones³. In contrast, the third enzyme involved in the synthesis of noradrenaline, dopa decarboxylase (DDC), has a more general distribution and is not specifically regulated by NGF².

It is not clear whether the promotion of axon outgrowth and the accompanying increased formation of neurotubules results from specific induction of subunit synthesis⁴ or from regulation of the assembly of subunits. The purpose of this study was to resolve this question by examining simultaneously the effect of NGF on tubulin subunit content and TH activity.

NGF was prepared as the 2.5S subunit⁵ from mouse submaxillary gland. A single subcutaneous injection (10 mg per kg body weight) was administered to 5-d-old rats weighing 10–25 g. At appropriate times the animals were killed by a blow to the head and the superior cervical ganglia were removed under a binocular dissecting microscope. For determination of enzyme activity and protein content one pair of ganglia were homogenised in 0.5 ml 0.005 M Tris buffer containing 0.1% Triton in a power-driven glass homogeniser. The homogenates were centrifuged for 20 min at 10,000g at 4° C. Proteins⁶, TH⁶ and DDC⁷ were assayed; both TH and DDC assays were modified as described by Oesch *et al.*⁸. To determine tubulin subunit content 4–7 pairs of ganglia were homogenised in 0.5 ml of 0.05 M Na-pyrophosphate, 0.2 M NaCl, 1 M sucrose, pH 7 and supernatants were prepared as above. The presence of 1 M sucrose does not affect the absolute value of the colchicine binding activity in the extracts, but does increase the half life of that activity to approximately 220 h (ref. 9).

Figure 1a shows the time course of the changes in TH activity and protein content per pair of ganglia after a single injection of 10 mg per kg of NGF. A statistically significant ($P < 0.05$) increase in TH activity compared with untreated controls was detectable as early as 12 h after injection. The

maximum increase (350% of control) was reached at 48 h. Thereafter, the activity decayed gradually to 130% at 120 h. In contrast, a statistically significant ($P < 0.05$) increase of the protein content was not reached before 48 h and amounted to only 20%. The induction of TH was completely abolished if the animals were injected subcutaneously with 1.6 mg per kg of actinomycin D, 3 h before administration of NGF, and were killed 24 h later.

In contrast to the activity of TH, that of DDC changed virtually in parallel with the protein levels. The same is true for tubulin which did not change its specific activity over the whole period of 120 h (Fig. 1b).

In a further series of experiments the animals were injected daily with 10 mg per kg of NGF given subcutaneously for 5 consecutive days and were killed 24 h after the last dose. Under these experimental conditions the total activity of TH increased to 750% of controls, the specific activity to 375%. The corresponding values for tubulin were 275 and 140% respectively (Fig. 2).

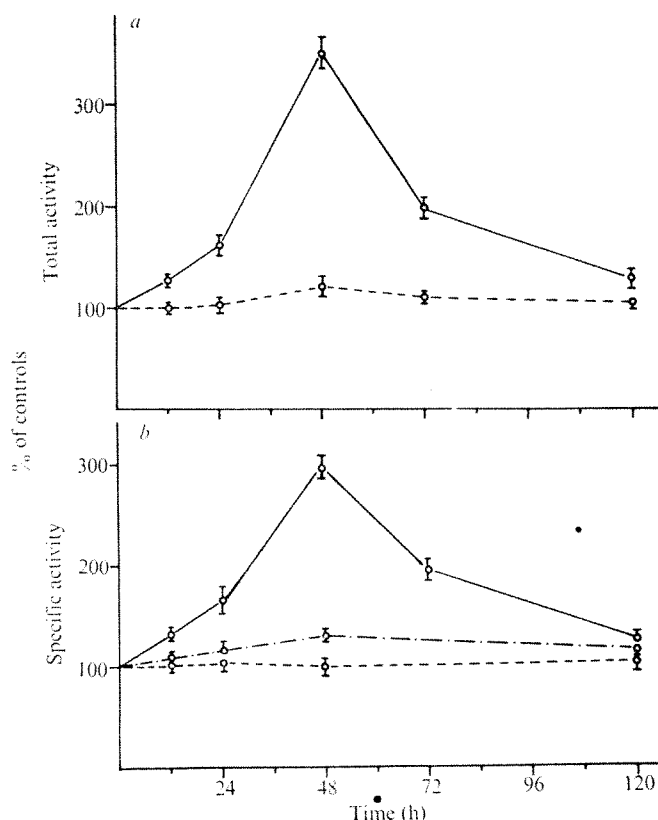


Fig. 1 Effect of a single dose of NGF on the levels of a, tyrosine hydroxylase (TH), ---, protein; b, tyrosine hydroxylase, ---, dopa decarboxylase (DDC), ----, tubulin in the rat superior cervical ganglia. The animals were injected subcutaneously with 10 mg kg⁻¹ of NGF 5 d after birth. TH activity and protein were determined in the supernatant after homogenising the ganglia in 0.005 M Tris-0.1% Triton X-100 and centrifuging the homogenate for 20 min at 10,000g. For the determination of TH activity the substrate (L-tyrosine) concentration amounted to 15 μ M, that of the cofactor (6, 7-dimethyl-5, 6, 7, 8-tetrahydropteridine) to 720 μ M. For the determination of DDC the substrate (L-³H-dopa) concentration was 1 mM, that of the cofactor (pyridoxal-5'-phosphate) was 0.24 mM and that of tranlycypromine was 1.2 mM. At the time of injection activity of TH was 0.3040 ± 0.019 nM dopa formed per h per pair of ganglia; that of DDC was 86.34 ± 6.04 nM dopamine formed per h per pair of ganglia. Tubulin was determined after incubation of the 10,000g supernatant with 8.3×10^{-6} M colchicine (2 Ci mmol⁻¹) for 90 min at 37° C by the filter assay of Borisy¹⁰. The specific activity of the tubulin in treated ganglia 48 h after injection was 3.6×10^{-5} d.p.m. bound per mg of protein. The values given represent the mean \pm s.e.m. of six or seven animals per group.

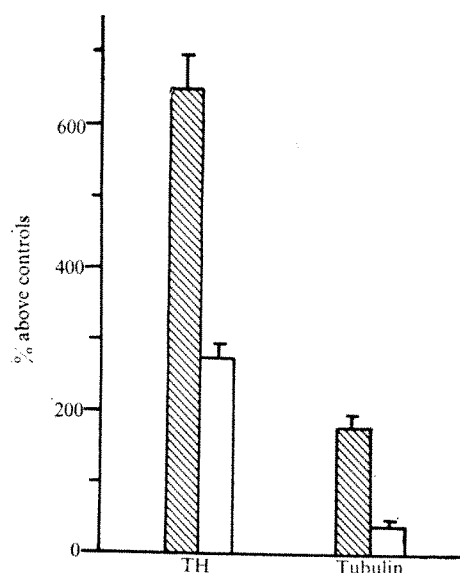


Fig. 2 Effect of repeated administration of NGF on level of TH and tubulin in the superior cervical ganglion. NGF was given subcutaneously on 5 consecutive days starting on the fifth day after birth. The ganglia were dissected 24 h after the last injection and treated as described in text. In the control animals the TH activity was 0.233 ± 0.007 nM dopa formed per h per pair of ganglia, the protein content was 14.8 ± 5.9 μ g per pair of ganglia, and the tubulin content was 5.52×10^{-4} d.p.m. bound colchicine per pair of ganglia. The values given represent the means \pm s.e.m. of six or seven animals per group. Shaded column, total activity; unshaded column, specific activity.

This study has shown that the effect of NGF on TH synthesis is quantitatively distinct after both single and multiple injections from the effect on tubulin synthesis. The dissociation of the appearance of increased numbers of neurotubules in electron microscopic pictures¹⁴ from any specific increase in the subunit content of the cells is consistent with the interpretation that NGF influences the assembly of preformed neurotubular subunits rather than enhancing their synthesis. This agrees with findings in other systems that outgrowth of cellular processes accompanied by the formation of microtubules does not require protein synthesis¹¹⁻¹³. Moreover, the induction of process formation in neuroblastoma cells by a glial-conditioned medium¹⁵ does not increase the tubulin content of the cells (F. S. and D. Monard, manuscript in preparation).

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¹ Levi-Montalcini, R., and Angeletti, P. U., *Physiol. Rev.*, **48**, 534 (1968).

² Thoenen, H., Angeletti, P. U., Levi-Montalcini, R., and Kettler, R., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1598 (1971).

³ Molinoff, P. B., and Axelrod, J., *A. Rev. Biochem.*, **40**, 465 (1971).

⁴ Bocchini, V., and Angeletti, P. U., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 787 (1969).

⁵ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randell,

R. J., *J. biol. Chem.*, **193**, 265 (1951).

⁶ Levitt, M., Gibb, J. W., Daly, J. W., Lipton, M., and Udenfriend, S., *Biochem. Pharmacol.*, **16**, 1313 (1967).

⁷ Hakanson, R., and Owman, C., *J. Neurochem.*, **13**, 597 (1969).

⁸ Oesch, F., Otten, U., and Thoenen, H., *J. Neurochem.*, **20**, 16 (1973).

⁹ Solomon, F., Monard, D., and Rentsch, M., *J. molec. Biol.*, **78**, 569 (1973).

¹⁰ Borisy, G. G., *Analyt. Biochem.*, **50**, 373 (1973).

¹¹ Seeds, N. W., Gilman, A. G., Amang, T., and Nirenberg, M. W., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 160 (1970).

¹² Hsie, A. W., and Puck, T. T., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 358 (1971).

¹³ Yamada, K. M., and Wessels, K. N., *Expl. Cell Res.*, **66**, 346 (1971).

¹⁴ Angeletti, P. U., Levi-Montalcini, R., and Calissano, P., *Adv. Enzymol.*, **31**, 51 (1968).

¹⁵ Monard, D., Solomon, F., Rentsch, M., and Gysin, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1894 (1973).

Reduced axoplasmic transport of choline acetyltransferase activity in dystrophic mice

MUSCULAR dystrophy has been regarded as a "primary degenerative myopathy"¹. But there is evidence from studies of both human muscular dystrophy and animal models of muscular dystrophy that the muscle disease may have a neural basis². The mechanism of the suspected neural disorder remains to be elucidated. Since there is independent evidence that in normal animals the trophic effect of nerve on muscle depends on intact axoplasmic transport³, we have initiated a study of axoplasmic transport in dystrophic mice. We have found a disturbance of axoplasmic transport of choline acetyltransferase activity in dystrophic mice.

Choline acetyltransferase (CAT) is the enzyme that catalyses the synthesis of the neurotransmitter acetylcholine⁴. In neural tissue, CAT is probably confined to cholinergic neurones⁵. It is likely that CAT, like other neural proteins, is synthesised in the nerve cell body and transported peripherally in the axoplasm to the nerve terminal^{6,7}. If a peripheral nerve is crushed by a ligature, CAT activity slowly increases on the proximal side of the constriction⁸. From the rate of accumulation of CAT activity, one can estimate the average velocity of proximo-distal axoplasmic transport of CAT⁹⁻¹¹. Here we present a study that shows a significant reduction in the average velocity of axoplasmic transport of CAT activity and a significant increase in the CAT activity in sciatic nerves of mice afflicted with an autosomal recessive form of muscular dystrophy¹². These findings may reflect a fundamental abnormality of axoplasmic transport of cholinergic neurones in dystrophic mice.

Male 129/ReJ dystrophic mice (dys/dys) with normal littermates (+/?), and normal retired breeders of known genotypes, (+/+) and (+/dys), were obtained from Jackson Memorial Laboratory, Bar Harbor, Maine. A ligature was placed on the right sciatic nerve, at mid-thigh level¹³. Animals were killed at various times following ligation and both sciatic nerves excised. One 1 cm length was cut from the middle of the non-ligated sciatic nerve. The 9 mm length of nerve immediately proximal to the ligature was divided into three 3 mm segments. The heat-labile CAT activity of the 8000g \times 10 min supernatant fraction of homogenised nerve segments was assayed with a simultaneous determination of the recovery of added purified mouse brain CAT activity. One unit of enzyme activity represents the production of 1 pmol of acetylcholine (ACh) from choline and ¹⁴C-acetyl coenzyme A per 3 mm length of nerve per min. We defined the accumulation of CAT activity as the increase in CAT activity within the 3-mm segment immediately proximal to the ligature. The initial CAT activity of the ligated nerve was estimated from the CAT activity of the non-ligated sciatic nerve. Values are presented as the mean \pm s.e.m. The statistical significance of values was determined by the Student *t* test.

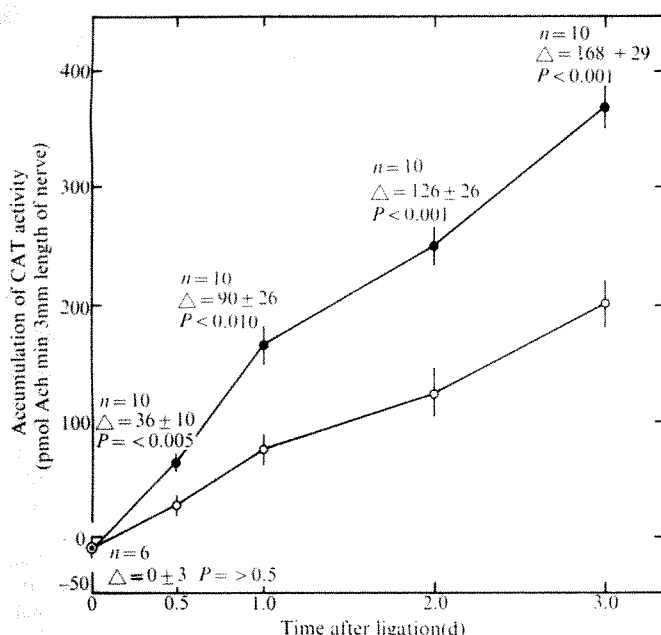


Fig. 1 Accumulation of CAT activity in sciatic nerves of 6 to 7-week-old dystrophic mice (○) and normal littermates (●) at various times after unilateral sciatic nerve ligations. The number of littermate pairs (n), and the statistical significance (P) of the difference ($\Delta = \text{mean} \pm \text{s.e.m.}$) between the accumulation of CAT activity of the littermate pairs are indicated at each time of killing. Nerve segments were homogenised (1 mm per 100 μl) in all glass Duall tissue grinders with 0.2 M Tris-HCl (pH 8.5), 0.5 M KCl, 1 mM EDTA, 1 mg ml^{-1} bovine serum albumin (BSA) and 1% butanol-1 (v/v); the homogenates were centrifuged at 8,000g for 10 min. CAT activity was assayed in a 20 μl reaction mixture with a final concentration of 100 μM ^{14}C -acetyl-coenzyme A (specific activity 53 mCi mmol^{-1} , New England Nuclear, Boston, Mass.), 15 mM choline iodide, 0.1 M Tris-HCl (pH 8.5), 0.5 M KCl, 1 mM EDTA, 0.1 mM eserine sulphate, 0.5 mg ml^{-1} BSA, 0.5% butanol-1 (v/v) and the 8,000g supernatant fraction of 0.083 mm of nerve. The CAT activity reaction mixture was incubated 6 min at 38°C, the reaction was stopped with 10 μl of 2 N formic acid, and the product was extracted into octanone and measured as described by Glover and Green²⁵. Occasionally in parallel experiments, the CAT reaction product from non-ligated and ligated nerve samples was further characterised by high voltage electrophoresis²⁶. There was quantitative agreement between the radioactivity that migrated with the R_f of acetylcholine and the radioactivity extracted into octanone. In the case of both dystrophic and normal mice (1) omission from the homogenisation solution of the KCl resulted in the failure to retain 80% of the homogenate CAT activity in the 8,000g supernatant fraction; (2) incubation at pH 7.0 instead of 8.5 resulted in 30% less CAT activity, and (3) omission from both the homogenisation and incubation mixture of the EDTA, BSA, or butanol-1 resulted in 80%, 35%, and 10% less CAT activity, respectively.

In both the dystrophic mice and their normal littermates, CAT activity accumulated as a linear function of time for at least 3 d after unilateral ligation of sciatic nerves (Fig. 1). But the accumulation of CAT activity in dystrophic mice was only $48 \pm 5\%$ of that in normal littermates whose nerves had been ligated for the same period of time ($n = 40$, $P < 0.001$). The relative accumulation of CAT activity was computed from (1) the accumulation of CAT activity in the ligated sciatic nerve of the dystrophic mouse as a percentage of (2) the accumulation

in the normal littermate, for the 40 pairs after time zero in Fig. 1.

The accumulation of CAT activity also may be described by equations fitted by the method of least squares to the data in Fig. 1: $y_c = 122t_c + 10$ ($n = 46$, $r = 0.93$, $P < 0.001$) and $y_d = 68t_d - 6$ ($n = 46$, $r = 0.77$, $P < 0.001$) for control and dystrophic mice, respectively, where y represents units of CAT activity, and t represents time in days. The average velocity of transport of CAT activity was computed from the time it took to increase the CAT activity in the ligated nerve by an amount equal to the initial CAT activity. Solution of the equations for $y_c = 202$ units and $y_d = 231$ units (see below) yielded $t_c = 1.6$ d and $t_d = 3.5$ d. Thus the average velocity of proximo-distal transport was 3 mm per 1.6 d = 1.9 mm d^{-1} for the controls and 3 mm per 3.5 d = 0.9 mm d^{-1} for the dystrophic mice.

The difference between the rates of accumulation of CAT activity in the sciatic nerves of dystrophic mice and normal littermates was not due to a difference in the spatial distribution of accumulated CAT activity. For at least 3 d after ligation, the accumulation of CAT activity was confined to the 3mm segment proximal to the ligature in both dystrophic and normal mice (Fig. 2).

The CAT activity per unit length of non-ligated sciatic nerves from dystrophic mice was 14% more than that of the controls. The non-ligated sciatic nerves of the dystrophic mice and normal littermates in Fig. 1 yield 231 ± 5 and 202 ± 6 units CAT activity, respectively ($\Delta = 29 \pm 5$ units, $n = 46$, $P < 0.001$). Though we do not know what fraction of the soluble proteins of nerves is axonal in origin, we found an increase in the soluble proteins in nerves of dystrophic mice that paralleled the increase in CAT activity. In companion experiments, the right sciatic nerves of 6 to 7-week-old dystrophic mice and normal littermates were assayed for CAT activity; the left nerves were weighed, and the 8,000g supernatant fractions were assayed for protein¹⁴. The weights of non-ligated sciatic nerves from dystrophic mice were not significantly different from those of the normals (1.1 ± 0.1 compared with 1.2 ± 0.1 mg cm^{-1} , $\Delta = 0.1 \pm 0.1$, $n = 8$, $P > 0.5$). Because Tris-HCl buffer and BSA interfered with protein determinations, 0.2 M KPO_4 buffer (pH 8.0) was substituted for Tris-HCl buffer in all homogenisation solutions, and the BSA was omitted from the homogenisation solutions used for measurement of protein content. The CAT activity was 19% greater in the nerves of the dystrophic mice (250 ± 9 compared with 210 ± 15 units, $\Delta = 40 \pm 13$, $n = 8$, $P < 0.02$), and the soluble protein was 18% greater in the nerves of the dystrophic mice (53 ± 2 compared with 45 ± 3 $\mu\text{g cm}^{-1}$, $\Delta = 8 \pm 2$, $n = 8$, $P < 0.01$).

The differences in initial CAT activity and in the accumulation of CAT activity were not due to differences in the content of endogenous activators or inhibitors of CAT activity. In every case, the recovery of purified mouse brain CAT activity added to the 8,000g supernatant fraction of corresponding nerve segments was the same for dystrophic mice and their controls. Table 1 shows the recoveries of added CAT activity from samples of nerve segments 3 d after unilateral sciatic nerve ligation.

Table 2 presents the results of several experiments with different ages and genotypes of 129/ReJ mice. In the sciatic nerves of 9 to 12-month-old normal retired breeder 129/ReJ mice, neither CAT activity nor accumulation of CAT activity differed between the genotypes (+/dys) and (+/+). Therefore, normal mice (+/?) probably constitute a reasonable control population for the study of dystrophic mice (dys/dys). As controls for this study, we chose normal mice which were also matched for

Table 1 Recovery of added purified CAT activity from 8,000g supernatant fraction of sciatic nerve segments

Group*	Non-ligated nerve	Ligated nerve (mm proximal to ligature)†		
		6	3	0
Dystrophic mice	$120 \pm 8\%$ ‡	$107 \pm 6\%$	$115 \pm 10\%$	$98 \pm 15\%$
Normal littermates	$117 \pm 11\%$	$112 \pm 15\%$	$106 \pm 10\%$	$103 \pm 11\%$

* The ten pairs of dystrophic mice and normal littermates killed 3 days after unilateral ligation of the sciatic nerve included in Fig. 1.

† The number refers to the distance between the ligature and the 3-mm segments of nerves (see Fig. 2).

‡ The recovery of added CAT activity was calculated as a percentage of the purified CAT activity added. CAT activity was purified 99-fold (final specific activity 0.13 $\mu\text{mol Ach min}^{-1} \text{mg}^{-1}$) from brains of 6 to 7-week-old C57 BL/10J mice from Jackson Memorial Laboratory, Bar Harbor, Maine²⁷.

Table 2 Initial CAT activity and accumulation of CAT activity 1 d after unilateral ligation of sciatic nerves in various groups of 129/ReJ mice

Group	Phenotype	Genotype	Weight (g)	Sciatic nerve CAT activity (pmol Ach per min per 3-mm length of nerve)	
				Initial	Accumulation
Eight 3 to 4-week-old littermate pairs	normal	+/?	9.7 ± 0.7	168 ± 10	204 ± 19
	dystrophic	dys/dys	7.1 ± 0.7*	214 ± 19 ⁺	111 ± 31 ⁺
Ten 6 to 7-week-old littermate pairs	normal	+/?	20.2 ± 0.6	185 ± 12	164 ± 20
	dystrophic	dys/dys	10.2 ± 0.5‡	214 ± 8*	74 ± 12 ⁺
Eight age-matched (9 to 12-month-old) pairs of retired breeders	normal	+/?	25.1 ± 1.1	498 ± 20	149 ± 30
	normal	+/?	30.0 ± 1.2	500 ± 26§	157 ± 18§

Significantly different from their normal littermates with * $P < 0.02$, $^+P < 0.01$, or $^{\ddagger}P < 0.001$.

§ Not significantly different ($P > 0.5$) from the age-matched controls.

age (littermates). Table 2 shows that the increase in CAT activity and the reduction in the accumulation of CAT activity were evident in 6 to 7-week-old dystrophic mice whether one uses normal controls matched for age (littermates) or normal controls matched for body weight (3 to 4-week-old normal mice).

The neuronal mechanisms which are responsible for the increase in CAT activity and the reduced rate of accumulation of CAT activity in sciatic nerves of dystrophic mice remain to be elucidated. The reduced accumulation of CAT activity probably reflects a decrease in the amount of CAT activity that moves down the nerve axon towards the nerve terminal each day. This interpretation is compatible with the recent autoradiographic evidence that there is a reduction in the amount of protein slowly transported down nerves of dystrophic mice¹⁵.

The reduced rate of accumulation of CAT activity in nerves of dystrophic mice could be explained by (1) a decrease in the number of cholinergic fibres in the nerves¹⁶⁻¹⁸; (2) a decrease in the amount of CAT available for transport from nerve cell bodies; (3) a decrease in the velocity or in the fraction of CAT transported down nerve axons. But of these alternatives, only the latter one, by itself, can also explain the increase in initial CAT activity we observed in nerves of dystrophic mice.

Other abnormalities of peripheral nerves in dystrophic mice have been reported. Nerves of dystrophic mice have areas of abnormal myelination¹⁹, there are differences between the gel electrophoresis patterns of proteins from nerves of dystrophic and control mice²⁰, and the nerves from dystrophic mice are less able than nerves from controls to support muscle regeneration^{2,21}. Our finding of reduced axoplasmic transport of CAT activity in dystrophic mice may be indicative of a primary neuronal abnormality. It is possible, however, that our results reflect a disturbance of neuronal function that is secondary to muscle disease.

With regard to the latter alternative, our observation of early changes in CAT activity and its transport suggests that these changes are at least not late consequences of the muscle disease (Table 2). Also, the loss of muscle cells that accompanies murine dystrophy seems an unlikely cause of the increased CAT in nerves of dystrophic mice because there is evidence that the absence of contact of muscle cells with neurones results in lower CAT activity of the neurones^{22,23}. It is also unlikely that the loss of muscle cells is the cause of reduced axoplasmic transport of CAT activity since we have recently shown that section of the sciatic nerve in normal mice does not reduce the rate of axoplasmic transport of CAT activity proximal to the sectioned end (unpublished results).

The CAT activity of dystrophic muscle has been shown to be reduced compared with normal muscle²⁴. The reduction in dystrophic muscle CAT activity may be related to the reduction in axoplasmic transport of CAT activity documented here. Whether reduced axoplasmic transport of CAT activity is actually involved in the pathogenesis of the muscle abnormalities in murine muscular dystrophy remains to be determined. The neural abnormalities in dystrophic mice may be independent of the muscle disease. But as previously mentioned, there is evidence that the trophic effect of nerve on muscle depends on intact axoplasmic transport³. Therefore, it is possible that the muscle disease in dystrophic mice may be related to a disturbance of the axoplasmic transport of motor (cholinergic) neurones.

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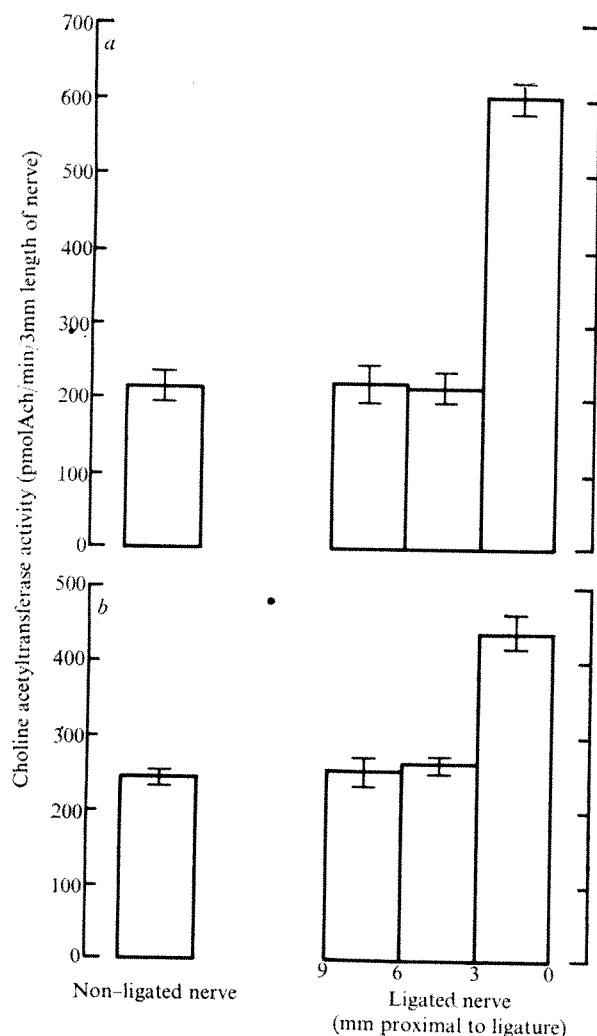


Fig. 2 Effect of unilateral ligation on sciatic nerve CAT activity in *a*, normal and *b*, dystrophic mice. Each value is the mean \pm s.e.m. CAT activity of nerve segments from the same ten pairs of dystrophic mice and normal littermates killed 3 d after unilateral ligation of the sciatic nerves included in Fig. 1.

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- ¹ Walton, J. N., *Br. Med. J.*, **1**, 1271-1274, 1344-1348 (1967).
- ² Gallup, B., and Dubowitz, V., *Nature*, **243**, 287-289 (1973).
- ³ Albuquerque, E. X., Warnick, J. E., Tasse, J. R., and Sansone, F. M., *Expl Neurol.*, **37**, 607-634 (1972).
- ⁴ Hebb, C., *Physiol. Rev.*, **52**, 918-957 (1972).
- ⁵ Kása, P., Mann, S. P., and Hebb, C., *Nature*, **226**, 814-816 (1970).
- ⁶ Hebb, C. O., and Waites, G. M. H., *J. Physiol., Lond.*, **132**, 667-671 (1956).
- ⁷ Hebb, C. O., and Silver, A., *J. Physiol., Lond.*, **169**, 41P-42P (1963).
- ⁸ Kása, P., Mann, S. P., Karcus, S., Tóth, L., and Jordan, S., *J. Neurochem.*, **21**, 431-436 (1973).
- ⁹ Wooten, G. F. and Coyle, J. T., *J. Neurochem.*, **20**, 1361-1372 (1973).
- ¹⁰ Oesch, F., Otten, U., and Thoenen, H., *J. Neurochem.*, **20**, 1691-1706 (1973).
- ¹¹ Saunders, N. R., Dziegielewska, K., Häggendal, C. J., and Dahlström, A. B., *J. Neurobiol.*, **4**, 95-103 (1973).
- ¹² Michelson, A. M., Russell, E. S., and Harman, P. J., *Proc. natn. Acad. Sci. U.S.A.*, **41**, 1079-1084 (1955).
- ¹³ Brimijoin, S., *J. Neurochem.*, **19**, 2183-2193 (1972).
- ¹⁴ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- ¹⁵ Bradley, W. G., and Jaros, E., *Brain*, **96**, 247-258 (1973).
- ¹⁶ McComas, A. J., and Mossaw, S. J., *Research in Muscular Dystrophy* (3rd Symposium on Muscular Dystrophy, London, 1965) pp. 317-341.
- ¹⁷ Harris, J. B., and Wilson, P., *J. neurol. Neurosurg. Psychiat.*, **34**, 512-520 (1971).
- ¹⁸ Harris, J. B., Wallace, C., and Wing, J., *J. neurol. Sci.*, **15**, 245-249 (1972).
- ¹⁹ Bradley, W. G., and Jenkinson, M., *J. neurol. Sci.*, **18**, 227-247 (1973).
- ²⁰ Salafsky, B., and Stirling, C. A., *Nature*, **246**, 126-128 (1973).
- ²¹ Salafsky, B., *Nature*, **229**, 270-273 (1971).
- ²² Fonnum, F., Frizell, M., and Sjöstrand, J., *J. Neurochem.*, **21**, 1109-1120 (1973).
- ²³ Giller, E. J. jun., Schrier, B. K., Shainberg, A., Fisk, H. R., and Nelson, P. G., *Science*, **182**, 588-589 (1973).
- ²⁴ McCaman, M. W., Stafford, M. L., and Skinner, E. C., *Am. J. Physiol.*, **212**, 228-232 (1967).
- ²⁵ Glover, V., and Green, D. P. L., *J. Neurochem.*, **19**, 2465-2466 (1972).
- ²⁶ Feigenson, M. E., and Saelens, J. K., *Biochem. Pharmac.*, **18**, 1479-1486 (1969).
- ²⁷ Potter, L. T., Glover, V. A. S., and Saelens, J. K., *J. biol. Chem.*, **243**, 3864-3870 (1968).

Haemocyanin synthesis and the branchial gland of *Octopus*

MUCH is known about the biochemistry of haemocyanin¹ but little about how or where it is made. It has recently been suggested² on morphological grounds that in cephalopod molluscs it is synthesised in the paired branchial glands. Here we present the first experimental evidence to support this hypothesis and throw doubt on earlier theories that these organs are endocrine³⁻¹⁰.

The gland cannot be an analogue of the adrenal medulla or cortex^{8,9}. It seems to be without nerves^{2,11} and pharmacological and fluorescence tests for monoamines are negative; it contains no lipids¹² or steroids¹³ and the well-developed endoplasmic reticulum (ER) is not smooth². Although transient cardiostimulation can be produced by crude extracts, this effect is not dose-dependent or specific to the gland, is much less than that of 5-HT and is not sustained as are the responses to extracts of the vena cava¹⁴. Furthermore, transplants are not effective in preventing death after bilateral extirpation of the glands.

The most remarkable features of the *Octopus* branchial gland are the extraordinary development of rough-surface ER and the presence of vacuoles within the cell containing granules that look like haemocyanin². These findings have been independently confirmed and extended to a squid¹⁵. Martin and Muzii (unpublished observations) have also

observed large intracellular inclusions of what appear to be macromolecules in crystalline array. These were of two sizes, one of which agreed with Fernandez-Moran's estimate¹⁶ for the cephalopod haemocyanin molecule.

It has also been shown¹⁷ that large amounts of labelled leucine injected into the blood disappear rapidly and are taken up by the branchial glands. If the glands are haematopoietic they should take up amino acids and the rate of uptake should be severely reduced after the elimination of the glands. We set out to establish this in our first experiments.

To impair the function of the glands, we applied slivers of dry ice directly to their surface and froze them. Subsequent examination under the light microscope and EM revealed extensive necrosis and we estimated that less than 10% of the total gland mass remained functional after freezing. This method was adopted because previous experiments had shown that complete, simultaneous, bilateral excision is generally fatal.

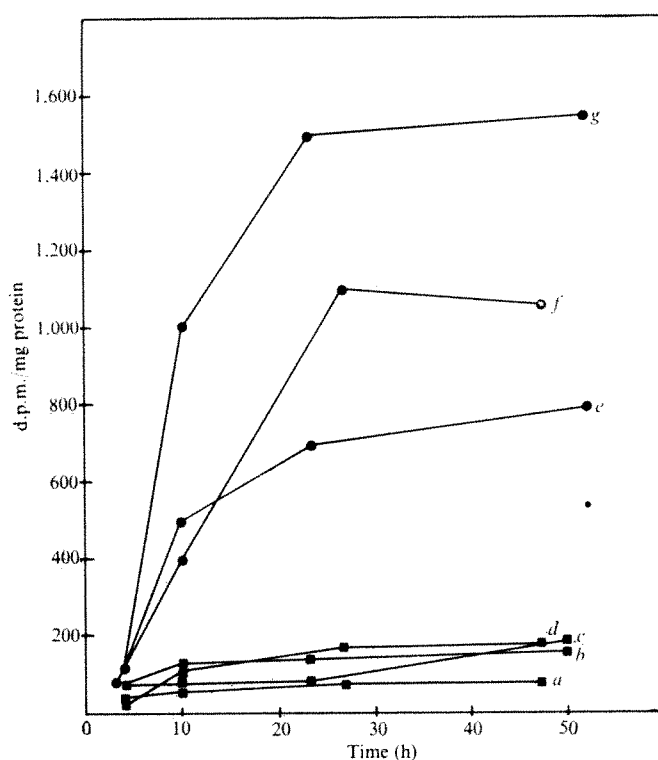


Fig. 1 *In vivo* L-¹⁴C-leucine incorporation into the haemolymph proteins of *Octopus vulgaris*. Experimental animals, (a, ♂ 230 g; b, ♀ 165 g; c, ♂ 160 g; d, ♂ 620 g) had their branchial glands frozen. Control animals, (e, ♀ 155 g; f, ♀ 180 g; g, ♂ 170 g) were anaesthetised only. Counts were corrected for background.

Healthy *Octopus vulgaris* were anaesthetised with 3% urethane, the mantle septum was cut and the mantle reversed to expose the visceral mass. Control operations ($n=3$) stopped at this point but the experimental group ($n=4$) had dry ice applied to the glands. The mantle was turned back and the animals allowed to recover in a stream of seawater.

Twenty-four hours afterwards, each animal was again anaesthetised and a single dose of 5.0 μ Ci of L-¹⁴C-leucine in 0.4 ml 1 M Tris-HCl buffer (pH 7.5) was injected into the lumen of the branchial heart. Haemolymph samples of 200 μ l were withdrawn under anaesthesia from the contra-lateral branchial heart at varying intervals over a 54 h period; blood cells were allowed to sediment overnight and the protein content of the supernatant determined by the

Biuret method¹⁸ using BSA as a standard.

Radioactivity was measured in aliquots of whole haemocyanin and compared with the radioactivity in TCA-precipitated haemolymph proteins. Samples of whole haemolymph (100 μ l) were dissolved in 10 ml of InstaGel scintillation cocktail (Packard, Chicago) and TCA-precipitated protein was collected on glass fibre disks¹⁹ and counted in a toluene-based scintillation fluid containing 5 g l⁻¹ PPO and 0.2 g l⁻¹ POPOP. Results from both methods were in excellent agreement.

Figure 1 illustrates the effect of freezing the glands on the uptake of labelled leucine. Experimental animals showed almost no increase in activity throughout the entire period of the experiment yet, in spite of considerable individual variation (not obviously related to size or sex), animals of the control group all exhibited a high uptake of radioactivity into the blood protein.

Although haemocyanin represents the major protein constituent of *Octopus* haemolymph, to confirm that the radioactivity reflected the synthesis of haemocyanin rather than some other protein, we resorted to an immunological characterisation, using the Ouchterlony double-diffusion method on an agar plate²⁰ followed by autoradiography of the dried plate. Haemocyanin antibodies were obtained by intravenously inoculating rabbits with 10 mg pure octopus haemocyanin three times at 10 d intervals; serum was collected 1 week after the last inoculation. In preliminary experiments anti-haemocyanin serum was tested against purified haemocyanin, whole haemolymph and concentrated haemocyanin-free haemolymph. A single precipitation line showing a complete arc of identity developed between serum and haemocyanin and whole haemolymph. No precipitation line was formed with haemocyanin-free haemolymph even when it was concentrated eight-fold.

In Fig. 2a is shown a double diffusion plate stained with Amidoschwarz in which haemolymph from control and operated animals (I and IV) gave a strong positive reaction, each yielding a single precipitation line that was identical to that of purified haemocyanin (II and III).

Autofluorography of the plate, by exposing the Kodak recording film to the dry gel plate for 2 weeks, revealed that the radioactivity was localised in a single line corresponding to the control animals' haemocyanin-antibody

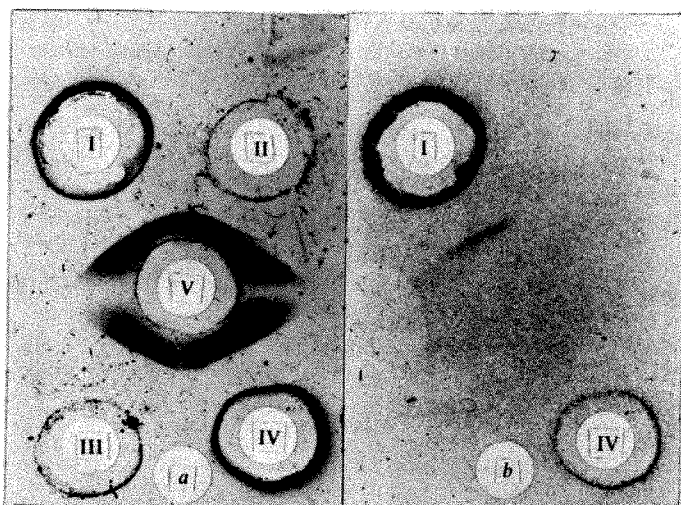


Fig. 2 Incorporation of L-¹⁴C-leucine into *Octopus* haemocyanin: immunoautoradiography. a, Amidoschwarz-stained immunodiffusion. Pure haemocyanin in wells II and III; radioactive haemolymph from control animal in well I; radioactive haemolymph from experimental animal in well IV; and anti-haemocyanin serum in well V. b, Autoradiography of a. Note the dark line indicating the radioactivity in the precipitation arc between wells I and V.

complex (Fig. 2b). There can be no doubt, therefore, that the amino acid is being incorporated into haemocyanin and that only in octopuses with intact branchial glands does labelled leucine get into the haemocyanin.

From this we conclude that the branchial gland of *Octopus* is undoubtedly involved in the biosynthesis of the respiratory pigment haemocyanin. Little is known about this process and it is hoped that the large size and accessibility of the branchial gland may make this gland invaluable for studying it.

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- ¹ Ghiretti, F., and Ghiretti-Magaldi, A., in *Chemical Zoology*, (edit. by Florkin, M., and Scheer, B. T.) 7, (New York, Academic Press, 1972).
- ² Dilly, P. N., and Messenger, J. B., *Z. Zellforsch.*, **132**, 193-201 (1972).
- ³ Grimpe, G., *Z. wiss. Zool.*, **104**, 531-621 (1913).
- ⁴ Turchini, J., *C. r. Ass. Anat.*, **18**, 477-478 (1923).
- ⁵ Hutchinson, G. E., *Nature*, **121**, 674-675 (1928).
- ⁶ Sereni, E., *Boll. Soc. ital. Biol. sper.*, **5**, 941-947 (1930).
- ⁷ Mitolo, M., *Arch. Sci. Biol. Napoli*, **24**, 33-58 (1938).
- ⁸ Taki, I., *Zool. Mag. Tokyo*, **52**, 91-92 (1940).
- ⁹ Taki, I., *J. Sci. Hiroshima Univ. Zool.*, **10**, 131-151 (1943).
- ¹⁰ Cazal, P., and Bogoraze, D., *Ann. Biol.*, **25**, 225-238 (1949).
- ¹¹ Young, J. Z., *Phil. Trans. B.*, **253**, 1-22 (1967).
- ¹² Baker, J. R., *Cytological Technique*, 4th ed. (London, Methuen, 1960).
- ¹³ Hanke, W., and Weber, K., *Gen. comp. Endocr.*, **4**, 662-672 (1964).
- ¹⁴ Berry, C. F., and Cottrell, G. A., *Z. Zellforsch.*, **104**, 107-115 (1970).
- ¹⁵ Schipp, R., Höhn, P., and Ginkel, G., *Z. Zellforsch.*, **139**, 253-269 (1973).
- ¹⁶ Fernández-Morán, H., van Bruggen, E. F. J., and Ohtsuki, M., *J. molec. Biol.*, **16**, 191-207 (1966).
- ¹⁷ O'Dor, R. K., and Wells, M. J., *J. exp. Biol.*, **59**, 665-674 (1973).
- ¹⁸ Gornall, A. G., Bardawill, C. J., and David, M. M., *J. biol. Chem.*, **177**, 751-766 (1949).
- ¹⁹ Mans, R. J., and Novelli, G. D., *Archs Biochem. Biophys.*, **94**, 48-53 (1961).
- ²⁰ Crowle, A. G., *Immunodiffusion* (New York, Academic Press, 1961).

Secretion-dependent uptake of extracellular fluid by the rat neurohypophysis

THERE is considerable evidence that hormone secretion from the neurohypophysis occurs by exocytosis of neurosecretory granules present in the nerve terminals¹⁻⁴. Exocytosis involves fusion of the granule membrane with the plasma membrane so that the entire soluble content of the granule is discharged into the extracellular space. Persistent hormone secretion does not lead to irreversible expansion of the plasmalemma of neurosecretory terminals, so the granule membrane must somehow be

retrieved after fusion. There is morphological evidence based on the use of electron dense markers in various preparations, that following exocytosis membrane is returned to the cell interior where it is present as microvesicles and larger cisternae⁶⁻¹⁰.

As large marker substances are taken into the nerve terminals, it seems likely that there ought also to be uptake of smaller molecules in proportion to their concentration in the external medium. If this is the case, the uptake of radioactive tracers might provide quantitative data on the magnitude of endocytosis occurring at actively secreting neuroendocrine terminals.

Neurohypophyses obtained from adult rats following decapitation were incubated in groups of four in 1 ml of oxygenated Locke solution¹¹ maintained at 37° C in a water bath. After 20 min, the solution was drawn off and immediately replaced by one containing: choline chloride, 150 mM; KHCO₃, 6 mM; CaCl₂, 2.2 mM; MgCl₂, 1 mM; glucose, 10 mM. ¹⁴C-Inulin (7.5 µCi ml⁻¹), ¹⁴C-mannitol (2.4 µCi ml⁻¹) or ¹²⁵I-albumin (2.4 µCi ml⁻¹, all three from Amersham) were present in the bathing fluid for the next 40 min. During the last 10 min of this second incubation, hormone release was evoked by raising the external KCl concentration to 56 mM, the choline chloride concentration being lowered to maintain constant osmolality. This incubation medium was then withdrawn and its oxytocin content determined in a rat bioassay¹². After stimulation, the preparations were washed for 150 min in Locke solution to remove the tracer molecules present in the extracellular spaces, the solution being renewed every 10 min. The neurohypophyses were then weighed, homogenised in 1 ml of HClO₄ 11% (w/v), centrifuged, and the radioactivity of the supernatant determined.

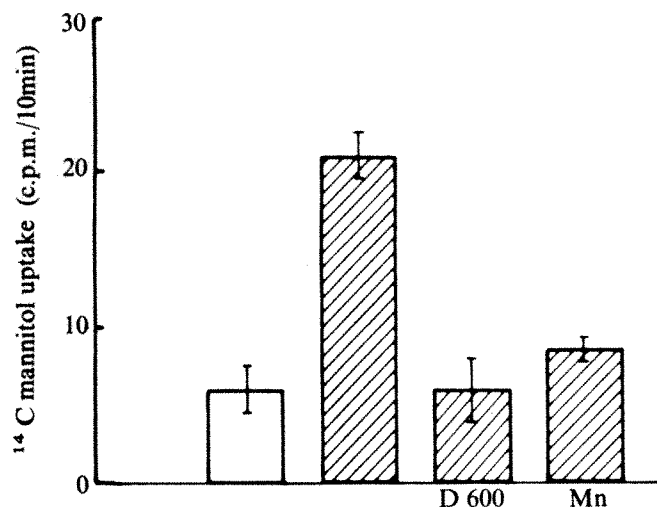


Fig. 1 Effects of calcium antagonists on mannitol uptake. Same experimental design as data of Table 1. □, Control uptake ($n = 4$); striped columns, stimulated preparations (56 mM KCl). Note absence of extra uptake of mannitol in preparations depolarised in presence of D600 (0.1 mM, third column $n = 6$) or Mn²⁺ (5 mM, fourth column, $n = 4$). Each column represents mean \pm s.e.m.

Exposure of neurohypophyses to the depolarising solution led consistently to an increased cellular uptake of extracellular marker substance. The results, summarised in Table 1, indicate that uptake is increased 3.0–5.4 fold in the stimulated preparations. The extra uptake of mannitol was greater than that of inulin or albumin but more experiments are needed to decide whether this difference is significant. The extra uptake could be caused either directly by a depolarisation-induced increase in the permeability to the tracer or indirectly as a result of secretion. The second hypothesis seems most likely because the extra uptake is abolished by including in the external medium either 5 mM manganous chloride or 0.1 mM of the organic calcium antagonist D600, (Fig. 1), both of which abolish

Table 1 Amount of radioactivity taken up by stimulated and control neurohypophyses

	Trapped radioactivity (c.p.m. per mg tissue)		Equivalent volume of extracellular fluid taken up during stimulation ($\mu\text{m}^3 \text{mg}^{-1}$)
	Stimulated preparations	Controls	
¹⁴ C-Mannitol	20.4 \pm 1.5 (8)	5.7 \pm 0.4 (4)	7.0 $\times 10^6$
¹⁴ C-Inulin	3.3 \pm 0.6 (6)	1.1 \pm 0.1 (5)	1.1 $\times 10^6$
¹²⁵ I-Albumin	4.1 \pm 0.2 (8)	0.75 \pm 0.1 (4)	1.1 $\times 10^6$

Preparations were kept 40 min in a Na-free Locke solution containing radioactive mannitol (0.015 mg ml⁻¹), inulin ($\sim 40 \text{ mg ml}^{-1}$) or albumin ($\sim 2 \text{ mg ml}^{-1}$). Stimulated neurohypophyses were incubated for the last 10 min in a high-K Na-free solution in presence of one of the extracellular marker substances. The Table shows the calculated uptake in control and stimulated preparations during the last 10 min of the incubation with radioactive marker. The difference in uptake between stimulated and control preparations is highly significant in all three cases (t test, $P < 0.001$). Each value represents mean \pm s.e.m. with the number of experiments in brackets.

calcium uptake induced by depolarisation of nerves¹³ and calcium-dependent hormone release from the neurohypophysis¹⁴. The view that uptake of extracellular tracer induced by depolarisation is secondary to secretion is supported by two other observations: first, both hormone release¹⁵ and tracer uptake are greater when secretion is evoked in a Na-free medium than when Na is present and, second, in Na-free media uptake of tracer induced by depolarisation increases linearly with tracer concentration in the external medium (Table 2).

These data are fully consistent with the hypothesis that, following exocytosis, membrane is retrieved by a process functionally similar to pinocytosis. On the assumption that uptake of tracer occurs without change in its concentration, it is possible to express the uptake in terms of a volume of extracellular fluid taken up and relate this to an equivalent volume of granules that must have undergone exocytosis. The data of Tables 1 and 2 give, for the three extracellular markers used, an average uptake of extracellular fluid equivalent to $3.1 \times 10^6 \mu\text{m}^3$ per mg tissue. In the same conditions, the amount of vasopressin and oxytocin released amounted to 90 milliunits, that is approximately 10% of the total neurohypophyseal hormone content of the glands believed to be contained in 2×10^{10} neurosecretory granules¹⁶. Assuming that release occurs solely by exocytosis, and taking 85 nm to be the mean radius of the core of the granules, the induced release should lead to a loss of $5.2 \times 10^6 \mu\text{m}^3$ per mg of tissue which is in surprisingly close agreement to the volume calculated from the uptake of extracellular markers.

If it is assumed, first, that both exocytosis and endocytosis involve spherical vesicles and, second, that membrane area is conserved, the relative volumes of fluid lost during exocytosis and gained during subsequent endocytosis should be in direct proportion to the ratio of the diameters of the vesicles involved in the two processes. Our data are consistent with membrane retrieval occurring through vesicles of diameter comparable to the granules undergoing exocytosis; but the data are not yet good enough to rule out other possibilities.

Morphological evidence supporting our conclusions was obtained by using the extracellular tracer horseradish peroxidase (HRP; Sigma, type II). HRP (2 mg ml⁻¹) had no effect on either resting levels of hormone secretion or secretion induced by depolarisation. In control experiments, following exposure to

Table 2 Dependence on the extracellular mannitol concentration of the secretion-dependent uptake of ¹⁴C-mannitol.

Mannitol concentration (mM)	Extra uptake in stimulated preparations (fmol mg ⁻¹)	Equivalent volume of extracellular fluid taken up ($\mu\text{m}^3 \text{mg}^{-1}$)
0.07	4.9 $\times 10^3 \pm$ 0.2 (8)	7.0 $\times 10^6$
1	67.7 $\times 10^3 \pm$ 9.7 (3)	6.8 $\times 10^6$
3	127.1 $\times 10^3 \pm$ 15.2 (3)	4.2 $\times 10^6$
5	317.4 $\times 10^3 \pm$ 11.7 (3)	6.3 $\times 10^6$

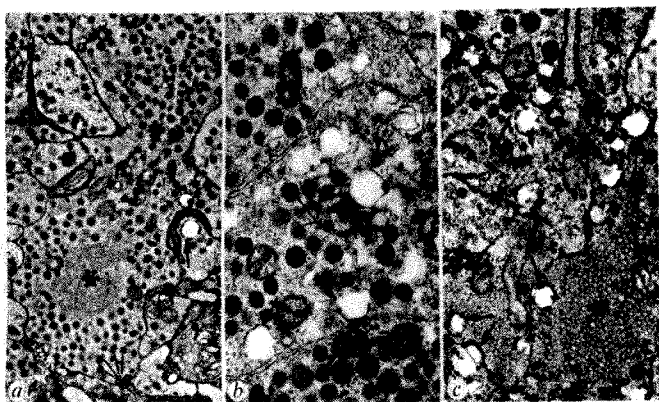


Fig. 2 Electron micrographs of neurohypophyses incubated in the presence, (a, c), or absence, (b), of horseradish peroxidase (HRP). Unfrozen sections (40 μ m thick) of glutaraldehyde-fixed specimens were treated to demonstrate sites of peroxidase activity¹⁷. a, Low magnification ($\times 7,500$) view of non-stimulated neurosecretory endings. Black HRP reaction product is present in the intercellular spaces, and in a few vacuoles within the cytoplasm (arrows). b, Stimulated neurohypophysis incubated in the absence of the tracer, to reveal endogenous peroxidase contained no HRP-tagged structures. The arrows indicate several membrane-bounded vacuoles ($\times 16,000$). c, Stimulated neurohypophysis. The arrows point to numerous peroxidase-labelled vacuoles. The size and location of the vacuoles are comparable to those shown in B ($\times 13,250$). * collections of microvesicles; sg, neurosecretory granules.

HRP for 40 min, the reaction product was mainly present in the extracellular space and very little was detectable in vesicles within the cytoplasm (Fig. 2a). In preparations that had been depolarised for 10 min (Fig. 2b and c) there was a variable degree of degranulation of neurosecretory endings and HRP reaction product was found in numerous membrane-bound vacuoles of a size (~ 100 nm radius) somewhat larger than that of the neurosecretory granules (~ 85 nm radius) seen within the endings. The vacuoles were preferentially localised near the plasmalemma. As previously reported⁵, microvesicles labelled with peroxidase, both coated and uncoated, could also be found but even after brief depolarisations their number was always low in proportion to the labelled vacuoles. As we found relatively few HRP-positive microvesicles, our results contrast with those of Nagasawa *et al.*⁵. We have no ready explanation for this discrepancy, but note that our experiments were performed in solutions free from sodium, leading thereby to a more powerful release reaction¹⁵. It may well be that two routes of endocytosis exist, and their relative importance may depend upon the intensity of secretion¹⁸.

Previous workers have described an extra uptake of calcium associated with depolarisation of the posterior pituitary^{14,19} and in view of our evidence for uptake of extracellular fluid it is clearly of interest to know how much of this extra entry of calcium might be accounted for by membrane retrieval and how much by other routes. In comparable conditions, the extra entry of calcium is 10–30 times as great as that of mannitol, indicating that only 3–10% of the calcium entry can be due to membrane retrieval.

Our results provide strong evidence that recovery from exocytosis includes membrane retrieval by a process that involves the indiscriminate uptake of extracellular fluid. The fate of the substances taken up is unknown; but it seems possible that the process of membrane retrieval may provide a route for the uptake of physiologically important substances into the neurone.

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- ¹ Douglas, W. W., Nagasawa, J., and Schulz, R., *Mem. Soc. Endocr.* **19**, 353–377 (1971).
- ² Dreifuss, J. J., Akert, K., Sandri, C., and Moor, H., *Brain Res.* **62**, 367–372 (1973).
- ³ Matthews, E. K., Legros, J. J., Grau, J. D., Nordmann, J. J., and Dreifuss, J. J., *Nature new Biol.* **241**, 86–88 (1973).
- ⁴ Nordmann, J. J., Dreifuss, J. J., and Legros, J. J., *Experientia*, **27**, 1344–1345 (1971).
- ⁵ Nagasawa, J., Douglas, W. W., and Schulz, R., *Nature*, **232**, 341–342 (1971).
- ⁶ Bunt, A. H., *J. ultrastr. Res.* **28**, 411–421 (1969).
- ⁷ Smith, U., *Tissue and Cell*, **2**, 427–433 (1970).
- ⁸ Heuser, J. E., and Reese, T. S., *J. Cell Biol.* **57**, 315–344 (1973).
- ⁹ Abrahams, S. J., and Holtzman, E. J., *J. Cell Biol.* **56**, 540–558 (1973).
- ¹⁰ Orci, L., Malaisse-Lagae, F., Ravazzola, M., Amherdt, M., and Renold, A. E., *Science*, **181**, 561–562 (1973).
- ¹¹ Douglas, W. W., and Poisner, A. M., *J. Physiol., Lond.*, **172**, 1–18 (1964).
- ¹² Bisset, G. W., Clark, B. J., Haldar, J., Harris, M. C., Lewis, G. P., and Rocha e Silva, M., *Br. J. Pharmac.* **31**, 537–549 (1967).
- ¹³ Baker, P. F., *Prog. Biophys. molec. Biol.*, **24**, 177–223 (1972).
- ¹⁴ Dreifuss, J. J., Grau, J. D., and Nordmann, J. J., *J. Physiol., Lond.*, **231**, 96–98p (1973).
- ¹⁵ Dreifuss, J. J., Grau, J. D., and Bianchi, R. E., *Experientia*, **27**, 1295–1296 (1971).
- ¹⁶ Morris, J. F., Tilly, G. N., and Hamilton, G., *Proc. third Eur. Anat. Cong.*, 68–70 (Manchester, 1973).
- ¹⁷ Graham, R. C., and Karnovsky, M. J., *J. Histochem. Cytochem.*, **14**, 291–302 (1966).
- ¹⁸ Castel, M., *Proc. seventh Conf. Eur. com. Endocr.*, (Budapest, 1973).
- ¹⁹ Douglas, W. W., and Poisner, A. M., *J. Physiol., Lond.*, **172**, 19–30 (1964).

Food vacuole membrane in nutrient uptake by *Tetrahymena*

ALTHOUGH the ciliate protozoon, *Tetrahymena pyriformis* was first cultivated in sterile broth more than 50 years ago¹, and in chemically defined media more than 20 years ago² its uptake of nutrients in solution is poorly understood. For example, the part played by the food vacuoles is still unknown. The vacuoles seem to be essential for rapid cell multiplication in some media^{3–5}, but calculations show that the volume of the food vacuoles formed per unit time cannot account for the observed uptake of either acetate⁶ or nucleosides (present report). Previously, it was proposed that the mucous layer might be able to concentrate nutrients and thus increase their uptake⁷. I here present an alternative hypothesis.

Inclusion of insoluble material in the medium shortens generation times and induces formation of food vacuoles in *T. pyriformis*^{3–5}. The generation times are more than 40 h in particle-free, sterile-filtered proteose peptone broth, but only 6 h in this medium in the presence of particulate material (ferric or aluminium hydroxides, heat-denatured egg albumin, polystyrene beads, or the precipitate formed by heat sterilisation of the proteose peptone broth). The slowly growing cells contain less than three vacuoles per average cell, whereas the fast growing cells have 20–30 (ref. 3). These results suggest that particles induce formation of food vacuoles and that formation of food vacuoles is required for rapid uptake of one or more essential nutrients.

Data from growth experiments in media of known composi-

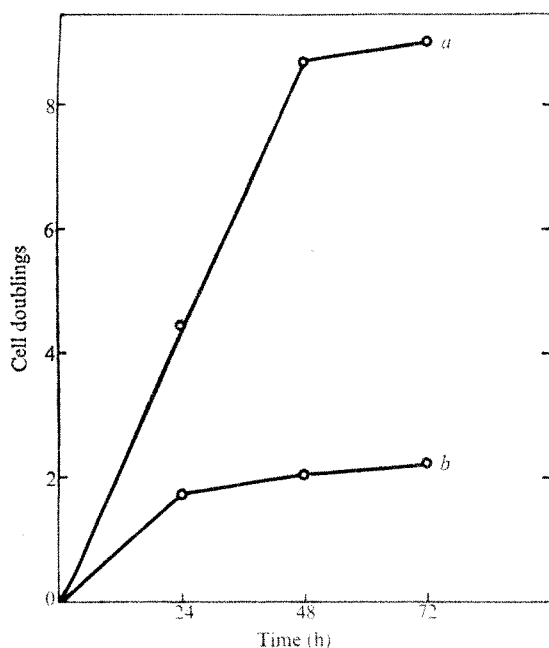


Fig. 1 Cell multiplication in populations of *Tetrahymena pyriformis* in the presence (a) and absence (b) of particulate material, Sephadex beads diameter approximately 1 μm . The nutrient medium was chemically defined except for addition of proteose peptone, final concentration 0.05%. The initial cell concentrations were 1,000 cells ml^{-1} . Each point represents the average of eight experiments. Figure reproduced from ref. 4.

tion allow the estimation of the contribution of the food vacuoles to the uptake of compounds which *Tetrahymena* cannot synthesise *de novo*, for example nucleosides⁸. Figure 1 shows cell doublings against time in the presence and absence of particles which induce the formation of food vacuoles. The estimate is based on a doubling time of 6 h in the presence of the particles, an observed rate of vacuole formation of one every 3 min, a measured vacuole diameter of 6 μm and a nucleoside concentration of 80 mg per l of medium⁴. From these values it has been calculated that in one generation time a cell of average volume about 27,000 μm^3 (ref. 9) makes vacuoles of which the total volume amounts to about 13,000 μm^3 . Assuming equal initial extracellular and intravacuolar concentrations, this volume contains about 10^{-12} g nucleosides. The cell requires, however, approximately 100×10^{-12} g nucleosides in order to double its content of nucleic acids¹⁰. Uptake of nucleosides through the cell surface is apparently insufficient to make up the difference, since cell multiplication is poor when food vacuoles are not formed due to the absence of particles (Fig. 1).

To account for the discrepancy, I propose that *Tetrahymena*, by some means other than uptake through its whole surface, can sweep nutrients from a volume of medium which is much larger than the sum of the volumes of the food vacuoles formed in the same time. It is unlikely that the stimulating effect of the particles is due to collision effects on the whole cell surface. This conclusion is based on results obtained in particle-containing media in which both cell multiplication and vacuole formation were greatly reduced in two sets of experimental conditions, namely in a mutant of *T. pyriformis*¹¹ containing no food vacuoles (unpublished experiments) and in the wild type after inhibition of vacuole formation with cytochalasin B (refs. 12, 13).

Since particles assist uptake of nutrients present in low concentrations, and neither the volumes of the vacuoles formed, nor entry through the whole cell surface can in any way account for the extent of this uptake, the major site of uptake under these conditions may well be the food vacuole membrane—especially while it is under formation and in open connection with the external medium. The oral structures maintain a strong current of fluid past the cytopharynx at the base of which the food vacuoles are formed. This current brings the membrane

of the forming food vacuole in contact with a relatively large volume of the extracellular medium and thus in contact with large amounts of nutrients. In support of this, it has been observed that food vacuoles collect Indian ink particles representing a medium volume at least 500 times as large as their own (L.R., H. E. Buhse jun., and K. Groh, in preparation).

Alternatively, nutrient uptake through the cell surface suffices for rapid multiplication in the absence of food vacuoles, when a generous supply of nutrients is maintained⁵.

The hypothesis presented here accounts for the experimental results obtained to date. Confirmation of its validity requires demonstration that food vacuole membrane is much more efficient than the rest of the cell surface in transporting such compounds as amino acids and nucleosides from dilute solution. This is possible in that reduced food vacuole formation is correlated with lengthened generation times in limiting media whether the scarcity of new vacuoles is due to lack of particles³⁻⁵, to inhibition of food vacuole formation by cytochalasin B treatment^{12,13} or to a temperature-sensitive mutation. The hypothesis reconciles the apparent conflict between vacuole volumes, nutrient concentrations and amounts of nutrients taken up. It also draws attention to a few so far neglected points in the discussion of the significance of the food vacuole in the uptake of dissolved nutrients by *Tetrahymena*.

This letter is dedicated to Professor Erik Zeuthen.

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- ¹ Lwoff, A., *C. r. hebd. Seanc. Acad. Sci. Paris*, **D176**, 928-930 (1923).
- ² Kidder, G. W., and Dewey, V. C., *Archs Biochem. Biophys.*, **66**, 486-492 (1957).
- ³ Rasmussen, L., and Kludt, T. A., *Expl Cell Res.*, **59**, 457-463 (1970).
- ⁴ Rasmussen, L., and Modeweg-Hansen, L., *J. Cell Sci.*, **12**, 275-286 (1973).
- ⁵ Rasmussen, L., *Expl Cell Res.*, **82**, 192-196 (1973).
- ⁶ Seaman, G. R., in *Biochemistry and Physiology of Protozoa*, **2** (edit. by Hutner, S. H., and Lwoff, A.), 91-158 (Academic, New York, 1955).
- ⁷ Nilsson, J. R., *C. r. Trav. Lab. Carlsberg*, **39**, 83-110 (1972).
- ⁸ Kidder, G. W., and Dewey, V. C., in *Biochemistry and Physiology of Protozoa*, **1** (edit. by Lwoff, A.), 323-400 (Academic, New York, 1951).
- ⁹ Chapman-Andresen, C., and Nilsson, J. R., *C. r. Trav. Lab. Carlsberg*, **36**, 405-432 (1968).
- ¹⁰ Zeuthen, E., and Rasmussen, L., in *Research in Protozoology*, **4**, (edit. by Chen, T. T.), 31 (Pergamon, Oxford, 1972).
- ¹¹ Orlas, E., and Pollock, N. A., *Progress in Protozoology*, Abstracts of fourth Int. Congr. on Protozoology, 468 (University of Clermont, Clermont, 1973).
- ¹² Nilsson, J. R., Ricketts, T. R., and Zeuthen, E., *Expl Cell Res.*, **79**, 456-460 (1973).
- ¹³ Hoffmann, E. K., Rasmussen, L., and Zeuthen, E., *J. Cell Sci.* (in the press).

Aerosol particles on tobacco trichomes

MARTELL¹ has described a plausible chain of events by which atmospheric radioactivity is incorporated into insoluble particles that are deposited in the lungs of tobacco smokers. The subsequent long exposure of the lungs to alpha activity is a strong candidate for the cause of bronchial cancer. Here we provide evidence for one of the steps in the sequence.

The accepted major sources of a radioactivity in the atmosphere are radon and its decay products, which condense by attaching themselves to sub-micron atmospheric particles. According to Martell, many such aerosols contact and are incorporated into the sticky tips of tobacco trichomes (spine-like protuberances on the leaves). To contact the trichome tips directly the smaller particles that carry most of the radioactivity must diffuse to the sticky, exudate-coated tips of the trichomes. Later, during tobacco curing, the sticky exudate polymerises to encapsulate the aerosols in highly insoluble particles. Thus,

when inhaled as part of tobacco smoke, the radioactive aerosols have long residence times in the deep lung.

Martell has shown that trichomes of cured tobacco are radioactive and that the most insoluble, filterable portion of tobacco smoke is the most radioactive. In support of Martell's inference that the radioactivity observed on trichomes comes from atmospheric aerosols, we present evidence that aerosols are on and inside trichome tips.

Trichomes from North Carolina flue-cured and Turkish air-cured tobacco leaves were examined in a Cwiskscan-100 equipped with a Kevex X-ray energy spectrometer. This instrument allows visual observation at high magnification and detection of elements heavier than neon in particles down to 250 Å in diameter. Silicon, sodium, chlorine, calcium and potassium were seen frequently (Fig. 1), iron and lead occasionally. Only potassium and calcium were seen regularly in all tips and stems equally. These known components of tobacco² mask attempts to observe them in sub-micron silicate aerosols.

Aerosols of > 0.5 µm diameter can be analysed individually on the surface of trichomes. These larger particles could be seen attached to most of the trichome tips of the North Carolina tobacco and half of those on Turkish tobacco. Of five individual particles analysed on North Carolina trichomes, four gave clear

signals from silicon; one was doubtful. Iron was detected in several particles. One 0.5 µm particle on Turkish tobacco contained chlorine. These compositions imply that the particles were silicate minerals and salt, respectively.

Trichome tips at positions where no exterior particles were visible showed silicon in half of the North Carolina samples. Portions other than the tips showed none. For Turkish tobacco sodium and chlorine were found on tips and stems. Silicon and lead were present in about half of the tips. Some of the silicon was associated with visible sub-micron particles.

In short, tips, like the particles on them, contain elements commonly associated with both continental and marine aerosols. The direct inference is that where individual particles are not seen, the signal comes from many tiny aerosols trapped within the exudate on the tips. This observation provides an experimental link in Martell's reasoning that radon daughter radioactivity precipitates on aerosols which diffuse to trichome tips and later become insoluble residues in tobacco smoke. Martell proved that the radon daughter radioactivity is present in trichomes; we have shown that the aerosols which normally carry this radioactivity are present in the tips.

We thank E. A. Martell and S. E. Poet for discussions and for the tobacco samples.

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¹ Martell, E. A., *Nature*, **249**, 215 (1974).

² Schmeltz, I. (ed.), *The chemistry of tobacco and tobacco smoke*, **30**, 54 (Plenum, New York, 1972).

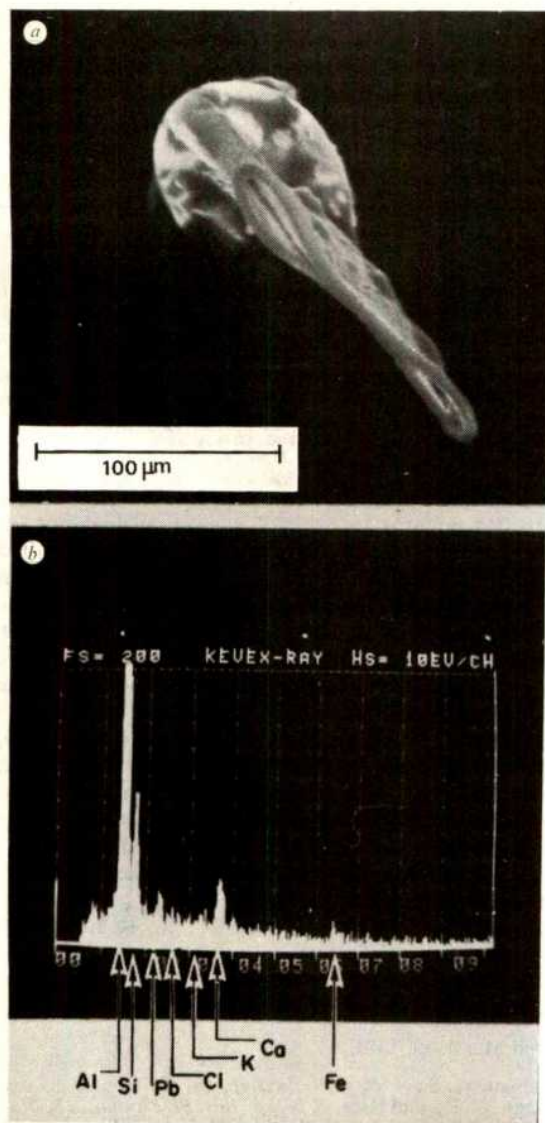
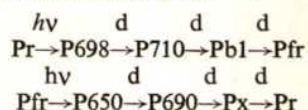


Fig. 1 *a*, Scanning electron micrograph of a cured tobacco trichome and *b*, X-ray spectrum of a trichome tip. Si, Fe, Pb, and Cl are elements commonly found in aerosols; K and Ca are components of the tobacco itself; and Al is a background signal from the sample holder.

Phytochrome intermediates in freeze-dried tissue

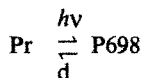
PHYTOCHROME is a plant photomorphogenetic pigment existing in two forms, Pr and Pfr, interconvertible by light, which have absorption peaks in the red and far-red regions of the spectrum respectively^{1,2}. Intermediates between Pr and Pfr have been studied by several techniques *in vivo* and *in vitro* including flash photolysis^{3,4}, low temperature spectroscopy⁵⁻⁷ and measurement of the dark (d) reactions of intermediates that accumulate in conditions of pigment cycling⁸⁻¹⁰. The pathways Pr→Pfr and Pfr→Pr have been shown to be different, but, both pathways involve an initial photoreaction of the chromophore, followed by a series of dark reactions^{10,11}:



P698, P710, P650, P690 are intermediates having peak absorbance at 698, 710, 650 and 690 nm respectively; Pb1 and Px are intermediates having relatively weak absorption bands. I now report a technique enabling phytochrome intermediates to be studied *in vivo* at 0° C using a dual wavelength spectrophotometer.

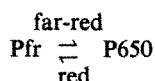
It has been known for some time that complete photoconversion of phytochrome is not possible in dehydrated tissue or samples of phytochrome dried on to gelatin films¹²⁻¹⁵. Measurements have been made, however, either by slowly

scanning a complete spectrum or by restricting measurements to the wavelengths 730 v 800 nm used routinely for the phytochrome assay. Figure 1 shows differential absorbance changes that occur in a sample of freeze-dried dark grown *Pisum* epicotyl tissue. Using wavelengths 735 v 800 nm it is clear that Pr cannot be photoconverted to Pfr on exposure to actinic red light. Observations at 698 v 800 nm, however, demonstrate that high absorbance after actinic red light disappears in darkness. Similar measurements at the peak absorbance of Pr, 665 v 800 nm, show an increase in absorbance in darkness after actinic red light. This is interpreted as the phototransformation of Pr to the intermediate P698 and its reversion to Pr in darkness^{6,10}.



This conclusion is supported by the lack of any absorbance changes after actinic far-red light and the fact that the kinetics of the absorbance decrease at 698 nm and the absorbance increase at 665 nm are identical. It is therefore concluded that in freeze-dried tissue, Pr undergoes isomerisation of the chromophore and that this event is reversed in darkness. This situation is similar to that observed at -70°C *in vivo*¹⁰.

A similar experiment carried out with tissue freeze-dried with phytochrome in the Pfr form shows that a photoreversible reaction occurs on exposure to alternate actinic red and far-red light. A difference spectrum for this reaction is shown in Fig. 2. Compared with the normal difference spectrum of phytochrome photoconversion in hydrated tissue the absorption in the red region of the spectrum is low and is similar to that of the intermediate P650 produced on exposure of Pfr to far-red light at -196°C (refs. 6 and 10). I conclude that this photoreaction of Pfr in freeze-dried tissue is restricted to the phytochrome chromophore.



The behaviour of P650 on exposure to actinic red light is clearly different from Pr which produces the unstable P698 under these conditions of dehydration.

The effect of dehydration on phytochrome seems to be similar to that of low temperature. Low temperatures, however, not only restrict the conversion of Pr to Pfr and *vice versa*, but shift the absorption peaks of Pr and Pfr to longer wavelengths

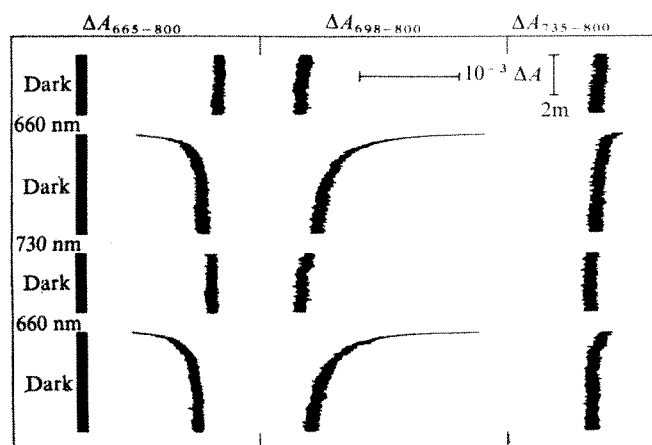


Fig. 1 Recordings of differential changes in absorbance in a sample of freeze-dried dark grown *Pisum* epicotyl tissue (3 mm path length) at 0°C using a Perkin-Elmer 156 dual wavelength spectrophotometer. They demonstrate the production of the intermediate P698 and its reversion to Pr after actinic red light. Sample consisted of 1 cm third internode sections of *Pisum sativum* cv. Alaska seedlings 7 d old grown in darkness at 25°C were frozen in liquid nitrogen, freeze-dried for 36 h and kept over P_2O_5 in a desiccator at -20°C for 5 d. All manipulations were carried out under a dim green safelight. Intensity of actinic red light (660 nm) $1.2 \times 10^3 \mu\text{W cm}^{-2}$, far-red light (730 nm) $1.3 \times 10^3 \mu\text{W cm}^{-2}$.

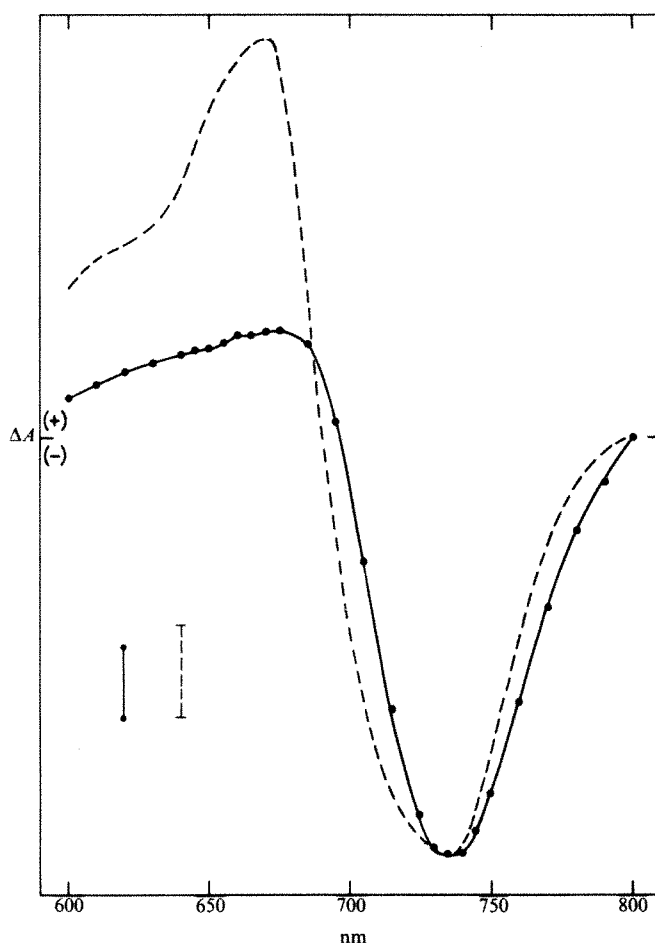


Fig. 2 ●—●, Difference spectrum for the photoreaction that occurs at 0°C in a sample of *Pisum* epicotyl tissue (3 mm path length) freeze-dried after a saturating exposure to red light (●—●, $2 \times 10^{-3} \Delta A$). It demonstrates the photoreaction between the intermediate P650 and Pfr. ---, Difference spectrum for the photoconversion of phytochrome in a freshly collected sample of *Pisum* epicotyl tissue at 0°C (l---l, $1 \times 10^{-2} \Delta A$).

(for example, the peak absorption of Pfr shifts from 732 nm at 0°C to 744 nm at -196°C)⁸. Dehydration does not seem to bring about such large wavelength shifts. This means that the absorption characteristics of intermediates measured in this way can be used for comparison with action spectra of physiological responses in which they have been implicated. The dehydrated tissue containing Pr and Pfr shows photoreactions which are interpreted as being restricted to the chromophore of phytochrome. Controlled rehydration experiments are being conducted to study the reactions of the isomerised chromophore and protein. The use of this method should enable a critical analysis of phytochrome intermediates to be made without the difficulties associated with the use of low temperatures. Furthermore, freeze-dried samples have been demonstrated to be stable even when stored for several days in darkness at room temperature.

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- Hillman, W. S., *A. Rev. Pl. Physiol.*, **18**, 301 (1967).
- Briggs, W. R., and Rice, H. V., *A. Rev. Pl. Physiol.*, **23**, 293 (1972).
- Linschitz, H., Kasche, V., Butler, W. L., and Siegelman, H. W., *J. biol. Chem.*, **241**, 3395 (1966).
- Cross, D. R., Linschitz, H., Kasche, V., and Tenenbaum, J., *Proc. natn. Acad. Sci., U.S.A.*, **61**, 1095 (1968).
- Pratt, L. H., and Butler, W. L., *Photochem. Photobiol.*, **8**, 477 (1968).

- ⁶ Spruit, C. J. P., and Kendrick, R. E., *Photochem. Photobiol.*, **18**, 145 (1973).
- ⁷ Kendrick, R. E., and Spruit, C. J. P., *Photochem. Photobiol.*, **18**, 139 (1973).
- ⁸ Briggs, W. R., and Fork, D. C., *Pl. Physiol. Lancaster*, **44**, 1081 (1969).
- ⁹ Kendrick, R. E., and Spruit, C. J. P., *Nature new Biol.*, **237**, 281 (1972).
- ¹⁰ Kendrick, R. E., and Spruit, C. J. P., *Photochem. Photobiol.*, **18**, 153 (1973).
- ¹¹ Kendrick, R. E., and Spruit, C. J. P., *Pl. Physiol. Lancaster*, **52**, 327 (1973).
- ¹² Kendrick, R. E., Spruit, C. J. P., and Frankland, B., *Planta*, **88**, 293 (1969).
- ¹³ Tobin, E. M., and Briggs, W. R., *Pl. Physiol. Lancaster*, **44**, 148 (1969).
- ¹⁴ Tobin, E. M., Briggs, W. R., and Brown, P. K., *Photochem. Photobiol.*, **18**, 497 (1973).
- ¹⁵ Balance, A. P., thesis, University of Rouen, France, (1973).

Chemical and biological degradation of waste plastics

BRITISH domestic refuse can mainly be divided into ash, cinders, paper, cardboard, glass, metals, plastics and organic matter^{1,2}. The relative amounts of each component have varied over the years: ash and cinders have decreased while paper, cardboard, glass, metals and plastics have increased, because of their widespread use in 'throwaway' packaging.

Cellulosic wastes present few problems during disposal, while glass and metal can be reused or recycled. Plastics, however, are less easily dealt with. They will not rot away in the soil or during composting, and they also present problems on incineration³⁻⁵. Some plastics waste can be recycled at source within industry but at present it would be uneconomical to reuse the plastics in town waste, and even in industry there is little demand for the reuse of plastics⁶.

It is generally agreed that most of the synthetic polymers in use today resist biodegradation but that the plasticisers, used to modify the physical properties of some plastics, are biodegradable⁷⁻⁹. As most of the plastics in town waste are unplasticised⁵, they remain unchanged after all but the most destructive disposal processes, the advent of plastics has called into question the idea of 'microbial infallibility'¹⁰. If a completely new biodegradable plastics were produced it would be more expensive^{11,12} than present plastics because of the limited scale of manufacture. On the other hand, the recent production¹³ of photodegradable plastics does not take into account the fact that the majority of town waste is still used for landfilling, and is therefore not exposed to sunlight.

Town waste at present contains 1.2% by weight (5% by volume) of plastics. About 70% of these plastics are derived from packaging: they comprise (by weight of packaging plastics): 66% polyethylene, 16% polystyrene, 8% polypropylene, 4% polyvinyl chloride, 3% thermosets, and 3% miscellaneous⁵. Our aim was to develop a process for converting waste plastics to protein. We hoped to do this by chemical degradation followed by fermentation, and a literature survey suggested that oxidation¹⁴⁻¹⁶ would be an effective degradation method. Indeed, previous experiments^{9,17} had shown that the oxidation products of polyethylene could support the growth of many thermophilic fungi and actinomycetes. We tried the effect of several oxidising agents on polyethylene, the commonest packaging plastic, and as a result of our experiments chose nitric acid (reaction conditions given in Figs 1-3). We found that the nitric acid could be recovered by distillation and reused. We were able to oxidise four 10 g batches of

polyethylene with one 250 ml batch of 86% v/v nitric acid.

We then took some discarded plastics packages, identified the plastics from their behaviour on burning⁵, and refluxed each of them with 86% v/v HNO₃. The polyethylene articles were completely dissolved after 23 h reflux, while polystyrene, polypropylene and polyvinyl chloride left residues.

We identified some of the oxidation products by gas chromatography. Oxidised polyethylene consisted of a mixture of straight-chain dicarboxylic acids containing from six to 12 carbon atoms; other components were present in trace amounts. Oxidised polystyrene consisted of two major components comprising 95% of the products. Oxidised polypropylene contained 14 components, seven of which were identified as dicarboxylic fatty acids containing 3,4,5,7,8,9 and 12 carbons. Polyvinyl chloride resisted oxidation by nitric acid.

We carried out experiments to determine whether the plastics oxidation products could support microbial growth. Nineteen thermophilic fungal species were incubated, in the conditions described in Fig. 1, with K⁺ salts of oxidised polyethylene as carbon source. (Previous experiments¹⁷ had shown that better growth was obtained on the K⁺ salts than on the dicarboxylic acids.) Little growth was obtained in the mineral salts alone, but several fungi grew well in the media containing the oxidised polyethylene. Three mesophilic fungal species cultured under the same conditions (but at 27° C) produced greater cell weights than did the thermophiles. Dry weights obtained per 50 ml of culture were: 34.2 mg for *Mucor* sp., 29.0 mg for *Penicillium* sp.,

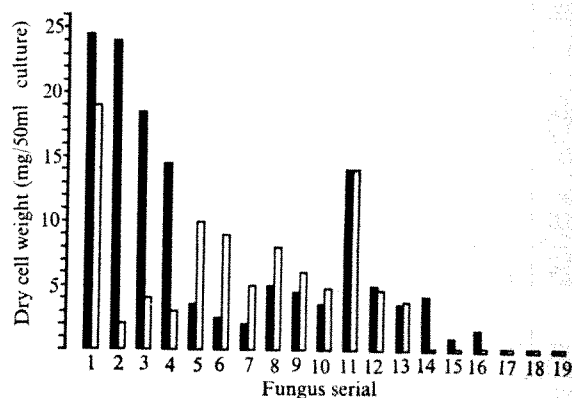


Fig. 1 Growth of thermophilic fungi on oxidised polyethylene. Polyethylene (10 g, ICI, Alkathene) was refluxed with 200 ml 60% v/v HNO₃ for 24 h. The oxidation mixture was cooled, diluted with water, then extracted into diethyl-ether. The ether extracts were bulked, washed with water, evaporated and the resulting dicarboxylic acids converted to their K⁺ salts by addition of 250 ml of 0.2 M KOH.

The K⁺ salts were incorporated at a 1.0% v/v level in 50 ml of sterile Eggins and Pugh¹⁸ mineral salts solution (without added yeast extract or L-asparagine) in 250 ml conical flasks. The medium was adjusted to pH 6.4 and inoculated with 2 mm disks of thermophilic fungi⁹ on agar. Two replicates were made at each temperature (40° C and 48° C), and after incubation, the mycelia were filtered off, washed with boiling distilled water and dried to constant weight. Controls consisted of fungi incubated with mineral salts without added oxidation products.

The figure shows the mean mycelial weight, minus the weight obtained in the controls, of the following fungi: 1, *Malbranchea pulchella* var. *sulphurea*; 2, *Cephalosporium* sp. 3, *Mucor pusillus*; 4, *Sporotrichum thermophile*; 5, *Humicola grisea* var. *thermoidea*; 6, *Humicola lanuginosa*; 7, *Mucor miehei*; 8, *Talaromyces duponti*; 9, *Talaromyces emersonii*; 10, *Thermoascus aurantiacus*; 11, *Aspergillus fumigatus*; 12, *Chaetomium thermophile* var. *coprophile*; 13, *Chaetomium thermophile* var. *dissitum*; 14, *Humicola insolens*; 15, *Stibella thermophila*; 16, *Myriococcum albobasidium*; 17, *Humicola stellata*; 18, *Thielavia thermophila*; 19, *Torula thermophila*. ■, 40° C; □, 48° C.

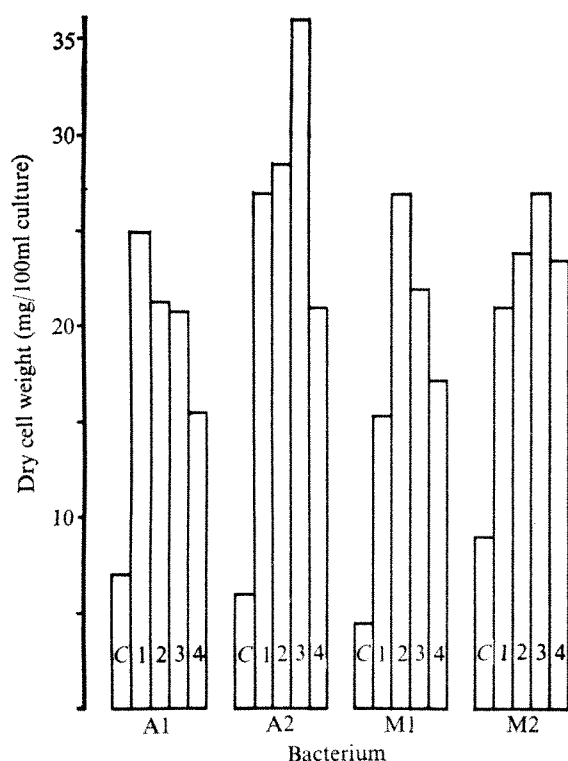


Fig. 2 Growth of mesophilic bacteria on the oxidation products of four plastics packages. Ten grams of each of the four plastics packages, 1, 'Marks and Spencer' white polyethylene bag; 2, 'Squezy' polyethylene detergent bottle; 3, 'Lancashire Hygienic Dairies' polyethylene milk bottle; and 4, 'Ovothrm' polystyrene (non-expanded) egg carton were refluxed for 24 h with 250 ml of 86% v/v HNO_3 . The oxidation mixtures were allowed to cool, then converted to their K^+ salts by adding the following volumes of 60% KOH: 1, 260 ml; 2, 260 ml; 3, 267 ml; 4, 210 ml. The K^+ salts of each oxidised plastic were incorporated at a 4.0% v/v level into a sterile nutrient salts solution after Millipore filtration. The culture medium contained: K_2HPO_4 1.0 g; KCl 0.5 g; $(\text{NH}_4)_2\text{SO}_4$ 0.5 g; MgSO_4 0.2 g; yeast extract 0.5 g; distilled water to 1.0 litre. Flasks containing 100 ml of this solution were inoculated with standard amounts of one of four bacterial cultures, A1, A2, M1, M2, and incubated at 25° C for 6 d with shaking in a Gallenkamp Orbital Incubator. Controls consisted of bacteria incubated with mineral salts without added oxidation products. After incubation, the bacteria were centrifuged off and their dry weights determined. The spent culture medium was examined for the presence of unused oxidation products (Fig. 3). C, Control; 1, polyethylene bag; 2, polyethylene detergent bottle; 3, polyethylene milk bottle; 4, polystyrene egg carton.

and 28.9 mg for *Dicoccum* sp.

Four mesophilic bacterial species (A1 and A2 isolated from the atmosphere; M1 and M2 isolated from soil) were cultured on the K^+ salts of the oxidation products of the plastics articles listed in Fig. 2. In every case the biomass exceeded that obtained in the controls, and examination of the culture medium showed that A1 and A2 removed all the polyethylene oxidation products, M1 removed 31% and M2 removed 21%. The low removal by M1 and M2 was improved by increasing the initial concentration of the oxidation products in the medium to 25% v/v: 7 d incubation then resulted in 82% and 83% removal by M1 and M2 respectively.

To obtain protein for analysis we grew 5 l batches of A1 and A2 on the K^+ salts of the oxidation products of a polyethylene milk bottle. During culture we removed a 100 ml sample each day and determined its content of oxidation products (Fig. 3) and bacterial cells: 95% of the oxidation products were removed, and the yield of bacteria reached a maximum, within 2 d. Average dry weights of 1.5 g per 5 l of each organism were obtained after 2 d growth, and the protein contents of A1 and A2 were 38.3% and 46.4% of their respective dry weights. The proteins (Table 1) contained all the essential amino acids except (for A2) methionine. They are potentially valuable as a food, although feeding trials and toxicity studies would be required to confirm this.

Higher yields of biomass were obtained from the oxidation products of polyethylene than from those of polystyrene or polypropylene. Yields (g dry weight per 100 g of plastic after 3 d growth) of A1, A2, M1 and M2 were: 43, 39, 28 and 23 respectively from polyethylene; 6, 13, 10 and 4 respectively from polystyrene; and 10, 7, 4 and 2 respectively from polypropylene. Biomass yields (g per 100 g of polyethylene) of 176, 120, 80 and 70 respectively were obtained from the first four thermophilic fungi listed in Fig. 1 after 19 d growth at 40° C. As sources of single-cell protein, fungi have three advantages over bacteria: they are easier to harvest, they have a lower nucleic acid content, and they lack a toxic lipopolysaccharide cell wall.

Although we have separated the plastics from other components of rubbish before oxidation, it is not necessary to do so. We have oxidised samples of mixed rubbish of similar composition to that of town waste, and have grown microorganisms on the oxidation products. We are not seriously proposing the oxidation of mixed town waste, but our experiments do indicate that nitric acid oxidation is effective even when the plastics have other materials mixed with them.

Table 1 Amino acid composition of protein obtained from bacteria A1 and A2 grown on polyethylene oxidation products—comparison with composition of other proteins

Protein	Essential amino acids (g/100 g protein)										Nonessential amino acids (g/100 g protein)						
	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val		Ala	Arg	Asp	Cys	Glu	Gly	Pro
Organism A1	2.1	5.6	9.5	6.7	0.4	3.6	5.5	4.0	7.7		11.7	5.6	9.3	—	10.3	9.3	4.9
Organism A2	1.9	6.3	9.1	6.5	—	3.9	5.5	4.0	8.6		12.8	4.9	9.5	—	10.5	9.5	5.5
Beef ²³	3.2	5.1	7.8	8.2	2.4	4.2	4.5	1.3	5.3		6.2	6.6	9.1	1.3	15.4	4.5	4.2
Cows milk ²³	2.7	6.2	9.9	7.8	2.4	5.1	4.6	1.4	7.0		3.7	3.7	8.2	0.8	22.2	1.9	9.8
FAO/WHO (ref. 23)	—	4.3	4.9	4.3	2.3	2.9	2.8	1.4	4.3		—	—	—	2.0	—	—	—

Protein was extracted from freeze-dried cells as follows^{20,21}. Approximately 100 mg of cells were weighed into a plastic 100 ml centrifuge tube, and 40 ml of 5.0% v/v trichloroacetic acid added. After standing for 30 min at 5° C the cells were centrifuged (all centrifugations carried out at 10,000 r.p.m. for 10 min in a Martin Christ High-Speed refrigerated centrifuge, model Zeta), and the supernatant discarded. The residue (R1) was incubated for 30 min at 40–50° C with 60 ml of 75% v/v ethanol/water, centrifuged, and the supernatant discarded. The residue (R2) was incubated for 15 min at 40–50° C with a mixture of 20 ml 75% v/v ethanol/water and 20 ml diethylether, centrifuged, and the supernatant discarded. The residue (R3) was incubated for 30 min at 100° C with 40 ml of 5% v/v trichloroacetic acid, centrifuged, and the supernatant once more discarded. The resulting residue (R4) was washed twice at room temperature, first with 40 ml of ethanol which had been acidified with sufficient HCl to give a final concentration of 5 m mol l⁻¹ then with 40 ml of diethylether. Each washing was removed by centrifugation. The final residue was dried in air, weighed, and calculated as a percentage of the dry weights of bacterial cultures A1 or A2. Amino acids were analysed using a JEOL JLC-6AH Aminoacid Analyser after hydrolysing 2.0 mg of each protein in 3.0 ml of 6.0 M HCl for 18 h at 105° C, in a sealed tube. Tryptophan (destroyed by acid hydrolysis) was determined by dissolving unhydrolysed protein in sufficient 0.1 M NaOH to give an extinction of 0.2–0.5 at 250 nm, then determining the absorption at four wavelengths²².

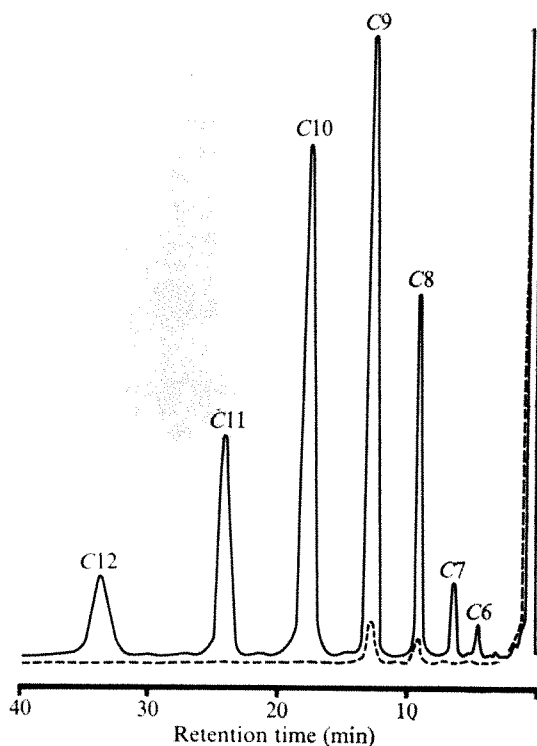


Fig. 3 Utilisation of polyethylene oxidation products by mesophilic bacterium A1. Four polyethylene milk bottles (44.5 g) were refluxed for 48 h with 890 ml 60% v/v HNO_3 , allowed to cool, and converted to their K^+ salts by addition of 60% KOH to give a final volume of 2.5 l. Two hundred millilitres of this solution were sterilised by Millipore filtration and added to 5 l of nutrient salts (Fig. 2) in a Biotec FL 110 fermenter (autoclaved at 15 lb in^{-2} for 20 min). The fermenter was inoculated with 2.0 ml of a 24 h broth culture of bacterium A1, and incubated with vigorous aeration for up to 6 d at 25° C. Each day a 100 ml sample was removed aseptically, centrifuged, and the bacterial cells dried and weighed. The supernatant was examined for the presence of unused polyethylene oxidation products as follows: it was acidified with concentrated HCl to pH 2.0, extracted with 2 × 100 ml diethylether, the ether extracts bulked, dried over anhydrous Na_2SO_4 , filtered, and evaporated to dryness. The dry oxidation products were converted to their dimethyl esters¹⁹ (for gas chromatography) by boiling for 3 min with 5.0 ml of 14% boron trifluoride-methanol complex (British Drug Houses). The reaction was stopped by addition of 20 ml water, and the esters extracted into 2 × 50 ml diethylether and evaporated to dryness. The esters were dissolved in 0.5 ml chloroform, and 1.0 μl samples injected on to the gas chromatograph. Gas chromatography was carried out on a Perkin Elmer F11 (Mark 2) dual column gas chromatograph equipped with dual flame ionisation detectors. The instrument was fitted with glass columns, 6 feet long and 3 mm internal diameter, packed with diethylene glycol succinate, 20% w/w on Chromosorb W, HMDS, 80–100 mesh. Chromatography was carried out isothermally at 165° C, with an injection port temperature of 270° C and a carrier gas (nitrogen) flow rate of 20 ml min^{-1} . Gas pressures used were: nitrogen, 40 lb in^{-2} (270 kN m^{-2}); hydrogen 20 lb in^{-2} (135 kN m^{-2}); air 30 lb in^{-2} (202 kN m^{-2}). Peak assignments were made by comparing their retention times with those of authentic dimethyl ester standards: dimethyl malonate and dimethyl sebacate (Sigma, London), dimethyl succinate, dimethyl glutarate and dimethyl suberate (Ralph N. Emanuel, Limited). —, 0 h; ---, 48 h.

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- ¹ Tinker, J., *New Scientist*, **54**, 389–390 (1972).
- ² Patterson, W., and Searle, G., *Packaging in Britain: a policy for containment* (Friends of the Earth, Ltd, 1973).
- ³ Rolfe, T. J. K., *Mon. Bull. Br. Coal Util. Res. Ass.*, **33**, (2), 28–31 (1969).
- ⁴ Cheater, G., *Publ. Cleans.*, **60**, (4), 187–199 (1970).
- ⁵ Staudinger, J. J. P., *Disposal of plastics waste and litter*, Monograph No. 35 (Society of Chemical Industry, 1970).
- ⁶ Mack, W., *First Nat. Conf. Composting—Waste Recycling*, 28–30 (Rodale Press, 1971).
- ⁷ Eggins, H. O. W., and Mills, J., *United Nations Industrial Development Organisation*, publication No. 1D/WG.96/4, 1–40 (1971).
- ⁸ Mills, J., Barnes, T. G., and Eggins, H. O. W., *Int. Biodeterior. Bull.*, **7**, 105–108 (1971).
- ⁹ Mills, J., *thesis*, University of Aston in Birmingham (1973).
- ¹⁰ Hughes, D. E., *Ind. Recovery* (1969).
- ¹¹ Eggins, H. O. W., Mills, J., Holt, A., and Scott, G., *Microbial Aspects of Pollution*, 269–279 (Academic Press, 1971).
- ¹² Lewin, R., *New Scientist*, **49**, 440–441 (1971).
- ¹³ Scott, G., *New Scientist*, **47**, 293 (1970); *Chem. Br.*, **9**, 267–272 (1973).
- ¹⁴ Whittaker, D., and Forsyth, J. S. A., *Br. Patent*, 581,279 (1946).
- ¹⁵ Beachell, H. C., and Nemphos, S. P., *J. polym. Sci.*, **21**, 113–124 (1956).
- ¹⁶ Alter, H., *Ind. Engng. Chem.*, **52**, 121–124 (1960).
- ¹⁷ Mills, J., and Eggins, H. O. W., *Int. Biodeterior. Bull.*, **6**, 13–17 (1970).
- ¹⁸ Eggins, H. O. W., and Pugh, G. J. F., *Nature*, **193**, 94–95 (1962).
- ¹⁹ Metcalfe, L. D., and Schmitz, A. A., *Analyt. Chem.*, **33**, 363–364 (1964).
- ²⁰ Khosrovi, B., *thesis* Univ. Manchester Inst. Science and Technology (1969).
- ²¹ Roberts, R. B., Cowie, D. B., Abelson, P. H., Bolton, E. T., and Britten, R. J., *Studies of biosynthesis of Escherichia coli*, 13–20 (Carnegie Institution, Washington DC, 1955).
- ²² Goodwin, T. W., and Morton, R. A., *Biochem. J.*, **40**, 628–640 (1946).
- ²³ *Documenta Geigy—Scientific Tables* (edit. by Diem, K., and Lentner, C.) seventh ed., 516 (J. R. Geigy, S.A., Basle, Switzerland, 1970).

Haidinger's brushes and predominant orientation of collagen in corneal stroma

WHEN a source of bluish light, such as the sky, is viewed through polaroid glass or other polarising material, a characteristic figure known as Haidinger's brushes is fleetingly seen at the fixation point by most observers. The 'brushes' are shaped like an hourglass, yellow and darker than the surround, and are orientated with their long axis at right angles to the transmission plane of the polaroid. On either side of the brushes are light blue areas which are brighter than the surround: by some observers they are seen more clearly than the brushes themselves. If the polaroid is rotated, the brushes rotate with it in the same direction, and this manoeuvre prevents the figure from fading.

It is generally agreed that Haidinger's brushes result from the absorption of blue light by the pigment of the macula lutea^{1–3}. If the macular pigment, which absorbs maximally in the wavelength range 430–490 nm (refs 2, 4) is dichroic radially, then linearly polarised light will be absorbed about the diameter of the macula which lies at right angles to its plane of polarisation, producing a yellow region of diminished brightness with intervening blue areas occurring as a contrast effect. Nevertheless, Shurcliff⁵ argued on the basis of the appearance of the brushes with circularly polarised light that the accepted explanation of their causation might be wrong. According to Shurcliff, right-handed, circularly polarised light produces an oblique upward-to-the-right orientation of the brushes in each eye and left-

handed light produces an upward-to-the-left obliquity irrespective of the orientation of the circular polariser. Brindley³ has suggested that the effect of circularly polarised light could be explained if any of the optical media of the eye such as the cornea were birefringent with axes vertical and horizontal.

I have repeated Shurcliff's observations using quarter wave plate compensators as a source of circularly polarised light, but have been unable to confirm his findings either in my own eyes or in those of any subject whom I have so far examined. I have, however, found that a quarter wave plate for yellow light ($\lambda = 575$ nm, retardation checked by the method of de Sénarmont and with a Babinet compensator) consistently produces in persons, including myself, who are able to see Haidinger's brushes clearly, a reversal of the figure so that its long axis lies at right angles to its previous direction. This reversal is most evident when the transmission plane of the polaroid is vertical or horizontal, and the $\frac{1}{4}\lambda$ plate is interposed between the polaroid and either eye with its slow direction in the upward-and-outward diagonal. When the $\frac{1}{4}\lambda$ plate is inserted accurately in the upward-and-inward diagonal the figure disappears. A $\frac{1}{2}\lambda$ plate, on the other hand, produces reversal in the upward-and-inward diagonal and disappearance in the upward-and-outward diagonal.

These findings are explained if the collagen in the corneal stroma is predominantly orientated in the upward-and-outward diagonal—a view which agrees with earlier work^{2,6} on birefringence in the visual pathway. Collagen is positively birefringent with its optical axis lengthwise, so that its slow direction is along the length of the fibre. It is the only substance in the optical path that is sufficiently birefringent to influence the orientation of the figure significantly. Reversal will occur when the combined retardation of collagen and wave plate is near to half the wavelength of blue light, while disappearance will result from the circular or near circular polarisation occurring when the combined retardation is approximately a quarter or three quarters the wavelength of blue light.

To estimate the actual retardation produced by the corneal collagen in the upward-and-outward diagonal in my own eyes, I observed the effects of introducing into the visual path one or more compensators made from thin commercial polythene with a retardation of $\frac{1}{12}\lambda$. With the slow direction of the compensator in the upward-and-inward diagonal, that is, in the subtraction position with respect to the corneal collagen, the definition of the figure was enhanced by a retardation of $\frac{1}{12}\lambda$ and returned to normal with a retardation of $\frac{1}{6}\lambda$. These findings are compatible with a retardation due to the collagen of approximately $\frac{1}{12}\lambda$ (48 nm). A $\frac{1}{12}\lambda$ compensator in the upward-and-outward diagonal should, then, give the same combined retardation ($\frac{1}{6}\lambda$) as a $\frac{1}{4}\lambda$ compensator in the upward-and-inward diagonal, and so cause the figure to disappear.

This was found to be the case. It is now possible to explain more fully the results obtained with $\frac{1}{4}\lambda$ and $\frac{1}{2}\lambda$ plates. A $\frac{1}{4}\lambda$ plate in the addition position produces a combined retardation of $\frac{1}{2}\lambda$ which is approximately $\frac{2}{5} \times$ the wavelength of blue light giving reversal of the figure, and in the subtraction position a combined retardation of $\frac{1}{6}\lambda$, that is, $\frac{1}{5} \times$ the wavelength of blue light causing disappearance. A $\frac{1}{2}\lambda$ plate produces in the subtraction position a combined retardation ($\frac{5}{12}\lambda$) equal to $\frac{1}{2} \times$ the wavelength of blue light giving reversal, and in the addition position a combined retardation ($\frac{7}{12}\lambda$) equal to $\frac{3}{4} \times$ the wavelength of blue light causing disappearance.

A class experiment was conducted to determine the prevalence of a preferential orientation of the corneal collagen. One hundred students were asked to visualise Haidinger's brushes using polaroid and to report the effect on their orientation of a $\frac{1}{4}\lambda$ plate made from three layers of polythene. Eighty-three students were able to see the brushes, and of these 83% (69 students) obtained reversal with each eye when the slow direction of the $\frac{1}{4}\lambda$ plate was in the upward-and-outward diagonal and disappearance with the plate in the opposite diagonal. Because the subjects were not used to making such observations, these results may be regarded as confirmation of a

predominant upward-and-outward direction for the collagen bundles in the cornea. Recent authors⁷⁻⁹ have stated that the corneal collagen is randomly arranged, although Kokott's¹⁰ reconstruction of the superficial layer of the stroma shows a mainly oblique direction with an apparent preponderance in the upward-and-outward diagonal. It may be significant that the predominant orientation shown by the present study is in the line of pull of the tendons of the superior and inferior oblique muscles of the eye.

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- ¹ De Vries, Hl, *et al.*, *Physica*, **19**, 419–432 (1953).
- ² Naylor, E. J., and Stanworth, A. J., *J. Physiol.*, **124**, 543–552 (1954).
- ³ Brindley, G. S., *Physiology of the Retina and Visual Pathway*, (2nd Ed.) (Arnold, London 1970).
- ⁴ Wald, G., *Science*, **101**, 653–658 (1945).
- ⁵ Shurcliff, W. A., *J. opt. Soc. Am.*, **45**, 399 (1955).
- ⁶ Boehm, G., *Acta ophthalm.*, **18**, 109–142 (1940).
- ⁷ Naylor, E. J., *Q. Jl microsc. Sci.*, **94**, 83–88 (1953).
- ⁸ Jakus, M. A., in *The Structure of the Eye* (edit. by Smelser, G. K.) 343. (Academic Press, New York 1961).
- ⁹ Smelser, G. K., and Ozanics, V., in *The Cornea World Congress* (edit. by King, J. H. Jun., and McTigue J. W.). (Butterworths London 1965).
- ¹⁰ Kokott, W., *Albrecht v. Graefes Arch. Ophthalm.*, **138**, 424–485 (1938).

The apparent heaviness of colours

EARLY this century, E. Bullough¹ showed that some combinations of colours, one above the other, are chosen as more 'natural' than other combinations, which tend to look top heavy. Various methods of measuring the apparent weight of colours were subsequently devised: Bullough's preference method, tests in which the weight of coloured blocks was judged either visually or directly by hand^{2,3,4}, and the 'weighing' of half-inch circles of coloured paper at either end of a simulated balance arm with an adjustable fulcrum⁵. There was general agreement that red and blue were the heaviest colours, yellow the lightest. But no statistical evaluation was used in the earlier work; and as the colours were surface-illuminated, the effect of colour was easily confounded with that of brightness. In fact, most investigators considered that brightness was probably a crucial factor. In the present study, an adaptation of Monroe's procedures, the effects of colour and brightness were investigated separately using larger transilluminated stimuli, with brightness carefully controlled. Our results show that the effect is independent of brightness. Coloured circles, equal in subjective brightness, differ considerably in apparent weight, while achromatic stimuli which differ in brightness are not consistently different in weight.

The display as seen by the subjects is shown in Fig. 1. Two circular holes, 10 cm in diameter with 30 cm between centres, were cut in a matt black screen. The holes were covered by ground glass, on to which the stimuli were back-projected by two slide projectors. Between the circles was a horizontal slit along which the subject could move a small luminous pointer to the 'balance point', by turning a knob below the board. The display was positioned vertically in front of the subject in a dark cubicle, so that his eyes were about level with the stimuli and the control knob was within easy reach. Movements of the pointer were recorded on an oscilloscope screen, unseen by the subject.

We tested the effects of colour in the absence of brightness differences, and the effects of brightness in the absence of colour differences. In order to simplify the procedure, each of the test stimuli was individually 'weighed' against a white stimulus of constant brightness. For the colour experiment five colours were used: Red, Orange, Yellow, Green, and Blue (Kodak

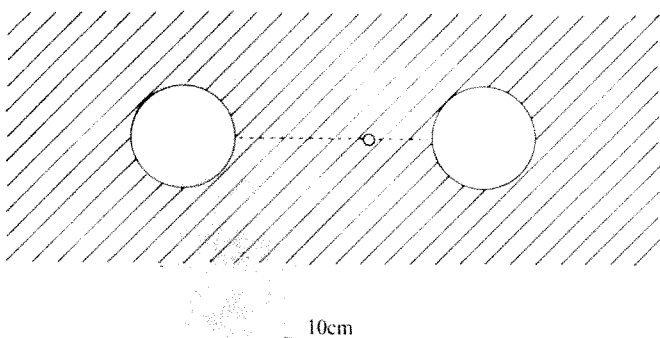


Fig. 1 The display as seen by the subjects.

Wratten filters, Nos 25, 22, 12, 58 and 38A, respectively), all adjusted to be equal in subjective brightness to the standard. For the brightness experiment white stimuli of four different brightness levels were used, covering a 25-fold range in physical intensity.

Each colour was presented eight times and each brightness level four times in the course of a testing session on one subject, giving 56 'weighings'. The brightness stimuli were mixed in amongst the colour stimuli and the whole order was randomised, with the constraint that each stimulus should appear equally often on the left and right of the display. The order was changed for different subjects to eliminate possible order effects. Medians of the judgements made to each stimulus type were used in the comparison.

Ten men and ten women took part in the experiment. All were Cambridge undergraduates, none of whom had any previous knowledge of the phenomenon being studied. Each subject was given the following printed instructions at the start of the session.

"The apparent weight of colours. Pictures are often said to have a centre of gravity, perhaps determined by the way the different colours are arranged. Early this century, those investigating the psychology of aesthetics had the idea that colours have weight. This is an experiment to test that idea.

Imagine the slit joining the two circles to be a rigid bar connecting two heavy illuminated spheres, and supported by the luminous pointer as a fulcrum. By turning the knob, move the pointer along the slit to a position about which the two spheres appear to be exactly balanced in weight. There are no right or wrong answers, so please do not feel that you need to take a long time to make these judgements."

No practice examples were given, though the subjects were encouraged to spend more time over the first few judgements so that they should get the idea. When the subject indicated, for each stimulus pair, that he was satisfied with the position of the pointer, this was recorded, and the next pair presented.

With the coloured stimuli most subjects had little difficulty in making these unusual judgements, although a few said that they did not accept the metaphor of 'weight', and were simply placing the pointer where it looked best. With the stimuli of different brightness the subjects appeared more unsure of what to do, and their judgements were rather less reliable.

To evaluate the results, the position of the pointer was expressed in terms of the displacement from the mid-point towards the test stimulus, positive displacements thus indicating

Table 1 Median displacement of pointer from mid-point towards the test stimulus

Colour	Yellow	Green	Blue	Orange	Red
	0.9	1.9	1.9	2.1	3.8 cm
Brightness*	-0.8 0.2	-0.4 0.4	+0.3 0.2	+0.7 0.3 cm	

*Brightness given in log-foot-lamberts relative to standard

Table 2 Average ranks attributed to each colour

	Red	Blue	Green	Orange	Yellow
Men	0.70	1.65	2.25	2.10	3.30
Women	1.05	1.75	1.65	2.50	3.05
Total	0.87	1.70	1.95	2.30	3.17

0, heaviest; 4, lightest.

increasing 'heaviness' relative to the standard. The medians of the 20 subjects' median judgements for each test stimulus are given in Table 1.

All the colours were regarded as heavier than the standard, with red the heaviest, yellow the lightest and the other three clustered in between. A Friedman two-way analysis of variance by ranks indicates that the effect of colour was highly significant ($P < 0.001$). On a Wilcoxon matched pairs test, yellow comes out as significantly lighter than all the other colours ($P < 0.05$ or better), and red as significantly heavier than green, orange and yellow.

The average ranks (from 0 as the heaviest to 4 as the lightest) attributed to each colour are shown in Table 2. Though the rank ordering of the different colours was generally consistent across subjects, there was considerable variation in the absolute distance to which the pointer was displaced, some subjects tending to stick close to the mid-point, while others used nearly the full length of the slit (the inter-quartile range for red extended from 1.7–5.7 cm). Men and women gave essentially similar results.

The results for brightness showed no significant effects of any kind.

No plausible explanation has yet been offered for why people should see any equivalence between colour and weight, nor can we offer one. Bullough suggested an explanation in terms of landscape associations and aerial perspective; but he himself considered this argument *ad hoc* and unconvincing. Indirect associations, of the kind 'red=important=heavy', seem more likely. Red is commonly regarded as a particularly striking colour; moreover, in tests of colour preferences, red and blue are generally considered the most pleasant colours, yellow the least pleasant. A correlation between saliency or colour preference and apparent weight, however, if it exists, has little explanatory power. The reasons for colour preferences are themselves unclear. Whatever the explanation, the consistency with which people make such peculiar 'synaesthetic' judgements about the affective value of colours is remarkable.

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- ¹ Bullough, E., *Br. J. Psychol.*, **2**, 111–152 (1907).
- ² De Camp, J. E., *J. exp. Psychol.*, **2**, 347–370 (1917).
- ³ Taylor, C., *J. gen. Psychol.*, **4**, 229–246 (1930).
- ⁴ Payne, M. C., *Am. J. Psychol.*, **71**, 725–730 (1958).
- ⁵ Monroe, M., *Am. J. Psychol.*, **26**, 192–206 (1925).

Male sterility induced in barley by photoperiod

THE continuous exposure of barley plants to short photoperiods leads to marked increases in the numbers of primordia and grains per ear^{1–4}. But little is known of the effects of brief exposures to short days and here we describe the distinctive effects of such

treatments given at different stages of ear development, on the fertility of the florets of ears of barley.

Seeds of barley (cultivar 'Proctor') were sown four per 4 inch pot containing John Innes compost No. 2, in a greenhouse in March 1973. After 10 d the resultant seedlings were thinned to give a uniform stand of two plants per pot. Except when given the short day treatments, plants were grown in a 16 h photoperiod comprising 8 h of daylight (0900h–1700h) in the greenhouse and 8 h of incandescent light (1700h–0100h) of an intensity of approximately 400 lx at pot height, in adjacent photoperiod chambers. The experimental layout consisted of eight randomised blocks, with each block taking up a single trolley. The trolley positions within both the greenhouse and photoperiod chambers were randomised daily to minimise block differences.

Immediately after the appearance of double ridges on the main shoot apex, the first of four transferences of plants into short days was carried out, 21 d after sowing (treatment A). The other groups of plants were transferred 35 d (treatment B), 42 d (treatment C) and 56 d (treatment D) after sowing. These plants received a photoperiod of 10 h for 14 d, this consisting of 8 h of daylight in the greenhouse (0900h–1700h) and 2 h of incandescent light (1700h–1900h) in photoperiod chambers situated next to the ones accommodating the plants receiving long days.

During the treatment period, those plants receiving the short photoperiods were removed from the different blocks and carried on a single trolley. After the 14 d treatment period, the pots were returned to their original positions, within blocks, on the various trolleys. At the beginning of each treatment, eight pots were selected at random and the plants dissected to determine the stage of development of the main shoot apex. These stages were the double ridge stage (treatment A), stamen initial stage (treatment B), awn initial stage (treatment C) and the final stage of ear differentiation with the awns longer than the spike (treatment D). The progress of ear development in the main shoot of the treated and control plants was determined by examining the apices of 16 plants sampled at weekly

intervals; the apices were fixed in 1:3 acetic alcohol and kept for subsequent examination. Sixteen pots were kept in long days throughout to serve as controls. The treated and control plants reached ripe-harvest maturity in a 2 week period at the end of July. At maturity, the numbers of ears per plant and of fertile and infertile florets for the individual ears were recorded. In the latter context the number of florets was counted from the first floret above the collar. Floret fertility is calculated as grain number divided by floret number and expressed as a percentage.

The short day treatments did not affect ear numbers per plant (Table 1). When imposed during the later stages of ear development, however, they markedly reduced the numbers of grains set per plant (Treatments C, D, Fig. 1a). The reductions were due to a significant depression of floret fertility rather than to any decrease in the numbers of florets produced per plant (Figs. 1b and c).

The reductions in floret fertility shown by treatments C and D were not distributed evenly between the various ears of the plant. Thus, when short days were imposed from the awn-initial stage (treatment C) the ear of the main shoot became almost wholly sterile, the ears in the axils of leaves 1 and 2 were unaffected and the ear in the axil of leaf 3 showed a small increase in fertility. In contrast, treatment D, which started when the awns of the main shoot apex were longer than the spike, caused almost total sterility of the axillary ears with the main shoot ear being hardly affected (Fig. 2).

Cytogenetic studies of main shoot ears of plants which had received treatment C showed pollen development to be abnormal. Thus 1 week before anthesis the young pollen grains were aggregated together and showed thick walls and irregularities in both size and shape; there was also a high frequency of nuclear aberrations with the occurrence of many bi- and multinucleates and micronuclei. At ear emergence, the anther contents had completely aborted and anthers were small and shrivelled. It seems likely, therefore, that male sterility was a major cause of floret sterility in the main shoot ears given treatment C.

Dissections of ovaries did not bring to light any obvious effect of treatment C on the development of the embryo sac. But this could not be regarded as conclusive proof that female development was unaffected as dissecting the ovary often led to fragmentation of the embryo sac into its constituent cells so as to make accurate comparisons difficult. Further evidence was therefore obtained from another experiment in which the florets on one side of the main shoot ears of plants which had received treatment C were cross pollinated with pollen from plants grown throughout in long days. The florets on the other side of these ears were not cross pollinated, so that any grain which set would be the result of self pollination. Grain numbers on the self pollinated side of the ear were very low, whilst those

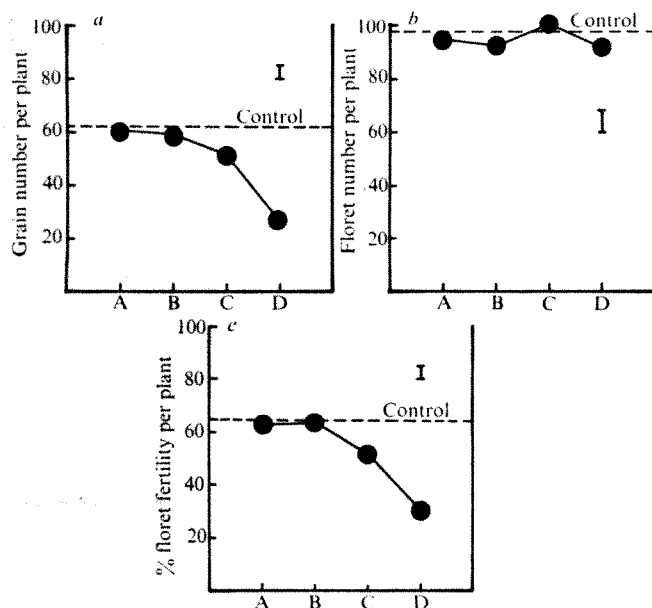


Fig. 1 The effects of a 2-week exposure to short days imposed at the double ridge stage, A; stamen-initial stage, B; awn-initial stage, C; and the later stages of awn differentiation, D on a, grain number per plant, b, floret number per plant and c, % floret fertility per plant. (Bar, least significant difference at $P < 0.05$.)

Table 1 Effect of photoperiod at different stages of ear development

Character	Control	A	B	C	D	L.S.D. $P < 0.05$
Ear number per plant	3.87	3.72	3.44	3.87	3.59	0.35
Total number of grains per plant	62.87	60.25	58.91	51.78	27.63	5.63
Total number of florets per plant	97.62	95.69	92.41	101.37	91.84	8.07
% fertile florets per plant	65	63	64	51	30	5
% floret fertility, main shoot	90	76	70	15	67	9
% floret fertility, leaf 1 tiller	59	67	30	53	14	16
% floret fertility, leaf 2 tiller	68	63	70	66	5	8
% floret fertility, leaf 3 tiller	55	61	77	68	6	9

A 2-week exposure to short days (10 h) was imposed at the double ridge stage, A; stamen-initial stage, B; awn-initial stage, C; and the later stages of awn differentiation, D.

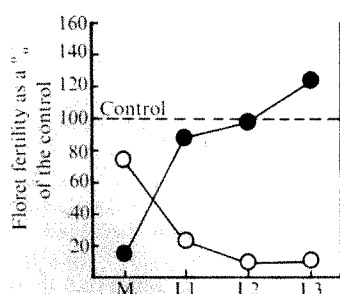


Fig. 2 The effects of a 2-week exposure to short days imposed at the awn-initial stage C (●—●) and the later stages of awn differentiation D (○—○) on the fertility of the florets of main shoot ears M, and the leaf 1, 2 and 3 tiller ears (L1, L2, L3). Fertility in all ears is calculated as (Grain No.)/(Floret No.) \times 100 and is plotted as a percentage of the control value.

on the cross pollinated side were considerably higher (Table 2). It seems, therefore, that the short day treatment had not affected the development of the embryo sac and that its effect on floret fertility was exerted by the induction of male sterility.

It is intriguing to consider why main shoot ears were affected by treatment C but not treatment D. It seems unlikely that the plants became unreceptive to the short day stimuli during treatment D, as the fertility of the florets of the lateral ears was markedly reduced by such treatment. It would seem more likely that pollen development is sensitive to short days only at certain stages and that in the main shoot ears these coincided with the exposure to short days in treatment C but not treatment D. The actual stages of pollen development covered by treatment C range from mitosis of the pollen mother cells at the start of treatment to the first prophase of meiosis at the end of treatment. As male and female meiosis have been shown to be synchronous in barley⁵, the embryo sac mother cell would have been at a comparable stage at the end of the treatment period.

Although any stage in the span of development covered by treatment C could be the critical process which is sensitive to short days, it may prove to be the stage of premeiotic interphase which is disrupted. This phase, in which the mitotic pollen mother cells are synchronised before entering meiosis, only occurs in male gamete formation, and it has been shown to be susceptible to changes in other environmental factors which induce varying degrees of floret sterility⁶⁻⁸.

No dissections of lateral ears were carried out but it was observed that the general development of these was later than that of main shoot ears, and it is likely, therefore, that the development of pollen in their florets lagged behind that in the main shoot ears. If this occurred, then the reduction in floret fertility in the lateral ears induced by treatment D, but not treatment C, would be consistent with the hypothesis that there is a stage during which pollen development is sensitive to short days and that this is the same for all ears.

The drastic reduction in floret fertility caused by exposure to short days is a new phenomenon. The mechanism of this effect is not clear, but phytohormones may be involved. There is evidence that levels of phytohormones in plant tissues change in

response to day length^{9,10}, and that such substances can affect floret fertility. Thus application of ethrel decreases floret fertility in both barley¹¹ and wheat¹², whereas gibberellins increase fertility in male sterile lines of tomato¹³ and barley¹⁴.

The results described here imply that the use of physiologically induced male sterility may well be feasible in the controlled hybridisation of barley varieties, particularly where a rapid assessment of heterotic combinations is needed in the choosing of parents for the production of hybrid barley varieties.

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- Nicholls, P. B., and May, L. H., *Aust. J. biol. Sci.*, **16**, 561-571 (1963).
- Aspinall, D., and Paleg, L. G., *Bot. Gaz.*, **124**, 429-437 (1963).
- Kirby, E. J. M., *Fld Crop Abstr.*, **22**, 1-7 (1969).
- Tingle, J. N., Faris, D. G., and Ormrod, D. P., *Crop Sci.*, **10**, 26-28 (1970).
- Bennett, M. D., Finch, R. A., Smith, J. B., and Rao, M. K., *Proc. R. Soc. Lond.*, **B183**, 301-319 (1973).
- Bennett, M. D., and Hughes, W. G., *Nature*, **240**, 566-568 (1972).
- Bayliss, M. W., and Riley, R., *Genet. Res.*, **20**, 201-212 (1972).
- Dover, G. A., *J. Cell Biol.*, **11**, 699-771 (1972).
- El-Antably, H. M. M., Wareing, P. F., in *Cellular and Molecular aspects of Floral Induction* (edit. by Bernier G.), 285-303 (Longman, London, 1970).
- Evans, L. T., *Aust. J. biol. Sci.*, **13**, 429-440 (1960).
- Law, J., and Stoskopf, N. C., *Can. J. Pl. Sci.*, **53**, 765-766 (1973).
- Hughes, W. G., Bennett, M. D., Bodden, J. J., and Galanopoulou, S., *Ann. appl. Biol.*, **76**, 243-252 (1974).
- Sawney, V. K., and Greyson, R. I., *Can. J. Bot.*, **51**, 2473-2479 (1973).
- Kasembe, J. N. R., *Nature*, **215**, 668 (1967).

Fluidisation as a feeding mechanism in beach flies

WE have seen groups of small (4-5 mm long) grey flies belonging to the species *Lipochaeta slossonae* Coquillett, 1896, standing, shaking and apparently feeding, or flying for short distances on stretches of wet sandy beach in La Jolla, California and San Felipe, Mexico. When undisturbed, they walked sideways or stood and shook their bodies, diagonally forward and downward, and backwards and upwards, at an estimated frequency of 5 s⁻¹. We guessed that they were fluidising the wet sand under their feet and thereby loosening some of the interstitial microflora which could then be sucked up as a kind of soup. This was confirmed by laboratory examination of the guts of several specimens, which contained the remains of large numbers of cells of dinoflagellates and diatoms. (The species of dinoflagellates were unfortunately unidentifiable since there were no cell wall remains, but they probably belonged to the genus *Amphidinium*, which comprises some of the commonest unarmoured interstitial dinoflagellates on this beach. The diatom species, however, could be readily identified by their silica walls. There were at least 10 genera, all typical of the marine interstitial community, including various species of *Navicula*, *Nitzschia*, *Pinnularia* and *Amphora*.)

The genus *Lipochaeta* has only one known species, *L. slossonae*: it is a poorly known fly, adults having been hitherto recorded only from Florida, Texas and from southern and central California^{1,2}. Its larvae and pupae are apparently still undescribed (B. A. Foote, personal communication). Very little is known about its biology

Table 2 Effects of cross-pollination with pollen from plants grown in long days throughout

	P: Cross-pollinated	Q: Self-pollinated	L.S.D. $P < 0.01$
Grain No. (one side of ear)	4.89	0.86	2.28
% floret fertility (one side of ear)	41.82%	7.26%	19.24%

The recipient plants were exposed to 2 weeks of short days at the awn-initial stage. The florets on one side of the ear (P) were cross pollinated using pollen taken from plants kept entirely in long days, while those on the other side (Q) were self-pollinated. Figures are the mean of 25 ears.

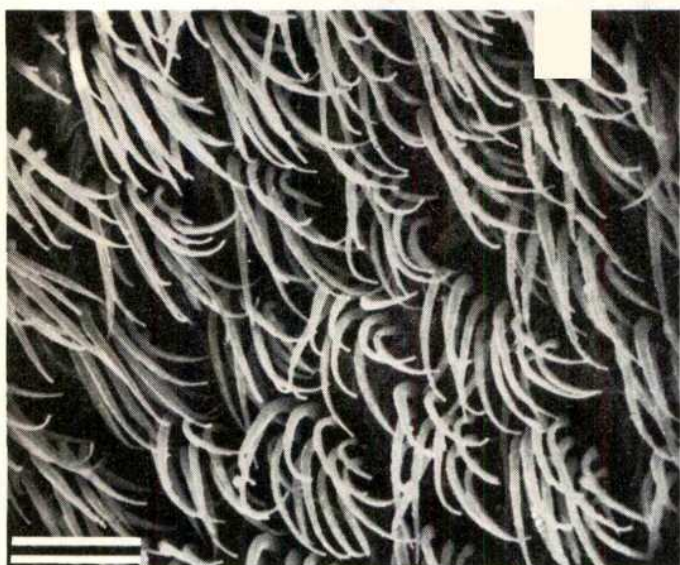


Fig. 1 Stereoscan electron micrograph taken on Cambridge S-4 SEM of portion of surface of clypeus, showing recurved hairs in bundles of 12-15. (Scale bar = 10 μ m.)

apart from the fact that adults are commonly found on moist sand of ocean beaches. They are not strong fliers, but they seem to be more wary and less easily caught than the kelp flies present in the same habitat.

The head, somewhat resembling that of a fish, is flattened dorso-ventrally, with an almost flat ventral clypeus covered by small recurved hairs grouped in bundles of 12-15 (Fig. 1). This is probably an adaptation for preventing wetting of the head while the fly is feeding. The antennae, which are extremely small and lack an arista, are inserted in pits spaced far apart (Fig. 2). The mouth parts are of the normal dipteran type except for the labella, which are modified to form a filtering apparatus: it has thickened ridges approximately 8 μ m apart and 10 μ m long (Fig. 3), leaving pores of a size suitable for the admission of small diatoms and other algae while excluding sand grains. Further feeding adaptations of these otherwise bristle-less flies² may be the long, stout spines along their legs and the strong apical tarsal claws which enable them to stand on wet sand without wetting the velvety hair cover of their legs and body.



Fig. 2 Lateral view of head of *Lipochaeta slossonae*, showing flattened ventral clypeus, eye, and small antenna in pit. (Scale bar = 500 μ m; stereoscan electron micrograph taken on Cambridge S-4 SEM.)

Many adult ephydriids, like their larvae, are known to feed on microscopic algae³. Analyses of algae found in the guts of a considerable number of species are presented by Deonier⁴, who also reviewed several earlier references on the feeding habits and diets of adult ephydriid flies. A significant observation on the feeding behaviour of adult *Scatella subguttata* (Ephydriidae) is that of Brauns⁵, who refers to pressure by the anterior, boat-shaped portion of the proboscis as being somehow involved in the "licking" of food organisms from sand grains. But the phenomenon of sand fluidisation, which we observed in the feeding behaviour of *Lipochaeta*, does not seem to have been reported elsewhere. Deonier⁶ merely reported that the adults of certain species of *Hydrellia* (Ephydriidae) "exhibit peculiar behaviour while feeding, e.g., *H. biloxiae* rhythmically pushes its body up and down", without offering further explanation of this unusual behaviour. Ocypodid crabs are known to feed on interstitial microflora by rolling wet sand into pellets with their maxillipeds and then sucking out the suspension of fine organic detritus, including the microalgae⁷. Apparently some flies can exploit the same dietary niche.

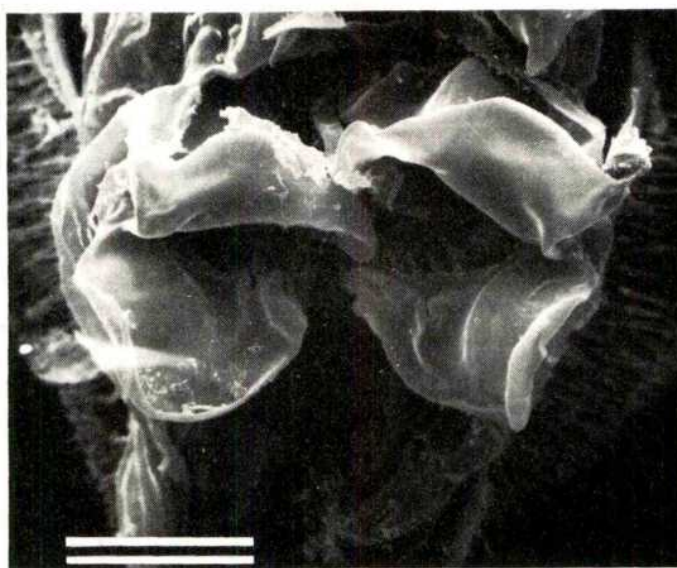


Fig. 3 Ventral view of portion of proboscis showing 'pores' on labella and small internal teeth. (Scale bar = 50 μ m; stereoscan electron micrograph taken on Cambridge S-4 SEM.)

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¹ Cole, F. R., *The Flies of Western North America*, 392 and 404 (University of California Press, 1969).

² Usinger, R. L., *Aquatic Insects of California*, 470 (University of California Press, 1971).

³ Dahl, R. G., *Opuscula Entomol. Suppl.* 25, 1-225 (1959).

⁴ Deonier, D. L., *Ohio J. Sci.*, 72 (1), 22-29 (1972).

⁵ Brauns, A., *Zool. Anzeiger*, 126, 273-285 (1939).

⁶ Deonier, D. L., *Smithsonian Contr. Zool.*, 68, 21 (1971).

⁷ Silas, E. G., and Sankarankutty, C., *Proc. Symp. Crustacea India*, ser. 2, part 3, 1008-1025 (1967).

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By **L. V. Skurkovich**, *Meditsina Publishing House, Moscow*.

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June 1974

154 pages

£7.50

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THE SEA: Volume 5: Marine Chemistry (The Sea)

Edited by **Edward D. Goldberg**, of *Scripps Institution of Oceanography*.

Based on the work of Lars Gunnar Sillén whose quest to understand the chemical reactions that determine the composition of seawaters laid a new foundation for investigation in marine chemistry. Provides both an assessment of knowledge relative to the problems proposed by Sillén and studies of unexplored realms of marine chemistry.

June 1974

912 pages

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BIOLOGICAL CLOCKS IN MARINE ORGANISMS: The Control of Physiological and Behavioral Tidal Rhythms

By **John D. Palmer**, *New York University*.

A survey of bioclock-controlled tidal rhythms—those organismic rhythms that persist over periods of approximately 12.4 h in constant conditions in the laboratory. First provides familiarity with the basic subject matter, then describes experiments with tidal rhythms and the properties derived from these laboratory manipulations. Finally, uses some of these properties—and those of circadian rhythms—to draw a rough sketch of the clockworks that underly persistent rhythms.

June 1974

186 pages

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NUTRITION AND FETAL DEVELOPMENT

Edited by **Myron Winick**, *Columbia University College of Physicians and Surgeons*.

Nine original articles focus on the most recent research conducted on prenatal malnutrition in both human and animal populations. Considers how maternal malnutrition affects foetal brain development and subsequent behaviour in animals, tells how in humans, maternal undernutrition affects the cellular growth of the placenta and reproduces biochemical signs of malnutrition in foetal serum and white cells. Given data show that many of these changes can be reversed by supplementing the diet of pregnant women. (*Current concepts in Nutrition, Volume 2*).

June 1974

200 pages

£7.70

THE THEORY OF RATE PROCESSES IN BIOLOGY AND MEDICINE

By **Frank H. Johnson**, *Princeton University*, **Henry Eyring**, *University of Utah*, and **Betsy Jones Stover**, *Universities of North Carolina and Utah*.

Outlines the conceptual basis of modern reaction-rate theory—the theory of absolute reaction rates—and applies the net results of this theory to representative rate processes in biology and medicine in an effort to achieve a better understanding of the phenomena involved.

June 1974

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Proof of natural selection

The Triumph of the Darwinian Method. By M. T. Ghiselin. Pp. 287. (University of California: Berkeley, Los Angeles and London, May 1973.) £1.50 paper.

IN 1909, the fiftieth anniversary of the publication of *On the Origin of Species* and the centenary of Charles Darwin's birth, two large volumes of essays on Darwin's work appeared. *Darwin and Modern Science*, edited by A. C. Seward (Cambridge University Press) and *Fifty Years of Darwinism* (Holt, New York) contained essays from many prominent scientists. Two similar volumes, *A Century of Darwin*, edited by S. A. Barnett (Heinemann, London) and *Darwin's Biological Work*, edited by P. R. Bell (Cambridge University Press) appeared for the centenary of the *Origin* in 1959. Taken together, these four volumes represented until now the most comprehensive evaluation of Darwin's scientific interests and their later influence. The problem is that each contributor to these books considered only one aspect of Darwin's thought. Quite often, contributors disagreed vigorously among themselves about Darwin's methods and significance. The result was a very disjointed appreciation of Darwin's work.

Now Michael Ghiselin has written a much needed and very different kind of book. He read Darwin carefully with the aim of elucidating the unity of conception in his major writings. Dividing Darwin's work into the themes of natural history, geology, zoology, evolution, botany, and psychology, Ghiselin convincingly demonstrates that Darwin approached each with the same general methodology. He also shows clearly how all of Darwin's diverse biological investigations, after he turned from geology, were related to his conception of evolution by natural selection. Thus the monographs on barnacles, the books on worms, climbing plants, and psychology, are seen as parts of a single conception. Ghiselin's argument, though stretched thin in some places, is important and compelling. Indeed, the book might best be titled "The Unity of Darwin's Thought".

In other ways, Ghiselin's book is less successful. His title is drawn from his answer to the question: why did Darwin attain such a remarkable success? Why did he, and not others,

write the classic works in evolutionary biology? After discounting intellect, zeal, labour, and theft from predecessors as the crucial factors, Ghiselin answers: "Darwin's success may readily be explained by a very simple hypothesis which seems not to have occurred to his critics: he thought. He reasoned systematically, imaginatively, and rigorously, and he criticized his own ideas" (page 232). In other words, the key to Darwin's accomplishments was that he "applied, rigorously and consistently, the modern, hypothetico-deductive scientific method" (page 4).

This thesis is deficient. A great number of intelligent biologists have used the same general method and have not enjoyed Darwin's success. In the final pages Ghiselin tempers his thesis by admitting that "Darwin's strength of character, his self esteem, and his ambition are in no small measure responsible for his success" (page 242); also implicated were Darwin's courage, his "remarkable talent for judging the appropriate", his ideals, and his value system. Another perhaps important reason why Darwin had greater success than his competitors is that he had the immense advantage of nearly twenty years of reflection and gathering of evidence when it came to the application of the central ideas of the *Origin* to diverse areas of biology. Even then he had no monopoly on the idea of natural selection. For example, H. W. Bates, A. R. Wallace, and Fritz Müller developed mimicry theory, which has been a mainstay of the theory of natural selection from the early 1860s to the present. Clearly the keys to Darwin's success are more complicated than Ghiselin suggests in his title and major thesis.

The picture Ghiselin paints is this: Darwin, exemplar of scientific virtue, almost singlehandedly develops the theory of evolution by natural selection and applies it to many areas of biology. Because of stupidity, belief in design or final causes, religious prejudices, and general lack of appreciation of his methods and results, Darwin's contemporaries and successors constantly misinterpret and attack the theory of natural selection. "To some extent", admits Ghiselin, "these contrary views were justified by the difficulties of the scientific problem"

(page 7). Only with R. A. Fisher's *The Genetical Theory of Natural Selection* (Oxford University Press) in 1930 is the great part of this misinterpretation cleared away, leading for the first time to a proper appreciation of Darwin's ideas. Ghiselin, himself, is finishing the mop-up of Darwin's critics begun by Fisher in 1930, and continued in his 1954 essay, "Retrospect of the Criticisms of the Theory of Natural Selection" in *Evolution as a process*, edited by Huxley, Hardy and Ford (Allen and Unwin, London). But this picture of the hiatus of proper understanding in the time between Darwin and Fisher is seriously misleading. Who can believe that all the great biologists of the late nineteenth and early twentieth centuries were too limited to understand Darwin's theories? August Weismann, Hugo de Vries, William Bateson, Wilhelm Johannsen, Jacques Loeb, Thomas Hunt Morgan, even Francis Darwin, to mention only a few: how could they have failed to appreciate Darwin's theory of natural selection when any smart college student can succeed today? Something is wrong with Ghiselin's analysis of Darwin and his critics.

Here is the problem. Ghiselin believes that the theory of natural selection is Darwin's most important contribution to knowledge. I agree. Ghiselin believes further that Darwin presented adequate verification for natural selection. I disagree, along with Darwin's successors. Noting that Darwin believed he could not see natural selection in action because of its slowness, Ghiselin refutes Darwin's critics of the nineteenth century with this argument:

"The problem is no longer with us, since the production of new species in the laboratory, by processes known to occur in nature, is a mere chore. However, direct observations on the process of speciation were by no means necessary: Darwin answered his critics by maintaining that he could verify his theory indirectly by its implication of a large number of readily ascertained facts. Actually, the attack (upon natural selection) was wholly unfounded and most unphilosophical" (page 62).

To this Ghiselin adds that "we have no 'direct' evidence for the truth of any scientific theory", implying that Darwin's critics needed no more evidence than they got, although Ghiselin himself invokes modern laboratory experiments.

The only examples of natural selection in action Darwin provided in the *Origin* were, as he clearly stated, "imaginary" (page 90 in facsimile of first edition, Harvard University Press, 1964). As even Ghiselin admits, Darwin's theory of pangenesis and his ideas about the origin and maintenance of heritable variability in natural populations were unconvincing; yet natural selection depended upon an exact understanding of this variability. There was ample room for criticism of natural selection. One need only read Thomas Hunt Morgan's 1903 critique of Darwin, *Evolution and Adaptation* (Macmillan, New York) to see why an intelligent biologist could read Darwin carefully and still reject crucial aspects of his idea of natural selection. By reading twentieth century Darwinism back into Darwin, torical proportion his book requires.

Despite these criticisms, this book is the best available analysis of Darwin's works, and it is sure to stimulate substantial further research. In addition, the book contains a wealth of other ideas. Ghiselin analyses aspects of scientific method, philosophy, religion, and history; he even has a comparison of human history with geology and palaeontology. His arguments are consistently bold, clear and provocative. Although readers will surely wish to challenge many of these arguments, they will find this carefully researched book a refreshing and stimulating change from the usual fare of Darwin literature.

WILLIAM B. PROVINCE

Collisions in gases

Partially Ionised Gases. By M. Mitchner and Charles H. Kurger jun. Pp. 518. (Wiley Series in Plasma Physics.) (New York and London: Wiley-Interscience, November, 1973.) £12.50.

THIS book is concerned with the theory of collision-dominated plasmas and its application to practical engineering problems, particularly magnetohydrodynamic (MHD) energy conversion. It can be used as a text for graduate level courses of different emphases, by choosing appropriate parts of the nine chapters. The authors have taught the material in this way and make suggestions based on their experience in the introductory chapter.

Chapter 2 begins the book proper, introducing the physical concepts needed to understand collisional and radiative processes. More than twenty graphs for selected collision cross-sections as a function of energy are given at the end of the chapter, so that the order of magnitude of many cross sections not included can be estimated from this collection.

Chapter 3 deals in more detail with plasmas at rest and has a section on diagnostics, which unfortunately includes the erroneous statement that ion temperatures can be deduced from probe curves. The references quoted would correct this. The magnetohydrodynamic equations of motion are derived in chapter 4, after particle motion in combined static E and B fields has been discussed. This leads to a treatment of MHD power generation and other phenomena in flowing magnetised plasmas.

Collision theory as applied to elastic collisions in plasmas is described in chapter 5, where Coulomb scattering and three-body recombination appear. Radiation from plasmas is treated instructively on a semi-classical basis in chapter 6. A more formal treatment of elastic collisions on the basis of a fuller kinetic theory appears in chapter 7. The Cartesian-tensor expansion of the Boltzmann equation is introduced here and applied in chapter 8 to the calculation of transport coefficients for plasmas of all degrees of ionisation.

Inelastic collisions, which determine the degree of ionisation, are considered in chapter 9 on ionisational non-equilibrium. Departures from the Saha equation due to escape of radiation from steady-state plasmas are discussed, as are non-Maxwellian distributions and flowing plasmas.

The material is clearly presented; obviously this textbook has grown out of genuine efforts to teach. A fine selection of numerical examples is provided with each topic, and a multitude of references after each chapter. This makes the book useful as a guide to the original work. Beginners in the field of high temperature gas dynamics would be helped by the discussion of techniques for carrying out useful approximate calculations, and by the data presented in graphical and tabular form throughout the book.

The book is well printed and bound—though my copy contains some optically thin pages—and the diagrams are excellent.

P. F. LITTLE

Reproductive handbook

Female Reproductive System. Edited by R. O. Greep. Part 1: pp. 658; part 2 pp. 375. (Handbook of Physiology: A Critical Comprehensive Presentation of Physiological Knowledge and Concepts. Section 7: Endocrinology: Vol 2.) (American Physiological Society: Washington DC; Distributed by Williams and Wilkins, Baltimore, 1973.) Part 1; \$44.50; part 2; \$25.00.

THIS latest volume of the American Physiological Society's "Handbook of Physiology" maintains the very high standard of its predecessors. Roy Greep has obtained a galaxy of stars to write the 50 chapters on central nervous

system-pituitary-ovarian interrelationships, effects of hormones on sexual behaviour, ovary, female reproductive tract, pregnancy, immunoendocrinology, and fertility control. Greep's approach might appear somewhat parochial in that only four of the chapters have been written by authors not attached to North American laboratories, but it is a pity that his modesty prevented the addition of a chapter under his own authorship.

The various sections made very stimulating reading, though the chapters on, for example, the biosynthesis of the ovarian steroids inevitably provided heavier reading than did other chapters. In general, although each chapter overflows with pertinent information, the provision of facts has not been permitted to obliterate the fundamental concepts that are discussed. As an excellent example of this I would cite Joan Hoffmann's chapter on "The Influence of Photoperiods on Reproductive Function in Female Mammals" in which she lucidly introduces her subject and then continues, to produce a clear diagrammatic summary of the possible interplay in the rat of photoperiodic inputs, hormone levels, and neural thresholds to permit, or otherwise, the pre-ovulatory surge of luteinising hormone. Richard Michael's chapter on "The Effects of Hormones on Sexual Behavior in Female Cat and Rhesus Monkey" is equally entertaining, though it was noticeable that one quarter of the 205 references came from Michael's own publications.

The high standard of production of this volume is reflected in the excellent reproduction of all figures. This standard of illustration is particularly of note in the chapter by S. R. M. Reynolds on "Blood and Lymph Vascular Systems of the Ovary" and the chapter by Arthur Hertig and Barbara Barton on "Fine Structure of Mammalian Oocytes and Ova".

I would quibble, though, with the use of numbers in the text instead of authors' names to indicate references. References are, however, now quoted with full titles, which is an improvement since the handbook first appeared in 1959. Inevitably, reference could in general only be made to works published up to early 1971, and, as a result, some areas of current interest such as those of releasing factors or prostatic glands are not up to date nor given much coverage.

I would, however, recommend unequivocally that anyone working in the field of reproductive physiology should invest in this volume. It certainly fulfils the stated intention of providing a reference 'bible' for predoctoral study, for preliminary orientation in preparation for research, and for preparation of lectures.

BAREND TER HAAR

obituary

I. Lakatos

IMRE LAKATOS, Professor of Logic at the London School of Economics, was born in Hungary in 1922 and died suddenly in London on February 2, 1974. He was a forceful and original thinker, with an equally forceful and original personality. His chief contributions lay in the philosophy and history of mathematics and of the physical sciences. To those who found the combination of philosophy with history a strange one, Lakatos replied (paraphrasing Kant), "Philosophy of science without history of science is empty; history of science without philosophy of science is blind".

Most people think that the history of mathematics, while a legitimate object of curiosity, is not essential to the understanding or philosophical evaluation of mathematics itself. In mathematics, they say, we begin with axioms and definitions, and prove theorems from them. But Lakatos asked how the axioms and definitions came to be proposed, and how the proofs came to be discovered. Answering these questions led him into the territory of mathematical discovery or mathematical heuristics. It is a strange territory, where theorems get proved and then refuted, where the theorem and its proof get elaborated to deal with the refutation, refuted again, and once more elaborated, until in the end something like an axiomatic theory emerges. Lakatos's brilliant 'Proofs and Refutations' (*Br. J. Phil. Sci.*, 1963) is a case study of this process. Its dialogue form is no accident, for according

to Lakatos, mathematical discovery is brought about, essentially, by critical debate. The dialogue sparkles, and comes nearer than any of Lakatos's writings to capturing the unique style of the man.

Working mathematicians who, after all, inhabit these regions may find Lakatos's topography of them familiar. And yet the mathematics student is still confronted with long and complicated 'definitions', which may be the end product of centuries of trial and error, and expected to understand them without knowing their history. All he can do in this situation is memorise the definitions parrot fashion. The implications of Lakatos's work for the teaching of mathematics have yet to be explored.

Philosophers of mathematics have a different reaction to Lakatos's work. They insist that what he describes is only the murky preamble to the placing of mathematical results on a firm, rigorous and certain foundation. They see nothing in it to alter their view, held since Plato, that mathematics is the repository of absolutely certain knowledge. In another important paper, 'Infinite Regress and the Foundations of Mathematics', (*Aristotelian Soc., supplementary volume*, 1962) Lakatos examined the most recent attempts to provide secure foundations for mathematical theories (Frege, Russell and Hilbert). He showed that all these failed, but failed gloriously: their so-called 'foundations of mathematics' actually turned out to be new and exciting mathematical theories (mathematical logic, set theory, and

recursive function theory, respectively).

Much of this carries over to Lakatos's more recent work in the philosophy and history of the physical sciences. Here, of course, Lakatos was heir to the critical philosophy of science of Sir Karl Popper. (Indeed, his philosophy of mathematics is in some respects an extension of Popper's views to mathematics, an extension which Popper himself never made but later welcomed.) Lakatos developed Popper's philosophy of science in two directions. One was the emphasis upon the importance of history for the rational evaluation of scientific theories. He insisted, against the dominant view in this field, that scientific contributions cannot be appraised independently of the historical development which produced them. His second main interest was in whether a critical philosophy of science, which eschews any firm foundations for scientific knowledge, can avoid scepticism and irrationalism. His 'methodology of scientific research programmes' was an attempt to show that it can. In it, he was sharply critical of both the irrationalist tendencies he detected in recent writers (notably Thomas Kuhn) and of some features of Popper's own views.

Like all original work, Lakatos's work raised more questions than it answered. He still had much to teach us about these questions, for he died at the height of his powers. All that remains is to hope that his unpublished work, which is considerable, will appear, and that the students he inspired will continue the 'research programme' which he initiated.

Announcements

Awards

Graham Higman has been awarded the De Morgan Medal and Paul M. Cohn the Senior Berwick Prize of the London Mathematical Society (corrected announcement).

Cecil A. Hoare has been awarded the Manson Medal and Alister Voller the Chalmers Medal of the Royal Society of Tropical Medicine and Hygiene.

Appointments

David Robinson has been appointed Professor of Food Science at the University of Leeds.

R. E. Cotton, B. D. Edwards, J. A. Scott and G. K. Williamson have been awarded Special Professorships at the University of Nottingham.

Erratum

In the article "Aftershocks caused by the Novaya Zemlya explosion on October 27, 1973" by Hans Israelson, Ragnar Slunga and Ola Dahlman (*Nature*, **247**, 450; 1974) the distance scale in Fig. 1 should read 500 km. See also *Nature*, **248**, 712; 1974.

International meetings

September 30–October 2, 1st Annual Philadelphia Symposium on Ageing (Dr Richard C. Adelman, Fels Research

Institute, 3420 North Broad Street, Philadelphia, Pennsylvania 19140).

October 1–4, 2nd International Colloquium on the Exploitation of the Oceans (Association pour l'Organisation de Colloques Océanologiques à Bordaux, B.P. No. 315–16, 75767 Paris, Cedex 16, France).

October 3–9, 14th International Congress of Pediatrics (Sr Secretario General, XIV Congreso Internacional de Pediatría, Casilla de Correo 3177, Buenos Aires, Argentina).

October 7–14, 1st International Colloquium on Physical and Chemical Information Transfer and Regulation of Reproduction and Ageing (Dr J. G.

Vassilleva-Popova, Secretary General, The First Colloquium on Physical and Chemical Information Transfer and Regulation of Reproduction and Ageing, Department of Biophysics, Bulgarian Academy of Sciences, 13 Sofia, Bulgaria).

October 20-26, **26th International Congress of Physiological Sciences** (Secretariat, 26th International Congress of Physiological Sciences, New Delhi 1974, Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110016, India).

October 20-26, **11th International Cancer Congress** (General Secretariat, Eleventh International Cancer Congress, Via G. Venezian, 1 20133 Milan, Italy).

October 21-23, **4th Conference on Chemical and Molecular Lasers** (Dr W. Q. Jeffers, McDonnell Douglas Research Laboratories, PO Box 516, St Louis, Missouri 63166).

October 21-25, **25th Annual Session American for Laboratory Animal Science** (Mr Joseph J. Garvey, Executive Secretary, AALAS, 2317 W. Jefferson Street, Suite 208, Joliet Illinois 60435).

October 23, **Recent Advances in Ultra-violet and Visible Radiation Effects on the Skin** (Dr C. Ramsay, Department of Photobiology, Homerton Grove, London E9 6BX).

October 23-26, **College Park Colloquia on Chemical Evolution: Chemical Evolution of the Giant Planets** (Dr Cyril Ponnampuruma, Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, College Park, Maryland 20742).

October 31-November 2, **Communication and Control in Social Processes** (American Society for Cybernetics, Suite 530, 1130 Seventeenth Street, NW, Washington DC 20036).

Reports and Publications

Great Britain

Department of Energy, Production and Reserves of Oil and Gas in the United Kingdom—a Report to Parliament by the Secretary of State for Energy, May 1974. Pp. 27. (London: HMSO, 1974.) 32p net. [225]

The Common Sense of Concorde. Pp. 24. (Weybridge, Surrey: British Aircraft Corporation, Ltd., 1974.) gratis. [235]

The British Industrial Biological Research Association. Annual Report for 1973. Pp. 56 + 7 plates. (Carshalton: The British Industrial Biological Research Association, 1974.) [235]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 276, No. 1260: Thermo-mechanics of Rubber-like Materials. By P. Chadwick. Pp. 373-403. (London: The Royal Society, 1974.) [235]

Research Into Tertiary Science Education. (A selection of papers from the Conference on Tertiary Science Education held at Thames Polytechnic, December 1972.) Introduced and arranged by the conference organisers, David E. Billing and John R. Parsonage. Pp. 100. (London: Society for Research into Higher Education, 1974.) £2.60. [245]

Research Using Transplanted Tumours of Laboratory Animals: a Cross-Referenced Bibliography—Z. By D. C. Roberts. Pp. 224. (Mill Hill, London: Research Data Unit, Imperial Cancer Research Fund, 1974.) [245]

The International Review of Psycho-Analysis, Vol. 1, Parts 1-2. Edited by Joseph Sandler in association with M. Masud R. Khan. Pp. 1-256. Published quarterly. Subscriptions. Annual subscription £8.50 (Canada and USA \$22.50); single parts (current volume only) £3.50 (Canada and USA \$9). This double issue £7 or \$18. (London: Baillière Tindall, 1974.) [305]

Agricultural Research Council. Letcombe Laboratory. Wantage—Annual Report for 1973. Pp. vi + 95. (London: HMSO, 1974.) £1.33. [305]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 276, No. 1261: Energy in the 1980s—a Discussion organised by Sir Peter Kent, FRS. Pp. 405-620. (London: The Royal Society, 1974.) £7.50. [305]

Biomedical Mass Spectrometry, Vol. 1, No. 1, February 1974. Edited by Dr. Brian Millard and Professor Catherine Fenslau. Pp. xviii + 1-82. Subscription:—Vol. 1 (6 issues)—£25; \$65; DM 175.00. (London: Hayden & Son Ltd, 1974.) [315]

Public Health Laboratory Service. Monograph Series No. 5: Laboratory Methods 1. By Joan R. Davies, E. J. G. Glencross, J. Marks, C. D. Plows and M. E. M. Thomas. Pp. vii + 35. (London: HMSO, 1974.) 50p net. [36]

The Age of Scarcity? By Sir Alan Cottrell. (Eleventh Sir Julius Wernher Memorial Lecture of the Institution of Mining and Metallurgy delivered on 4th June, 1974, on the occasion of the "Minerals and the Environment" Symposium.) Pp. 4. (London: Institution of Mining and Metallurgy, 1974.) [36]

Bulletin of the British Museum (Natural History). Entomology. Vol. 29, No. 8: Revisional Notes on African Charaxes (Lepidoptera: Nymphalidae), Part IX. By V. G. L. van Someren. Pp. 415-487 + 18 plates. (London: British Museum (Natural History), 1974.) £9.95. [66]

Rowett Research Institute. Annual Report of Studies in Animal Nutrition and Allied Sciences. Vol. 29, 1973. Pp. 146. (Bucksburn, Aberdeen: Rowett Research Institute, 1974.) 80p; \$2. [66]

The Computer: The Challenge for Science and Society By Professor S. J. Goldsack. (Inaugural Lecture, 5 June 1973.) Pp. 163-181 (London: Imperial College, 1974.) 75p. [66]

Heart Drugs: The Chemical Control of Heart Function. Pp. 14. New Protein. Pp. 16. Antibacterials: The Chemical Control of Infectious Diseases. Pp. 16. (London: Imperial Chemical Industries, Ltd., 1974. Two copies of each title can be obtained price £1 from The Kynoch Press, Thames House North, Millbank, London, SW1P 4QG.) [66]

A Natural History of Loch Lomond. Pp. 112. (Glasgow: University of Glasgow Press, 1974.) 50p net. [76]

Working with Radiation. Pp. 32. (Risley, Warrington: Public Relations Department, British Nuclear Fuels, Ltd., 1974.) [76]

Provisional Atlas of the Amphibians and Reptiles of the British Isles. Edited by Henry R. Arnold. Pp. 4 + 15. (Abbots Ripton, Huntingdon: Biological Records Centre, Monks Wood Experimental Station, 1973.) 25p. [76]

Microbial Protein Production for Developing Countries. Pp. 131 (Reading: Tate and Lyle, Ltd., Group Research and Development, 1974.) [76]

Environmental Health Engineering in Hot Climates and Developing Countries. (Proceedings of the Two Day Conference held in the Department of Civil Engineering, Loughborough University of Technology, September 1973.) Edited by John Pickford. Pp. 107. (Loughborough: John Pickford, Department of Civil Engineering, University of Technology, 1974.) £3. [106]

The Work of Aslib for the year ended December 1973. Pp. 56. (London: Aslib, 1974.) [106]

Science Research Council. Computer Networks. Pp. 22. (London: Science Research Council, 1974.) [106]

Science Research Council. Greenwich Time Report. Royal Greenwich Observatory Time and Latitude Service, 1973, July-September. Pp. 369-394. (London: Science Research Council, 1974.) [106]

University of Bristol, Department of Agriculture and Horticulture Report of the Long Ashton Research Station (The National Fruit and Cider Institute) for 1973. Pp. xxii + 213 (Long Ashton, Bristol; Long Ashton Research Station, 1974.) £1.75. [116]

Other Countries

United States Department of the Interior: Geological Survey. Professional Paper 783: Oligocene Stratigraphy, Tectonics, and Paleogeography Southwest of the San Andreas Fault, Santa Cruz Mountains and Gabilan Range, California Coast Ranges. By Joseph C. Clark and Jan D. Rietman. Pp. iii + 18. (Washington, DC: Government Printing Office, 1973.) \$1.70. [155]

CERN—European Organization for Nuclear Research. CERN 74-7: Calcul Numerique des Derivees Partielles Successives des Fonctions de Deux et de Trois Variables—Applications au Developpement de Taylor-Maclaurin. Par J. F. Barthelemy. Pp. v + 41. (Geneva: CERN, 1974.) [165]

National Research Council of Canada. Associate Committee on Scientific Criteria for Environmental Quality. Report No. 1 of the

Subcommittee on Biological Phenomena: Problems and Selection of Topics for Compilation of Cause/Effect Data. Compiled by C. Quading, R. M. D. Sutton and N. Khan. Pp. 51. (Ottawa: NRCC, 1973.) [165]

Royal (Ontario) Museum. Domestication of the Carp *Cyprinus carpio* L. By Eugene K. Balon. Pp. 37. Life Sciences Contribution No. 97: A Revision of the Longipinnate Ichthyosaurs of the Lower Jurassic of England, with Descriptions of Two New Species (Reptilia: Ichthyosauria). By C. McGowan. Pp. 37. \$1.50. (Toronto: Royal Ontario Museum, 1974.) [165]

Report of the International Pilot Study of Schizophrenia. Vol. 1: Results of the Initial Evaluation Phase. Pp. x + 427. (Geneva: WHO, London: HMSO, 1973.) Sw.fr. 56. [165]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1757-L: Aquifers in the Sokoto Basin, Northwestern Nigeria, with a Description of the General Hydrology of the Region. By Henry R. Anderson and William Ogilbee. Pp. v + 79 + plates 1-11. (Washington, DC: Government Printing Office, 1973.) \$6.75. [175]

Contributions to the Geology of Suriname, 3. Pp. 139. (Paramaribo, Suriname: Geologisch Mijnbouwkundige Dienst, 1974.) [205]

United States Department of the Interior: Geological Survey. Bulletin 1394-C: Gastineau Channel Formation, a Composite Glaciomarine Deposit Near Juneau, Alaska. By Robert D. Miller. Pp. iii + 20. 35 cents. Water-Supply Paper 1939-E: Geohydrology and Water Resources of the Tucson Basin, Arizona. By E. S. Davidson. Pp. iv + 81 + plates 1-7. \$6.40. (Washington, DC: Government Printing Office, 1973.) [215]

Limits to Growth? A Study by the Ad Hoc Committee on World Dynamics. Pp. 40. (Wellington: The Royal Society of New Zealand, 1973.) \$1.50. [215]

Gesellschaft für Strahlen- und Umweltforschung GmbH, München. Jahresbericht 1973. Kurzfassung. Pp. 236. (München: Gesellschaft für Strahlen- und Umweltforschung GmbH, 1974.) [215]

Office de la Recherche Scientifique et Technique Outre-Mer, Paris et Bondy. Travaux et Documents de l'ORSTOM, No. 27: Le Gisement de Vertébrés du Phnom Loang (Province de Kampot, Cambodge). Faune Pléistocène Moyen Terminal (Loangien). Par M. Beden et C. Cuérin. Pp. 97. (Paris et Boney: Office de la Recherche Scientifique et Technique Outre-Mer, 1973.) 30 francs. [225]

Smithsonian Contributions to the Earth Science, No. 12: An Atlas of Volcanic Ash. By Grant Heiken. Pp. iv + 101 (33 plates). \$2. Smithsonian Contributions to Zoology, No. 160: Systematics of the Subterranean Amphipod Genus *Stygobromus* (Gammaridae). Part I: Species of the Western United States. By John R. Holsinger. Pp. iii + 63. \$1.45. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [235]

Exploring Energy Choices—a Preliminary Report of the Ford Foundation's Energy Policy Project. Pp. 81. (Washington, DC: The Energy Policy Project, P.O. Box 23212, 1974.) 75 cents. [235]

United States Department of the Interior: Geological Survey Water-Supply Paper 1989: Water Resources of the Little River Basin, Louisiana. By M. W. Gaydon, J. E. Rogers and R. P. Smith. Pp. iv + 74. \$3.15. Professional Paper 384-C: The Disintegration of the Wolf Creek Meteorite and the Formation of Pecoarite, the Nickel Analog of Clinohydroxite. Pp. vi + 107-135. \$1.30. (Washington, DC: Government Printing Office, 1973.) [235]

United States Department of the Interior: Geological Survey. Bulletin 1331-C: Petrography and Stratigraphy of Glacial drift, Mesabi-Vermilion Iron Range Area, Northeastern Minnesota. By T. C. Winter, R. D. Cotter and H. L. Young. Pp. iv + 41 + 2 plates. \$2.25. Bulletin 1372-I: New Cretaceous Formations in the Western Wyoming Thrust Belt. By William W. Rubey. Pp. iii + 35. 50 cents. (Washington, DC: Government Printing Office, 1973.) [245]

Canada: Ministry of State—Science and Technology. Directory of Research and Development Establishments in Canadian Industry. Pp. 207. (Ottawa: Information Canada, 1974.) [76]

Bibliography of Society, Ethics and the Life Sciences 1974 edition. Compiled by Sharon Solitto and Robert M. Veatch. Pp. 92. (Hastings-on-Hudson: Institute of Science, Ethics and the Life Sciences, 1974.) [76]

BC Research. 30th Annual Report, 1973. Pp. 24. (Vancouver: BC Research, 1974.) [76]

CERN—European Organisation for Nuclear Research. CERN 74-11: Dynamic Orientation of Nuclei at Low Temperatures—a Study of the Mechanisms of Dynamic Polarization in Polarized Targets. By W. de Boer. Pp. 76. (Geneva: CERN, 1974.) [76]

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Atmospheric Physics, 1972/1973. Pp. 34. (Melbourne: CSIRO, 1974.) [106]

Australian Journal of Zoology. Supplementary Series. No. 24: A Revision of the Scarabaeine Dung Beetles of Australia. II. Tribe Scarabaeini. By E. G. Matthews. Pp. 211. No. 26: Revision of the Subgenus *Callohesma* Mcheneri (Apoidea: Colletidae). By Elizabeth M. Exley. Pp. 58. (East Melbourne: Editorial and Publications Service, CSIRO, 372 Albert Street, 1974.) [106]

Publications du Centre National pour l'Exploitation des Océans, CNEO. Résultats de la Compagnie Cinea-Charcot 1 (22 Janvier-15 Février 1971). Ouvrage mis au point par A. Thiriot. Pp. 188. (Paris-Cedex: CNEO, 1974.) [106]

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APPOINTMENTS VACANT

DIRECTOR

The Jackson Laboratory invites applications for the position of Director of the Laboratory, which will become open on October 1, 1975. Qualified applicants are invited to submit a curriculum vitae and summary of experience to one of the following:

Dr James D. Ebert, Chairman, Search Committee, Marine Biological Laboratory, Woods Hole, Massachusetts 02543;

Dr James F. Crow, Member, Search Committee, Department of Medical Genetics, University of Wisconsin, Madison, Wisconsin 53706;

Dr Douglas L. Coleman, Secretary, Search Committee, The Jackson Laboratory, Bar Harbor, Maine 04609.

AN EQUAL OPPORTUNITY EMPLOYER. (3)

POSTDOCTORATE FELLOW IN IMMUNOGENETICS

Biochemist/Immunochemist with Ph.D. degree needed to work on the genetics of man's immune response toward limiting doses of naturally inhaled protein antigens (highly purified components of pollens, etc.). Opportunity to join a group which has had 3 years experience in this new research area. Excellent facilities and stimulating environment. Candidate must have a sound background in protein purification and characterisation. Experience in radioimmunoassay and leukocyte culture techniques advantageous. Demonstrated creativity in previous research essential. Send detailed curriculum vitae, list of publications and names of two referees to: Dr David G. Marsh, Johns Hopkins University School of Medicine at Good Samaritan Hospital, 5601 Loch Raven Boulevard, Baltimore, Maryland 21239.

Equal Employment Opportunity Employer M/F. (92)

UNIVERSITY OF READING DEPARTMENT OF CHEMISTRY RESEARCH DEMONSTRATORS

required in inorganic and physical chemistry from October 1, 1974, for three year appointment. Candidates should have a good Honours Degree in Chemistry, or equivalent qualifications. Demonstrators help supervise practical classes and undertake research leading to a higher degree (M.Phil or Ph.D.). Salary in the scale £1,046 x 51 to £1,149 p.a. (Under review). Apply to Professor G. W. A. Fowles, Department of Chemistry, University of Reading, Whiteknights, Reading, from whom further information about research topics can be obtained. (Ref: T 52.) (147)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND FACULTY OF MEDICINE

A new medical school in Canada is expanding its faculty and will make post-doctoral and faculty appointments in the field of Human Biochemical Genetics. Candidates must be prepared to work on human biochemical variation in the context of collaborative population studies though this does not preclude the pursuit of other research interests. Interested individuals should submit applications accompanied by a detailed curriculum vitae to Dr K. B. Roberts, Associate Dean for Basic Medical Sciences, Memorial University of Newfoundland, St John's, Newfoundland, A1C 5S7, CANADA. (152)



**Public Service
Canada**

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Canada**

THIS COMPETITION IS OPEN TO BOTH MEN AND WOMEN

RESEARCH SCIENTISTS

Salary up to: \$24,262

Department of Environment, St. John's, Newfoundland

The Newfoundland Biological Station requires two scientists for research into the biology and population dynamics of pelagic fishes of the Newfoundland-Labrador area.

DUTIES:

- participating in planning, initiating and implementing field and laboratory studies;
- analyzing data and interpreting results for presentation in report of scientific manuscript form;
- keeping abreast of developments in fisheries research;
- assigning duties to and supervising technical staff.

QUALIFICATIONS:

Include a Doctorate degree, or a lesser degree with related independent research experience equivalent to that required for a Doctorate degree, with experience in an area related to the duties of the position. Good health and physical condition with ability to work at sea. Knowledge of the English language is essential for this position.

APPLICATIONS:

Curriculum vitae or "Application for Employment" form, accompanied by complete university transcripts and the names and address of three referees should be forwarded no later than AUGUST 6, 1974 to

THE MANAGER
NEWFOUNDLAND PERSONNEL OFFICE
DEPARTMENT OF ENVIRONMENT
P.O. BOX 5300
ST. JOHN'S, NEWFOUNDLAND.

Please quote competition number: 74-100-31, 44 () in all correspondence.

Appointments as a result of this competition are subject to the provisions of the Public Service Employment Act. (160)

POST DOCTORAL RESEARCH APPOINTMENT

The Forestry Commission offers a period appointment for 3 years for research work in the Physiology Branch at their Northern Research Station, Roslin, Midlothian.

Subjects

- (a) To investigate the internal and environmental control of root initiation in conifers.
- (b) To develop early-test methods—physiological screening techniques, for testing the characteristics of trees while still at the seedling stage.

Qualifications

Ph.D. in plant physiology or other plant science.

Age

Under 30 years on December 31, 1974.

Salary

Negotiable, within the range for Scientific Officer (£1,435 to £2,329) or for Higher Scientific Officer (£2,221 to £2,854)—depending on qualifications and experience.

Closing date for Applications

Application forms and further details may be obtained from Mr. F. M. Muggridge, Forestry Commission, Priestley Road, Basingstoke, Hants RG24 9NS. Completed application forms should be returned not later than July 31, 1974. (161)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF MATHEMATICS SEMICONDUCTOR THEORY

Applications are invited from recent graduates for a RESEARCH STUDENTSHIP and from persons completing postgraduate work (M.Sc. or Ph.D.) for a RESEARCH ASSISTANTSHIP. The work involves the application of quantum mechanics and statistical mechanics to problems of recombination in semiconductors with special reference to device physics (solar cells, light emitting diodes, etc.).

A good honours degree in mathematics, physics, engineering or equivalent is essential.

Please apply as soon as possible giving name of one referee to Academic Registrar, The University of Southampton SO9 5NH. (153)

UNIVERSITY OF MANCHESTER RESEARCH ASSISTANT DEPARTMENT OF CHILD HEALTH

Applications are invited from suitably qualified recent graduates for this post with a multidisciplinary research group concerned with growth and development of the brain. The Research Assistant will help with the management of biochemical, animal behavioural and clinical laboratories for which previous experience is not essential. Opportunities exist to prepare for a higher degree. Salary range p.a. £1,569-£1,791 (£1,758-£1,980 from October 1st.). Further particulars and application forms (returnable by July 15th) from the Registrar, The University, Manchester, M13 9PL. Quote ref: 149/74/N. (169)

PHARMACEUTICAL RESEARCH AND DEVELOPMENT

Our extensive research and development laboratories at Loughborough offer an excellent career opportunity for young graduates interested in contributing to our current research programme into respiratory and cardiovascular diseases.

These posts would particularly suit a recent graduate with a good honours degree or equivalent in pharmacology or pharmacy and the enthusiasm and ability to carve out a successful career in a large, progressive research environment.

An attractive competitive starting salary will be offered and excellent conditions of employment include a full range of fringe benefits and opportunities for movement and advancement throughout our R and D function.

Please write with brief details of qualifications held or expected, and information concerning research activities to date:

A.B. Johnston, Fisons Limited, Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire.



(70)

UNIVERSITY OF RIYADH MEDICAL SCHOOL SAUDI ARABIA (IN ASSOCIATION WITH THE UNIVERSITY OF LONDON)

Applications are invited from male honours graduates for the following posts in Pre-Clinical Medicine at the University of Riyadh Medical School. The vacancies have arisen as a result of the rapid expansion of the School:

ANATOMY—PROFESSOR/LECTURER/DEMONSTRATOR
BIOCHEMISTRY—ASSISTANT LECTURER/DEMONSTRATOR
PHYSIOLOGY—ASSISTANT PROFESSOR/LECTURER/DEMONSTRATOR

Applicants should have a higher degree and teaching experience.

The University of Riyadh is an independent University established in 1957. In 1968 the University established a Medical School in association with the University of London. The request by the University of Riyadh for assistance in this project is covered by a sponsorship agreement. The University of London advises on the curriculum, the form and conduct of examinations, the physical facilities for teaching, the appointment of academic staff and other matters. From the outset it has been anticipated that this assistance would continue over a period of at least 10 years possibly extending to 15 years. All teaching of medical undergraduates is in the English language.

Salary scales:

PROFESSORS—On range Saudi Ryals 6,200 to SR7,200 per month plus a housing allowance of SR12,000 per annum.

ASSISTANT PROFESSORS—On range SR5,200 to SR6,200 per month plus a housing allowance of SR10,000 per annum.

LECTURES—On range SR4,200 to SR5,200 per month plus a housing allowance of SR8,000 per annum.

DEMONSTRATORS—Salary and housing allowance will be negotiable in accordance with qualifications and experience.

CONSULTANCY POSTS—Additional allowances will be paid for appointees holding consultant posts at the Teaching Hospital.

Note: £1 sterling=Saudi Ryals 8.5.

Appointments are for 1 year or longer; renewable. Secondments would be considered.

Detailed applications (3 copies) including a curriculum vitae and naming three referees should be sent not later than July 26, 1974 to the Inter-University Council for Higher Education Overseas, 90-91 Tottenham Court Road, London W1P 0DT from whom further particulars are available. (150)

THE ROYAL VETERINARY COLLEGE (University of London) Division of Paraclinical Studies DEPARTMENT OF PATHOLOGY LECTURER IN VIROLOGY

Applications are invited from veterinary and science graduates for the newly-created post of Lecturer in Virology which is available from October 1, 1974.

Candidates should have considerable post-graduate experience in animal virology and possess a Ph.D. or equivalent degree. The appointee will be required to participate in teaching virology and virus diseases at the undergraduate and postgraduate levels. Strong research interests are essential; good facilities available, including supply of gnotobiotic animals. Prospective applicants are invited to contact Professor W. Plowright for further information.

Salary scale £2,118 to £4,896 plus London Allowance (under review) at present £162 per annum, plus threshold supplement. Initial salary according to qualifications and experience. Superannuation under F.S.S.U.

Application form and further details available from Assistant Secretary (Personnel) (N). The Royal Veterinary College, Royal College Street, London NW1 0TU. **Closing date for applications** August 19, 1974. (149)

INSTITUTE OF CANCER RESEARCH AND ROYAL MARSDEN HOSPITAL DIVISION OF PHYSICS APPLIED TO MEDICINE

Two vacancies will occur in September for Physicists to work in the diagnostic and therapeutic applications of ionizing radiation to medicine. The department is large and well equipped and covers both X-ray beam work, radioisotope work, diagnostic X-rays, and ultrasound. Candidates need not have worked in the medical field but should have a 1st or 2nd Class Honours Degree. Further particulars can be supplied on request. New entrants will be given a thorough training in all aspects of the work. The Institute is an integral part of the British Postgraduate Medical Federation which is a School of London University. Appointments will be made on M.R.C. Scales Grade 2 £1,830-£3,378+£162 London Weighting, or Grade 1 £3,543-£4,548+£162 L.W. depending on the candidate's qualifications and postgraduate experience in medical physics or a closely related field. Superannuation on F.S.S.U. but suitable candidates from N.H.S. can retain existing pension arrangements. Applications giving full curriculum vitae and names of two referees to the Secretary, 34 Sumner Place, SW7 3NU, quoting ref. 300/G/76. (154)

ASSOCIATION OF CLINICAL PATHOLOGISTS

Medical Microbiology for Non-Medically Qualified Microbiologists February 10-21, 1975.

This non-residential lecture course will be held in London at the Adelphi, 1-11 John Adam Street, W.C.2.

The fee payable is £40.

Applications are invited from graduates in science, who are making, or who intend to make a career in Medical Microbiology.

Further information and application forms may be obtained from: Miss Valerie Matt, Department of Microbiology, QUEEN CHARLOTTE'S MATERNITY HOSPITAL, 339 Goldhawk Road, London W6 0XG. (179)

THE UNIVERSITY OF MANCHESTER RESEARCH TECHNICIAN

required to assist with a laboratory investigation on the genetical and cytological basis of sex ratio distortion due to meiotic drive in the mosquito *Aedes aegypti*, supported for two years from a grant by the Science Research Council to Dr R. J. Wood. This post is suitable for a graduate in a biological subject with experience of genetical theory and techniques. Salary £1,704 rising to £1,758 p.a. after one annual increment. Applications, together with the names of two referees, should be sent as soon as possible to Dr R. J. Wood, Department of Zoology, The University, Manchester M13 9PL. (171)

CHARING CROSS HOSPITAL

Fulham Palace Road, Hammersmith W.6

BIOCHEMIST

DEPARTMENT OF MEDICAL ONCOLOGY

Applications are invited for a graduate biochemist to join a team isolating tumour antigens. It is essential that applicants have an interest in protein chemistry and a working knowledge of current separation techniques. Working under supervision, the successful applicant would be given his own project and therefore some previous research experience would be useful. An opportunity would be given to register for a higher degree.

Salary on scale £1,623 to £2,820, inclusive of London Weighting, according to qualifications and experience, plus threshold payment of £5.22 per month.

Further details available from Dr. G. T. Rogers, 01-748 2050 ext. 2087. Application forms from Miss Matthews, Personnel, ext. 2995. (155)

THE LONDON HOSPITAL (WHITECHAPEL)

GRADUATE RESEARCH ASSISTANT OR QUALIFIED TECHNICIAN

Required in the Professorial Gastro-Enterological Research Unit. The work will be concerned with radio-immuno-assay and experience in this technique is desirable.

Applications to R. C. Taylor, Assistant Secretary, The London Hospital (Whitechapel), Whitechapel, London E1 1BB. Tel: 01-247 5454 Extn. 388. (167)

COMMONWEALTH AGRICULTURAL BUREAU

Vacancy for

SCIENTIFIC INFORMATION OFFICER

at the

COMMONWEALTH BUREAU OF SOILS

ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, HERTFORDSHIRE

Duties: Preparing abstracts for the abstracting journal "Soils and Fertilizers", which covers world scientific literature on soils and soil-plant relationships, and dealing with scientific enquiries within these fields.

Qualifications: A degree in soil science, agricultural science or sciences basic to these. Ability to write good and concise English essential. The successful candidate may be required to learn a foreign language. *Experience or training in information work, including subject indexing, would be an advantage.

Salary: In scale £1,435 to £2,592 (bar), £2,798 to £3,478 (bar), £3,756 to £4,004, plus a compensatory allowance (taxable but not superannuable) of 41% to offset personal contribution to F.S.S.U. Starting salary according to qualifications, experience and age.

Application forms and full particulars from the Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN.

Closing date for applications: July 29, 1974. (184)

THE QUEEN'S UNIVERSITY OF BELFAST

Lectureship in Geology

Applications are invited for a lectureship in the Department of Geology in the general field of Palaeontology, preferably in Micro-palaeontology. The post is tenable from October 1, 1974 or such other date as may be arranged. Initial placing, which will depend on experience and qualifications will be made at one of the first three points on the scale for lecturers: £2,118, £2,241, £2,412 rising to £4,896 with contributory pension rights under the F.S.S.U. The appointment will be subject to a period of probation of up to three years in duration. Applications should be received by August 10, 1974. Further particulars may be obtained from The Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. (Please quote Ref. 74/N.) (168)

Research & Development with Roussel

PHARMACOLOGIST

IMMUNOPHARMACOLOGY

A Pharmacologist with an interest in immunopharmacology is required to join a team working in the field of allergic diseases. The position would suit a recent graduate or equivalent, preferably with 1 to 2 years relevant experience, who is looking for the opportunity to join a progressive and expanding Company.

Roussel Laboratories Ltd. is part of a large, international group researching, developing, manufacturing and marketing a wide range of pharmaceutical and allied products.

Conditions of service and fringe benefits are exceptional and in addition to a competitive salary, assistance with relocation expenses will be given where appropriate.

Please write with concise details of your qualifications and experience to:

Miss R. Curtis
Roussel Laboratories Ltd.,
Covingham, SWINDON,
Wilts. SN3 5BZ.

ROUSSEL

(192)

UNIVERSITY OF RIYADH MEDICAL SCHOOL SAUDI ARABIA

(IN ASSOCIATION WITH THE UNIVERSITY OF LONDON)

Applications are invited from male honours graduates for the following posts in the Medical School at the University of Riyadh. The vacancies which are for the pre-medical years have arisen as a result of the rapid expansion of the Medical School:

BIOLOGY—LECTURER/ASSISTANT LECTURER

BIOSTATISTICS—LECTURER

Applicants should have a higher degree and teaching experience.

Salary scale: Lecturers SR4,200 to SR5,200 per month plus a housing allowance of SR8,000 per annum; Assistant Lecturers—negotiable according to qualifications and experience. (£1 sterling=Saudi Ryals 8.80).

The University of Riyadh is an independent University established in 1957. In 1968 the University established a Medical School in association with the University of London. The request by the University of Riyadh for assistance in this project is covered by a sponsorship agreement. The University of London advises on the curriculum, the form and conduct of examinations, the physical facilities for teaching, the appointment of academic staff and other matters. From the outset it has been anticipated that this assistance would continue over a period of at least 10 years possibly extending to 15 years. All teaching of medical undergraduates is in the English language.

Appointments are for 1 year or longer; renewable. Secondments would be considered.

Detailed applications (3 copies) including a curriculum vitae and naming three referees should be sent not later than July 26, 1974 to the Inter-University Council for Higher Education Overseas, 90/91 Tottenham Court Road, London W1P 0DT from whom further particulars are available. (151)

CELLULAR IMMUNOLOGIST

Research appointment available for independent studies in the field of tumor immunology. Current ongoing programmes include studies on the mechanism of tumor cell destruction and the development of in vitro assays for estimation of cell mediated immune responses.

Preference given to experienced Ph.D., but active consideration given to imminent or newly graduated Ph.D. with strong immunology background. Salary open. Send curriculum vitae and names of three references to:

Dr C. S. Henney
The O'Neill Research Laboratories
Johns Hopkins University School of Medicine
at the Good Samaritan Hospital
5601 Loch Raven Blvd.
Baltimore, Maryland 21239, U.S.A.
An equal opportunity employer. (183)

STRANGEWAYS RESEARCH

LABORATORY WORT'S CAUSEWAY CAMBRIDGE CB1 4RN CELL BIOLOGIST

A postdoctoral position is available to work on the basic mechanisms of connective tissue metabolism. The candidate should have a sound background in cell biology but experience in connective tissue research is not essential.

The starting salary is £2,223 per annum with F.S.S.U. benefits. The appointment is for one year initially, but may be renewed for up to three years.

Please send curriculum vitae and names of two referees to Dr. J. J. Reynolds, Tissue Physiology Department, Strangeways Research Laboratory, Wort's Causeway, Cambridge CB1 4RN. (164)



Imperial Chemical Industries Limited

AGRICULTURAL DIVISION

Billingham, Cleveland.

A vacancy exists for a

MATHEMATICIAN

to join an existing team of eight graduates at Billingham. ICI Agricultural Division operates a complex of very large plants incorporating much new Technology originating from within, or developed by the Division. The mathematical Section in Research and Development Department deals with a wide variety of problems in chemical engineering theory, in the support of plant design and operation, and in some areas of mathematical planning. Candidates should have a good honours degree and an interest in applications such as reactor theory and fluid mechanics or statistics and operational research. Although a mathematics degree is preferred, candidates qualified in mathematical physics or in chemical engineering with a strong theoretical bias would be considered.

The section has good computer facilities, and candidates should possess or be ready to acquire competence in modern computing methods and programming.

The Company operates house purchase, profit sharing and contributory pension schemes and offers financial assistance towards removal expenses for married men.

Write giving brief details of age, qualifications and experience to:—

Mr M A J W Pegg

Personnel Department

Imperial Chemical Industries Limited

Agricultural Division

PO Box No 1 Billingham, Cleveland.

(205)

BASIC GRADE BIOCHEMIST

for Biochemistry Laboratory. Well equipped department offering good training facilities including radioimmunoassay techniques. Good Honours degree in Chemistry or Biochemistry required. Whitley Council conditions apply. Please reply with full details and names and addresses of 2 referees to Top Grade Biochemist, West Park Hospital Laboratories, Epsom, Surrey KT19 8PB. (158)

UNIVERSITY OF NATAL DEPARTMENT OF PHYSICS DURBAN

Applications are invited from suitably qualified persons for appointment to two posts of

LECTURER

In addition to teaching, successful candidates will be expected to take part in the experimental or theoretical activities of one of the research groups of the Department. The fields of interest are—

- (i) Magnetosphere Physics
- (ii) Laboratory Plasma Physics
- (iii) Electron Microscope studies of crystal defects.

Previous experience in one of these fields will be an advantage.

The salary scale attached to the posts is: R4,800 by R300 to R6,900 per annum plus 15% pensionable allowance.

The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant. In addition, an annual vacation savings bonus up to R260 for married men and R130 for women and single men is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, South Africa, with whom applications, on the prescribed form, must lodged not later than August 31, 1974, quoting Ref. Adv. 75/74. (162)

THE ROWETT RESEARCH INSTITUTE

BUCKSBURN, ABERDEEN AB2 9SB
FIELD INVESTIGATIONS OFFICER

Applications are invited from suitably qualified graduates for a post to examine the incidence and severity of trace metal deficiencies in cattle and sheep. Trials are being planned on farms in the northeast of Scotland, and these may later be extended. A graduate with a good honours degree in agriculture, or agricultural chemistry or a degree in veterinary science, is required to help to organise and to participate in this work. The appointment is for a period of 3 years in the Nutritional Biochemistry Department.

Salary ranges (which are currently under review): Scientific Officer £1,435 p.a. rising to £2,329 p.a. Higher Scientific Officer £2,221 p.a. rising to £2,854 p.a. Superannuation under F.S.S.U. with a non-pensionable allowance of 4½% of basic salary to offset contributions.

Salary placing will be in accordance with qualifications and experience but for entry to the higher grade, candidates should have had at least 2 years' appropriate post-qualifying experience.

Applications, with full details, together with the names of 2 referees, should be addressed to the Secretary of the Institute, from whom further particulars are available, by August 17, 1974. (163)

UNIVERSITY OF READING

Two Research Assistants required in Department of Soil Science. Applications are invited:

(a) From graduates in Physical Chemistry, Soil Science or agricultural chemistry to work on mechanisms of phosphate sorption by soil minerals.

(b) From graduates in Physics or Physical Chemistry to join a research group concerned with studies of Pore Space in soils and its effect on gas and water flow.

Position (a) is supported by a grant from A.R.C. and position (b) by a studentship awarded by M.A.S.S. Applicants should have obtained first or upper second class honours and will be expected to register for a higher degree.

Both positions provide support at current research council studentship rates plus fees.

Further details may be obtained from Professor D. J. Greenland, Department of Soil Science, University of Reading, London Road, Reading, Berks. (Ref: MN 29). (177)

UNIVERSITY OF BRISTOL

The University invites applications for the appointment of **DIRECTOR** of the Long Ashton Research Station, which will become vacant on August 1, 1975, on the retirement of Professor J. P. Hudson, M.B.E., G.M., M.Sc., Ph.D. The Director is a Professor of the University and Head of the Department of Agriculture and Horticulture. The post is graded in the rank of Deputy Chief Scientific Officer in the Agricultural Research Service at a salary of £7,279 to £8,080.

Suitably qualified candidates are invited to submit applications, together with the names and addresses of three referees, by October 21, 1974. Further particulars of the appointment may be obtained from the Secretary of the University, Senate House, Bristol BS8 1TH. (176)

THE GRASSLAND RESEARCH INSTITUTE

HURLEY, MAIDENHEAD, BERKSHIRE SL6 5LR

HEAD OF

AGRONOMY DEPARTMENT

Applications are invited for the above post which will become vacant from January 1, 1975. The Institute is grant-aided by the Agricultural Research Council and is an Associated Institution of the University of Reading.

The research programme of the Department is directed towards an understanding of how to grow and utilise grass and forage crops. The rôle and importance of the Department in the Institute is to be extended and will in future include work with grazing animals, and the assessment of management factors under a range of climatic and soil conditions.

Candidates should have a special knowledge of grass and forage crops, and experience of conducting and organising research in this field, and have a desire to translate scientific knowledge into agricultural practice.

The post is graded Senior Principal Scientific Officer on the scale of £6,300 to £7,141 per annum.

Superannuation under F.S.S.U. with a non-pensionable allowance of 4½% of salary to compensate for personal contributions.

Further information may be obtained from the Secretary to whom applications should be submitted before September 15, 1974. (175)

**ROTHAMSTED
EXPERIMENTAL STATION
HARPENDEN, HERTS. AL5 2JQ
SCIENTIFIC OFFICER**

required to help with the design, construction and development of specialised apparatus to be used in studies on coconut breeding.

The Overseas Development Administration is currently supporting work on mechanical systems for the extraction and processing of coconut pollen as part of a mass pollination programme aimed at improving coconut production. This temporary post (initially for a period of 1 year from September 1, 1974) has been provided to support work now being done at Rothamsted Experimental Station.

Qualifications: Pass degree, H.N.C., H.N.D., or equivalent. Practical ability in use of workshop equipment essential.

Salary in scale £1,435 to £2,329 per annum (under review) according to qualifications and experience. Superannuation with non-pensionable allowance of 5½% to offset personal contributions.

Applications to the Secretary quoting Ref. 2292 by August 22, 1974. (165)

**SURREY EDUCATION
COMMITTEE
EWELL COUNTY TECHNICAL
COLLEGE
REIGATE ROAD, EWELL, SURREY
DEPARTMENT OF BIOLOGICAL
SCIENCES**

Required as soon as possible:

LECTURER 1 to teach **CELL BIOLOGY** and/or **BIOCHEMISTRY** for H.N.C. in **MEDICAL LABORATORY SUBJECTS**. The College is an approved centre for this course. Some experience of medical **MICRO-BIOLOGY** or **HISTOPATHOLOGY** and an interest in research and problems of learning in the biomedical field would be advantageous.

Applicants with suitable experience will have the opportunity to teach to M.I.Biol. (Honours B.Sc.) level.

Salary: £1,800 to £2,874 per annum—for good honours graduate the scale is extended to £3,045 per annum. £118 London Allowance payable per annum. Generous relocation expenses and assistance with house purchase in approved cases.

Further information and application forms available from the Vice-Principal at the College upon receipt of a stamped addressed foolscap envelope. Applications to be returned within 14 days of the appearance of this advertisement. (173)

**THE UNIVERSITY OF MANCHESTER
RESEARCH ASSOCIATE IN
CHILD HEALTH**

Biochemist required with post-graduate research experience for this post in the Department of Child Health with a team investigating brain growth and development. Salary p.a. according to experience, up to £2,388 (£2,580 from October 1). Applications (quoting telephone number if possible) to Dr John Dobbing, Department of Child Health, Stopford Building, Manchester M13 9PT by July 20. Quoting ref: 152/74/N. (186)

**THE UNIVERSITY OF
LANCASTER**

Department of Biological Sciences

Applications are invited for a **LECTURESHIP** in either freshwater ecology or physiological ecology in the fields of plant/water relations or ion-uptake by plants.

Further particulars may be obtained (quoting reference L.832/D) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW, to whom applications (five copies), naming three referees, should be sent not later than July 28, 1974. (189)

Fisheries Research

Applications are invited for a permanent and pensionable post in the Fisheries Research Laboratory of the Department of Agriculture based at Coleraine, Co. Londonderry.

The successful applicant who may be appointed at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level will be required to undertake investigations primarily concerned with the rational exploitation of commercial species and the development of marine fish farming, particularly shellfish. A knowledge of population dynamics, culture of shellfish, and fish biology would be an advantage. Ability to drive would be an asset.

Senior Scientific Officer: £2,798-£3,895 (under review).

Over 25 and under 32 years of age with a first or second class Honours Degree in Zoology or Biology and at least 4 years relevant postgraduate experience, preferably in Fisheries Research.

Higher Scientific Officer: £2,221-£2,854 (under review).

Under 30 years of age with an Honours Degree as above and at least 2 years relevant post-graduate experience.

Scientific Officer: £1,435-£2,329 (under review).

Under 27 years of age with an Honours Degree as above.

Grading and commencing salary will be related to qualifications and experience.

Please write or telephone for an application form, quoting Ref. SB 186/74/N to Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26).

Completed forms must be returned to arrive not later than 25 July, 1974. (174)



**NORTHERN IRELAND
CIVIL SERVICE**

North East London Polytechnic

Faculty of Science

Department of Biological Science

Applications are invited for the following four

Research Assistant posts:-

- (1) To work under the supervision of Dr D. Bryce on the ecology of Diptera larvae in woodland soil.
- (2) To work under the supervision of Dr D. Bryce on the ecology of Chironomidae in a polluted river system.
- (3) To work under the supervision of Drs S. Ball and J. Humphreys on enzyme variation in coccidial parasites.
- (4) To work under the supervision of Dr K. J. Adams on investigations into the mechanisms of control of cell division.

For posts (1) and (2) candidates should have or expect to obtain a good honours degree or equivalent qualification in Biology, for post (3) a similar qualification in Biochemistry or Biology and for post (4) candidates should hold a good honours degree in the area of Cell/Molecular Biology or in Electronics and have an interest in the application of electronics to biological problems.

In each case the appointment will be initially for two years at a starting salary of £1,427 per annum and the successful applicant may be permitted to register for a higher degree of the C.N.A.A.

Application form and further details from:—

**Academic Staffing Officer (2),
North East London Polytechnic,
Forest Road, London, E17 4JB. Telephone: 01-527 2272. Ref: S/CF 132.**

Closing date: 25 July, 1974.

(181)

**UNIVERSITY COLLEGE DUBLIN
RESEARCH POSTS IN GENETICS
OF ANIMAL DEVELOPMENT**

A Postdoctoral Fellow and a Research Assistant are required for a 3-year project sponsored by the National Science Council, on the inheritance of appetite, and of muscle and fat development in animals. The project will involve research on mice and sheep in University College, Dublin and in the Agricultural Institute at Belclare, Co. Galway. Knowledge of quantitative genetics is essential.

Full details from:

**Dr J. C. McCarthy,
Faculty of Agriculture,
University College,
Glasnevin,
Dublin, 9,
IRELAND.**

(191)

S.U.N.Y. AT STONY BROOK

Several faculty positions at the Assistant and Associate Professor levels are available, beginning June, 1975, in the newly established Department of Pharmacological Sciences. Candidates with research interests in neurobiology, molecular biology, biochemistry or biophysics are sought. A minimum of two years postdoctoral experience is required. Closing date September 1, 1974. Send resume and references to Dr. Arthur P. Grollman, Chairman, Department of Pharmacological Sciences, S.U.N.Y. at Stony Brook, Stony Brook, New York 11790. An affirmative action, equal opportunity employer.

(182)

Research & Development Director £10,000 plus.

The Fertilizer Division of Fisons Limited produces and markets fertilizers throughout the UK and has a current annual turnover approaching £80m.

The Research & Development Director to be appointed will be directly responsible to the Managing Director for all research and development activities in process technology, chemistry and agronomy. He will control a main research unit and two outstations employing in total 350 people, of whom 80 are of graduate standard. Preferred age is 35-45. A five figure salary is envisaged plus car, good pension scheme and significant additional benefits. The appointment is based at Levington, near Ipswich, Suffolk.

The successful candidate will have a proven record of success at a senior level in the research and/or development field, not necessarily in a commercial organisation. Degree subject is less important than the ability to manage a multi-discipline R & D activity. A doctorate is preferred.

Please write briefly and in confidence to Mr. J. Mugliston, Divisional Director, Fisons Limited—Fertilizer Division, Felixstowe, Suffolk IP11 7LP.



(261)

BIOCHEMISTRY DEPARTMENT CHAIRMAN

STATE UNIVERSITY OF NEW YORK AT STONY BROOK

Nominations and applications for this appointment are invited. Qualifications should include an outstanding record of achievement in research, teaching and appropriate administrative experience.

The Department serves the educational needs of undergraduates in the College of Arts and Sciences, medical, dental and other students in the School of Basic Health Sciences and graduate students enrolled in PH.D. programs.

The State University of New York at Stony Brook is an Affirmative Action—Equal Opportunity Employer and encourages applications from women and members of American minority groups.

Appointment title and salary are dependent upon qualifications and experience. Send nominations and inquires to:

Dr Vincent P. Cirillo,
Chairman, Search Committee,
Department of Biochemistry,
State University of New York,
Stony Brook, New York 11794

(217)

BRENT AND HARROW AREA HEALTH AUTHORITY BRENT HEALTH DISTRICT

A vacancy occurs for a Technician or Junior Technician in the Cytology Section of the Histopathology Laboratory. Some experience preferred. This Laboratory deals with Gynaecological and non-Gynaecological Cytology as well as Chromosomal Examinations.

Further information from Chief Technician 01-965 5733 Ext. 385. Application forms from and returnable to:

Personnel Department (Ext. 610), Central Middlesex Hospital, Park Royal, London NW10 7NS. (180)

UNIVERSITY OF OXFORD POSTDOCTORAL BIOCHEMIST or CELL BIOLOGIST

required to work on mechanism of chromosome condensation at mitosis on grant from Cancer Research Campaign.

Applications and enquiries to Dr J. M. Barry, Department of Agricultural Science. (194)

UNIVERSITY OF READING DEPARTMENT OF MICROBIOLOGY

Applications are invited for a Postdoctoral Research Appointment to work on the purification and characterisation of the Tetramethylammonium Mono-oxygenase system of Bacteria. Candidates should be biochemists who have had experience in the techniques of Anzymology, protein purification and microbiology. Salary: £2,118 x £129 - £2,247 p.a. plus F.S.S.U.

A research assistant is also required to work on the biochemistry of the utilization of Ethors by micro-organisms. Applicants should be graduates in biochemistry or microbiology. The successful applicant will be required to isolate and culture micro-organisms, prepare and purify cell-free extracts and undertake biochemical studies on the enzymes of ether utilization. Salary scale £1,435 - £1,846 p.a.

These appointments are both for two years from October 1974 and are supported by S.R.C. grants.

Apply with curriculum vitae and the names of two referees, as soon as possible, to Dr. L. J. Zatman, Department of Microbiology, University of Reading, London Road, Reading, RG1 5AQ. (Ref: MN30). (199)

UNIVERSITY OF BRISTOL SCHOOL OF CHEMISTRY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a Science Research Council Postdoctoral Research Assistantship to work on the calculation of scattering cross sections in gas phase molecular collisions. The work will be directed towards the interpretation of crossed molecular beam experiments which examine inelastic collisions. A background in collision theory or computing experience will be an advantage.

The appointment, which is for one year in the first instance, with a possible extension to two years, is available from October 1974. Salary range up to £2,247 per annum.

Applications, with curriculum vitae and names of two referees, to Dr. G. G. Balint-Kurti, School of Chemistry, Bristol University, Bristol BS8 1TS, as soon as possible. (203)

THE EAST OF SCOTLAND COLLEGE OF AGRICULTURE invites applications for the post of HEAD OF CROP HUSBANDRY ADVISORY AND DEVELOPMENT DEPARTMENT

Applicants should have knowledge of R. & D. and Advisory aspects of crop production.

Salary scale (under review) £4,341 to £5,994.

F.S.S.U. with non-pensionable pay supplement to offset contributions.

Further particulars and application form from the Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (190)

**UNIVERSITY OF OXFORD
MICROBIOLOGY UNIT
DEPARTMENT OF BIOCHEMISTRY**

A Postdoctoral Research Assistantship is available, from October 1, 1974, to work on the synthesis of ribosomes by a mutant of *Escherichia coli*. The post is financed by the Medical Research Council and the initial salary is a present £1,821 p.a. The project is directed by Dr D. G. Wild from whom further information may be obtained and to whom applications should be sent. (193)

**HAWKER SIDDELEY AVIATION
LIMITED
Broughton Chester
FINANCIAL DIVISION
ASSISTANT FINANCIAL
ACCOUNTANT**

There exists an interesting position for a suitably qualified accountant or finalist (ACA or ACCA) to head our final accounts department. He will report directly to the Financial Accountant and be involved in the computation of weekly/monthly management reports, the preparation of final accounts, annual and four year forecasts and also be responsible for the nominal ledger and capital assets section.

This position is a monthly staff appointment and big Company employee benefits include contributory pension scheme with free life assurance, four weeks holiday after qualifying period, canteen facilities. A five day 37½ hour week is in operation. Please apply giving details of age experience and qualifications to Eryl V. Hughes, Employment Officer, Hawker Siddeley Aviation Limited, Broughton, Chester. Or telephone Chester 24646 Ext. 34. (200)

**JUNIOR TECHNICAL OFFICER
(HORMONES & BLOOD PRODUCTS)**

Applications are invited from recently qualified graduate CHEMISTS or BIOCHEMISTS for a post within the Division of Hormones & Blood Products. The successful applicant will join a group working on the isolation and identification of peptides and enzymes and on the development of chemical methods to complement bioassay techniques.

Salary on scale £1,407 - £2,073 including London Weighting. Salary award pending. The Institute is situated in pleasant surroundings close to Hampstead Underground Station. There is an active Sports & Social Club and Superannuation benefits.

Please apply quoting Ref. 0026 giving brief details (an application form will be sent to you) to R. S. Dunn, Personnel Officer, National Institute for Biological Standards & Control, Holly Hill, Hampstead, NW3 6RB. Tel: 435 2232 (198)

**THE QUEEN'S UNIVERSITY
OF BELFAST**

**CHAIR OF
PHYSIOLOGY**

Applications are invited for the second Chair of Physiology from 1st October, 1974 or such later date as may be arranged. The successful applicant will be responsible for the day to day running of the section of the department dealing with teaching and research in Histology. Candidates who have medical qualifications would be considered for a joint appointment with the Eastern Health & Social Services Board with a University salary of £7,125 with contributory pension rights under the F.S.S.U.; an additional salary is paid by the Eastern Health & Social Services Board on a sessional basis for hospital services undertaken over and above University duties. Candidates who are not medically qualified would hold the University Chair with a salary of £6,810, with F.S.S.U.

Applications should be received by 31st August, 1974. Further particulars may be obtained from The Personnel Officer, The Queen's University of Belfast, BT7 INN, Northern Ireland. (Please quote Ref. 74/N). (206)

Opportunity Overseas

St. Helena

Fisheries Adviser

To be fully associated with the operation of a Pilot Fishing Survey to be carried out over 1-2 years with the objective of increasing the country's fish supply to its domestic market and to train a Counterpart. Applicants should have a degree in a Natural Science and have 5 years postgraduate experience. Experience in pelagic and demersal tropical fishery especially with tunny and crayfish also required. Appointment for 3 years.

Salary in scale £3,750 to £5,250 p.a. plus a tax free overseas allowance in scale £150 to £670 p.a.

Other benefits include free family passages, paid leave, children's education allowances free accommodation and medical attention.

Applicants should normally be citizens of, and permanently resident in the United Kingdom.

For full details, application form and booklet about St Helena, please apply giving age and brief details of qualifications and experience to:

Appointments Officer

Ministry of Overseas Development

Room E301, Eland House
Stag Place, London, SW1E 5DH

(243)



UNIVERSITY OF CAPE TOWN



CHAIR OF MARINE GEOSCIENCE

Applications are invited for the newly created Max Sonnenberg Chair of Marine Geoscience, which the University hopes to fill at an early date. The salary scale is R8 100 x 300 - R9 900 per annum plus a 15% pensionable allowance.

Applicants should submit a full curriculum vitae indicating their teaching and research experience and interests, present salary, and the earliest date on which they could assume duty, and should furnish the names and addresses of three referees whom the University may consult.

Further information should be obtained from The Registrar, University of Cape Town, Private Bag, Rondebosch, C.P.

Applications must reach the registrar by 30th September, 1974.

Appointment will be subject to a satisfactory medical certificate and the University reserves the right to appoint a person other than one of the applicants or to make no appointment. (213)



Research Opportunities in Pharmacology

Smith Kline and French Laboratories Limited carry out a key rôle in an international research effort at laboratories based in Welwyn Garden City. Owing to expansion in various areas of our research programme we have vacancies in our Pharmacology Department for

A Senior Pharmacologist

preferably with at least 10 years postdoctoral experience in industrial or academic research in the general field of inflammation.

Post Doctoral and Graduate Pharmacologists

with Ph.D's, first degrees or the equivalent in pharmacology or a related subject and with an interest in research into aspects of the pharmacology of the cardiovascular, central nervous, digestive or reproductive systems or into aspects of toxicology of immunology.

Junior Pharmacologists

with H.N.C., O.N.C. or the equivalent, as well as those with lesser qualifications but with an interest in pharmacology. Day release will be allowed to enable suitable qualifications to be obtained at the company's expense.

You can expect to benefit from being part of a large organisation both in remuneration and career opportunities. Salary will depend on qualifications and experience in each post.

Those interested should write with brief career details to:

**A. Maltby, Personnel Officer,
Smith Kline and French Laboratories Limited,
Mundells,
Welwyn Garden City, Herts.**

(260)

A member of the worldwide **SmithKline Corporation**

The Lister Institute of Preventive Medicine

(University of London) Elstree, Herts.

requires a

BACTERIOLOGIST

to undertake investigative and developmental work on bacterial vaccines and therapeutic sera. This post is one of responsibility and offers plenty of scope for interesting and rewarding research; it is suitable for a worker with a doctoral degree or of comparable seniority. Salary in accordance with experience and qualifications. Superannuation under F.S.S.U.

Applications to:

Secretary, Lister Institute of Preventive Medicine, Elstree, Hertfordshire.

(239)

SOUTH GLAMORGAN AREA HEALTH AUTHORITY TENOVUS INSTITUTE FOR CANCER RESEARCH

HEATH, CARDIFF

SENIOR SCIENTIFIC OFFICER

required for the Supra-regional Steroid Assay Service Laboratory of the above Institute. Experience in steroid analysis necessary and knowledge of automated techniques useful.

SALARY: £2,964 to £3,843 according to age, qualifications and experience

APPLICATIONS: Forms available from and further particulars regarding the post may be obtained from Professor K. Griffiths, Institute Director (Tel. 755944, Ext. 2579).

(223)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY DEPARTMENT OF MATHEMATICS RESEARCH ASSISTANT

Applications are invited for the temporary post of Research Assistant in Theoretical and Experimental Polymer Rheology. The Department has a strong Group undertaking research in the Mechanics and Physics of Solids and Fluids, and it possesses excellent facilities for experimental investigations in Rheology. There is direct access to extensive computer facilities in the University and the Institute. The successful candidate will be either a mathematician who wishes to test theories experimentally or an experimental scientist, and he will normally be expected to pursue researching leading to a higher degree of the University.

Salary—£1,764 per annum with F.S.S.U. rising to £1,839 per annum for the second and any subsequent years.

Applications, giving details of education, experience and research interests should be addressed to Dr. A. Kaye, Mathematics Department, U.M.I.S.T., Sackville Street, Manchester, M60 1QD, not later than 26th July, 1974. (201)

INSTITUTE OF OPHTHALMOLOGY (UNIVERSITY OF LONDON)

Judd Street, London WC1H 9QS

RESEARCH ASSISTANT (GRADUATE)

required to join team concerned with pharmacological aspects of ocular inflammation. Salary £2,118 p.a. plus £162 London Allowance. Applications to Professor E. S. Perkins at above address, with names and addresses of two referees. (218)

THE LONDON HOSPITAL

and the

NORTH EAST THAMES REGIONAL HEALTH AUTHORITY

have vacancy for a Non-resident SENIOR REGISTRAR in the GERIATRIC SERVICE for The London Borough of Tower Hamlets. A large part of the successful candidate's time will be spent in the geriatric/psycho-geriatric assessment unit at The London Hospital (Mile End). He/she will also be a member of the Department of General Medicine and will have opportunities for further education in a special field. The post gives an opportunity for teaching in a comprehensive district geriatric service.

Applications (no forms provided), giving the names and addresses of three referees, should be received by July 27, 1974 and addressed to: The Medical Staffing Office, The London Hospital, Whitechapel E1 1BB. Further particulars can be obtained from Dr Silver, Mile End (01-980 4855). (219)

THE QUEEN'S UNIVERSITY OF BELFAST

DEPARTMENT OF CHEMISTRY

Applications are invited for two posts, tenable from 1st October, 1974, of RESEARCH ASSOCIATE (postdoctoral), and RESEARCH ASSISTANT for research in polymer chemistry. The work will be concerned with the use of ¹³C nmr spectroscopy to elucidate the structure of polymers, and with the preparation and use of isotactic polypropylenimine as a template polymer. Appointments will be made for one year in the first instance. Salary, at appropriate points according to experience, will be on the scales Research Associate £2,118 to £2,247 to £2,412; and Research Assistant £1,239 to £1,818.

Applications, giving the names of two referees, should be received by the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland, Not later than 31st July 1974. (207)

Fermentation Microbiologists

Shell Research Limited have a number of vacancies in the Fermentation Division of their Woodstock Laboratory at Sittingbourne, Kent for scientists and technical assistants to work in a multi-disciplinary team on process research and development towards the manufacture of single cell protein.

Senior Microbiologist

A Senior Microbiologist is required to lead a team engaged in isolation and characterisation of new cultures, monitoring fermentation systems and studying interactions in mixed cultures. The work will involve the use of the laboratories' extensive continuous culture facilities and will play a central role in developing commercial processes. A mature graduate is required for this post, probably over 30 years of age, with proven ability and, preferably, with experience in microbial screening or taxonomy.

Microbiologists

There are two vacancies for graduates in microbiology to work on isolation and characterisation of cultures and to study symbiotic interaction. You should have a

good honours degree in microbiology; preference will be given to candidates with proven research ability.

Technical Assistants

Two Technical Assistants are required to work on microbial taxonomy and microbial control in fermentation processes. You should have an H.N.C. in applied biology or the equivalent in a relevant subject, basic training in microbiology and practical experience in standard microbial techniques. A further vacancy exists for a technical assistant to work on bench scale continuous fermentation systems. Preference will be given to those candidates with one or more 'A' levels in relevant subjects or equivalents who have had some basic training in microbiology and, preferably, experience with fermentation equipment.

The levels of appointment will depend on background and experience, and the salaries offered will be competitive.

Please write, giving full details regarding personal background and experience, indicating for which position you wish to apply, to Shell Research Limited, Recruitment Division (N), PNEL/34, Shell Centre, London SE1 7NA.



(226)

PHARMACOLOGISTS/ TOXICOLOGISTS

Honours Graduates with some experience required for Research and short and long term toxicological studies of compounds of potential therapeutic importance. Excellent opportunities for advancement in modern, well-equipped laboratory. Pension and Assurance Scheme. Application Forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (209)

UNIVERSITY OF MANCHESTER DEPARTMENT OF MEDICAL BIOCHEMISTRY RESEARCH ASSOCIATE

A postdoctoral biochemist is required to work under the direction of Dr. S. Itzhaki on a research project supported by the Cystic Fibrosis Research Trust, studying factors affecting the uptake of macromolecules by cells. Appointment for one year initially from October 1st, at a salary of £2,118 p.a. Further particulars and application forms (returnable by July 29th) from the Registrar, The University, Manchester M13 9PL. Quote ref: 155/74/N. (215)

Technician-R&D

G. D. Searle are one of the world's leaders in medical research. Our Research and Development department at High Wycombe is engaged in fundamental work in the areas of Immunology, Biochemistry and Molecular Biology. A vacancy has arisen for a Technician to assist in the extraction and assessment of macromolecules for various biological activities.

Ideally you should have experience of working in a hospital or biochemical laboratory, and have a qualification up to HNC/IMLT level. Anyone about to leave school/college and who is keen to pursue a technical career, should also apply. Full training will be given, including the facility for part-time study.

An attractive commencing salary will be offered, and conditions of employment include 4 weeks' holiday in each full calendar year of employment, Pension Fund, Restaurant and an active Social Club. Assistance with moving will be given in appropriate circumstances.

Initially please contact H W Cooke, Personnel Manager, Research and Development, G D Searle & Co Ltd, Lane End Road, High Wycombe, Bucks, OR telephone him on High Wycombe 21124 ext. 130, quoting reference T/119.

The SEARLE logo, consisting of the word "SEARLE" in a bold, sans-serif font, enclosed within a black rectangular box.

(262)

Chemist/ Biochemist

for pollutants research up to £2,500p.a.

The expansion of the Department of Environmental Physiology has created the need for a young chemist or biochemist to join a team studying the toxicology of a variety of inhaled substances.

They will be responsible for the generation and monitoring of experimental test atmospheres and expected to develop new and improved systems of generation for a variety of gaseous and particulate pollutants. Therefore previous experience in the fields of particle size analysis, mass monitoring techniques, and quantitative analysis would be a distinct advantage. Salaries up to £2500 per annum are offered together with a pleasant working environment close to Huntingdon within easy reach of Cambridge and fast rail and road links to London, excellent fringe benefits including 4 weeks holiday and an active sports and social club.

Further information and application form can be obtained from:

Mrs. Nancy McCree, Recruitment Officer,
Huntingdon Research Centre,
Huntingdon, PE18 6ES,
Telephone Woolley 431, Ext. 251.

H.R.C.

(210)

THE UNIVERSITY OF MANCHESTER RESEARCH ASSOCIATE IN CHILD HEALTH

Biochemist required with postgraduate research experience for this post in the Department of Child Health with a team investigating brain growth and development. Salary p.a. according to experience, up to £2,388 (£2,580 from October 1st). Applications (quoting telephone number if possible) to Dr. John Dobbing, Department of Child Health, Stopford Building, Manchester, M13 9PT, by July 20th. Quote ref. 152/74/N.

(214)

UNIVERSITY OF RHODESIA FACULTY OF SCIENCE CHAIR BIOCHEMISTRY

Applications are invited for appointment to the Chair and Headship of the Department of Biochemistry.

Salary Scales and Conditions (Approx. Stg. equiv.): £7,430 by £277 to £8,812, (Approx. US\$ equiv.): \$18,008 by \$670 to \$21,359.

Family passages and allowance for transport of effects; installation loan; travel allowance for Sabbatical and Contact Visits; superannuation and medical aid schemes. For persons recruited from outside Rhodesia, unfurnished accommodation on or near the campus and within easy reach of good schools is guaranteed for up to three years.

Applications: Six copies, giving personal particulars (including full names, place and date of birth), qualifications, experience, and publications, and naming three referees, should be sent by **August 31, 1974** to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP.167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. Applicants from outside Southern Africa should send an additional copy to the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further information may also be obtained. (212)

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Paddington, London W2 1PG

Applications are invited from engineering and physics graduates and chartered engineers, with not less than four years postgraduate training/experience in applied electronics research, for an appointment in Bio-Engineering Department with team conducting applied research leading to design and development of novel instruments and devices for use in a variety of specialties. Current activities include projects in surgery, obstetrics and gynaecology, urology and bacteriology. Excellent facilities. Starting salary on scale the maximum of which is £3,285 plus £162 L.A. p.a. Further details from Dr R. E. Trotman. Apply, The Secretary. (197)

TECHNICIAN or JUNIOR TECHNICIAN

required to undertake general laboratory duties in new M.R.C. Mammalian Development Unit. Experience in histology, photography, or in vitro embryo culture desirable. Applicants should preferably have experience in a biological research laboratory, or hold a University degree in a relevant subject. Salary according to age and experience. Applications to Dr M. L. Buehr, M.R.C. Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE. (225)

GRADUATE IN BIOCHEMISTRY

or related fields required to join research group studying effects of diet on metabolism of carcinogens. It is hoped that the successful applicant will work for a degree of the University of London. Applications, giving full details of qualifications, to The Secretary, The Medical College of St Bartholomew's Hospital, West Smithfield, London EC1A 7BE quoting reference 661. (222)

Avon Area Health Authority (Teaching)

Area Physicist

Salary scale: £5190—£5919 plus responsibility allowance

The Avon Area Health Authority (T), in co-operation with the South Western Regional Health Authority, propose to appoint a Top Grade Physicist, with special responsibility allowance, to act as Head of the Avon Area Medical Physics Service and as Adviser on Medical Physics to the South Western Regional Health Authority.

The Appointment is consequent upon the retirement of Dr. H. F. Freundlich later this year.

Whitley Council Terms and Conditions of Service apply.

Applications, giving the names of two referees, should be sent by the **8th August, 1974**, to The Personnel Officer, Avon Area Health Authority (T), National Westminster Court, Little John St., Bristol, BS1 2EE (Tel. Bristol 293541), from whom further particulars may be obtained.



(238)

JUNIOR TECHNICIAN

required for a research project in the Department of Surgery. Apply in writing, stating age and giving details of education and experience, if any, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. D.S.3. (195)

**GLASSHOUSE CROPS RESEARCH
INSTITUTE
SCIENTIFIC OFFICER**

required for Entomology Department to join team studying INSECT VIRUSES in relation to crop protection, involving careful, detailed laboratory work. Applicants should be over 21 with degree or equivalent qualification in virology or biochemistry. Salary within scale £1,435 to £2,329 (under review) according to qualifications and experience. Contributory superannuation scheme with additional allowance of 54% of salary to offset contributions. Further particulars from Secretary of the Institute, Worthing Road, Rustington, Littlehampton, Sussex, to whom applications giving full biographical details should be sent by 31 July. (208)

GUY'S HOSPITAL MEDICAL SCHOOL

Applications are invited from medical graduates for the post of Junior Lecturer or Lecturer (full time or part time) in the Department of Physiology. The duties include teaching practical physiology, with man as a subject, and with emphasis on relevance to medicine.

The person appointed will have opportunity to follow his own line of research or to join in with current work. There are excellent facilities for co-operative research with other departments, both academic and clinical. For a recently registered graduate there are good facilities for preparation for the examinations for higher diplomas.

Salary (October 1974 rates, full time) for Junior Lecturer £2,580 to £3,285 and for Lecturer £3,285 to £4,896, plus £162 London Allowance and F.S.S.U.

Application forms are obtainable from the Dean, Guy's Hospital Medical School, London Bridge SE1 9RT and should be returned not later than July 29, 1974. (221)

UNIVERSITY OF BIRMINGHAM

Applications are invited for the post of

CURATOR

of the University Botanic Gardens.

Candidates should possess the relevant horticultural qualifications and good practical experience. Previous experience in the organisation of botanic gardens for teaching and research purposes is desirable but not essential. Salary is from £2,817 to £3,201 according to experience and qualifications. Rented accommodation is available.

Please apply to:

The Senior Assistant Secretary,
University of Birmingham,
P.O. Box No. 363,
Birmingham B15 2TT.
Reference: 133/B/288.

(224)

**NATIONAL COUNCIL FOR
SCIENTIFIC RESEARCH**

Applications are invited for an immediate vacancy for a:

**SENIOR PRINCIPAL PROFESSIONAL
OFFICER IN ANIMAL PRODUCTION,
ANIMAL PRODUCTIVITY
RESEARCH UNIT**

Applicants must hold postgraduate University degrees in agriculture, animal science, veterinary science, or animal nutrition, animal physiology or animal management. Candidates should have extensive practical research experience. The successful candidate will be responsible for developing, in relation to the Animal Productivity Research Programme, policy on animal production research and supervising the work of the Research Unit. He would be required, under the direction of the Council to collaborate with Government Departments and parastatal organisations.

The salary scale is in the range of K7,000 by K200 to K7,600. A gratuity of 20 to 30 per cent, depending on the length of service is paid to non-Zambians. Travel and education allowances are available for minor dependent children. Comfortable accommodation with basic furniture will be provided at low rental.

Applications giving full personal details, qualification and experience and naming three referees should be sent to:

The Secretary-General,
National Council for Scientific Research,
P.O. Box CH. 158,
Chelston,
LUSAKA,
Zambia.

(228)

Graduates & Technicians

Metabolism & Pharmacokinetics with the international leaders

We are Europe's largest research unit operating in the field of Life Sciences. As a result of our rapid and continuing expansion there are new vacancies for graduates and technicians in our Department of Metabolism and Pharmacokinetics.

Candidates should possess or be studying for an appropriate qualification (HNC, MIBiol, GRIC, BSc, PhD). The work will involve studies of the metabolic fate and pharmacokinetics of labelled drugs, pesticides and food additives using radioisotopes. Relevant experience would be desirable but is not essential. Proven practical ability is of prime importance.

These vacancies provide an ideal opportunity for suitably qualified scientific staff to join one of the leading drug metabolism research groups in the UK. Attractive career opportunities based on merit exist. The centre is located near Huntingdon in an attractive part of the country with good access to London by road and rail. We offer excellent working conditions together with progressive general benefits, a competitive salary and the usual pension and other fringe benefits.

Please write or telephone for an application form to

**Mrs. Nancy McCree, Recruitment Officer,
Huntingdon Research Centre,
Huntingdon PE18 6ES.**

**Tel: Woolley (STD 048 086) 431,
quoting reference number 301.**

H.R.C.

(211)

Lister Institute of Preventive Medicine

(University of London)

Elstree, Herts.

PRODUCTION MANAGER

The Lister Institute requires a Manager to take charge of the production for human use of bacterial vaccines, virus vaccines and therapeutic sera at its Elstree Laboratories. Applicants must have considerable experience in the organisation and day to day running of a unit producing biological materials; and the person appointed will play a major part in planning and implementing a considerable expansion in production. The salary will be in accordance with experience and qualifications and will be appropriate to a post of this seniority. Superannuation under F.S.S.U.

Applications to: **The Secretary,
Lister Institute of Preventive Medicine,
Elstree, Hertfordshire.**

(240)

CSIRO**AUSTRALIA****DIVISION OF COMPUTING RESEARCH
CANBERRA****RESEARCH SCIENTIST**

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD:**COMPUTER
STIMULATION OF
BIOLOGICAL SYSTEMS**

GENERAL: The Division of Computing Research, with headquarters in Canberra, A.C.T., has recently installed a CDC 76 (commonly called a 7600 computer). The capacity and expansion potential of the configuration provide an unsurpassed tool for research and development projects. A rapidly expanding network of mini computers at CSIRO research centres enables the concentration of interactive and batch work in Canberra. Currently twelve batch terminals and more than one hundred interactive terminals are connected.

Major research studies at present include simulation techniques, operating systems, computer languages, data-base management and artificial intelligence. A consulting service is also provided to other CSIRO Divisions covering all aspects of computer use including applications packages, data logging, numerical analysis and the methodology of modelling and simulation.

An increasingly important part of the Division's activities is concerned with computer modelling and simulation of systems having biological components. Projects of interest include insect and parasite control, plant and animal growth, nutrient cycling in grazed pastures, aquatic ecosystems and arid-zone ecosystems. Areas of research cover: continuous, discrete and specialised simulation languages, numerical methods for use with such languages, probabilistic processes, and hierarchical techniques for the modelling and optimisation of complex systems.

DUTIES: To conduct research on improved methods for the computer modelling and simulation of complex systems, with special reference to biological systems.

QUALIFICATIONS: A Ph.D. degree in an appropriate biological field or equivalent qualifications together with demonstrable research ability. Considerable computational skill and ability to develop computer models are desirable.

SALARY: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a.

TENURE: The position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 900/251, should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64-78 Kingsway,
LONDON,
WC2B 6BD

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

by the 9th August, 1974.

(244)

**UNIVERSITY OF WESTERN
AUSTRALIA
PERTH**

CHAIR OF GEOLOGY

Applications are invited for the above-mentioned appointment which will become vacant on the retirement of Professor R. T. Pridar on December 31, 1975. No specific field of interest has been assigned to the position and specialists in any of the major areas of Geology will be considered. Policy regarding headships of departments in the University is currently under review, but it is intended that the appointee to the Chair will be Head of the Department of Geology for an initial term of at least three years.

The salary for a Professor is \$A19,614 (currently about £12,325) p.a. Benefits include superannuation similar to F.S.S.U., fares to Perth for appointee and dependent family, removal allowance, study leave and long service leave and housing loan scheme. Further information including conditions of appointment may be obtained from the Staffing Officer in the University, or from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications in duplicate stating full personal particulars, qualifications, experience and the names and addresses of three referees should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by August 17, 1974. (230)

**UNIVERSITY OF
NEWCASTLE UPON TYNE
DEPARTMENT OF PHARMACOLOGY
RESEARCH ASSOCIATE
(CLINICAL NEUROPHYSIOLOGIST)**

Applications are invited from medically qualified or other suitably qualified candidates, with experience in clinical neurology, for the post of Research Associate in Neurophysiology in the Department of Pharmacology to join a multidisciplinary research team investigating the actions of psychotropic drugs in man. The appointment will begin on October 1, 1974 (or earlier if possible) and will be for one year in the first instance, renewable up to a maximum of three years in all. Commencing salary according to age, qualifications and experience, will be within the following ranges:

Medically qualified £3,021 to £3,363 p.a.

Non-Medically qualified £2,118 to £2,931 p.a.

Applications giving the names of two referees should be submitted within three weeks of the appearance of this advertisement to Professor J. W. Thompson, Clinical Pharmacology Unit, 13 Framlington Place, Newcastle upon Tyne NE2 4AB from whom further information may be obtained if required. (232)

**UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF BIOLOGY
POSTGRADUATE RESEARCH
ASSISTANT**

Applications are invited for an M.R.C. funded Research Assistantship in a group studying the control of gene expression during blood cell differentiation, under the supervision of Dr N. Maclean. Applicants should have a good honours degree in biology, biochemistry or genetics, and may be permitted to register for a higher degree. In the latter case the salary will be appropriately reduced.

The appointment will be for three years from October 1, 1974 (one year in the first instance) and salary will be in a range up to £1,700 per annum.

Applications giving a curriculum vitae and the names of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH, to arrive not later than July 22, 1974. Please quote reference Na/253/R. (233)

**UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY
TECHNICIAN—GRADE 5
(PROTEIN CHEMISTRY)**

A TECHNICIAN will be required from October 1, 1974 in connection with research (Professor W. V. Shaw) into the structure, function, and evolution of proteins. The techniques used will include conventional and novel methods of peptide purification, and automated approaches to primary sequence determination. Applicants must have experience in Protein Chemistry.

Please write to Chief Technician, Department of Biochemistry, University of Leicester, Leicester LE1 7RH by August 15, 1974. (236)

Royal Hospital for Sick Children

Department of Pathology

Applications are invited for two vacancies

(1) **GRADUATE**, preferably postdoctoral for research into glycoprotein production by cultured cells in relation to Cystic Fibrosis. Previous experience in this field not essential.

(2) **GRADUATE AS TECHNICIAN** or **TECHNICIAN** for research on the lysosomal enzymes of cultured cells in inborn errors of metabolism. Previous experience in this field not essential.

Applications in writing, giving names and addresses of two referees to the Sector Administrator, Royal Hospital for Sick Children, 1 Billbank Terrace, Edinburgh EH9 1LN.

(227)

ST GEORGE'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

Tooting, London SW17 0QT

DEPARTMENT OF PHYSIOLOGY

HISTOLOGY TECHNICIAN GRADE 3

Applications are invited for the post of Technician Grade 3 to assist with an M.R.C. programme of research on experimental lung disease with emphasis on neurohistology and mucus production. This recently formed department has an active interest in light microscopy, histochemistry and electronmicroscopy of the lungs. Some experience in these techniques is preferable but is not a necessary requirement.

Salary scale £1,650 to £1,920 (under review) plus London Allowance, depending on experience.

Applications should give full details of qualifications, experience and present employment, and be sent to Professor J. G. Widdicombe at above address.

(237)

NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited from suitably qualified persons for the post of:

BIOMETRICIAN

Applicants must have post-graduate degree or experience in mathematics or statistics with considerable research experience in sampling and experimentation. Experience in sample survey techniques, and biometrics research is desirable.

The Unit is primarily a consulting group and collaborates with various research units of the Council in fields such as animal productivity, forestry and insect ecology. It also advises and assists organisations outside the Council on statistical problems.

Salary according to qualifications and experience on the salary scales indicated below but a higher salary would be offered to exceptionally well-qualified and experienced senior scientists.

Senior Professional Officers K4,680 by K240 to K5,640.

Principal Professional Officers K5,840 by K240 to K6,800.

For Zambians there is a superannuation scheme. Non-Zambians will be paid a gratuity of 25% of aggregate basic salary earned during service of not less than thirty months.

Accommodation will be provided on an economic rental basis but not exceeding 10% of basic salary. Hard furniture will be provided. For non-Zambians travel and educational allowances are available for minor dependent children attending school outside Zambia.

Applications giving full personal details, qualifications, experience and naming three referees should be submitted to

The Secretary-General,
National Council for Scientific Research,
P.O. Box CH. 158,
Chelston,
LUSAKA,
Zambia.

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CSIRO

AUSTRALIA

DIVISION OF COMPUTING RESEARCH CANBERRA

RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD:

OPERATING SYSTEMS

GENERAL: The Division of Computing Research has a Control Data Cyber 76 Computer (commonly called a 7600) in Canberra. With processor capable of averaging fifteen million instructions per second, central memory exceeding three million characters, disc capacity of twelve hundred million characters, and with substantial expansion capacity, the configuration provides an unsurpassed tool for research and development projects.

The Cyber 76 computer is currently linked to a network of batch terminals by a Control Data 3600 Computer. This will be replaced by a pair of more powerful "front-end" computers in 1975.

A rapidly expanding network of mini computers at CSIRO research centres is linked by Australian Post Office data services and enables the concentration of interactive and batch work in Canberra. Currently, about thirty batch terminals and about two hundred interactive terminals are connected.

DUTIES: To work as part of a team engaged in maintenance and development of operating system software for both the Cyber 76 and the new front end computers. Members of the team are expected to take some responsibility for day-to-day system maintenance but will also need the ability to undertake original and major development projects without detailed supervision or guidance.

Areas of likely development include the provision of interactive access to the Cyber 76 and front end computers, job scheduling, archival file storage and connection to communication networks.

QUALIFICATIONS: A Ph.D. degree (or equivalent) in computing science, together with demonstrable research ability. Previous experience in operating systems work is essential.

SALARY: Appointment will be made within the ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a.

TENURE: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 900/248, should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64-78 Kingsway,
LONDON WC2B 6BD

by August 9, 1974.

Applications in U.S.A. and Canada should be sent to:

The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(246)

CSIRO**AUSTRALIA****DIVISION OF ANIMAL GENETICS****NORTH RYDE NSW**

RESEARCH SCIENTIST/ SENIOR RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD:

POPULATION AND QUANTITATIVE GENETICS, POULTRY

GENERAL: The Division of Animal Genetics is concerned with research in genetics which has immediate or potential application to animal production. It has field stations and laboratories at which sheep, beef and dairy cattle work is in progress, and a poultry unit located at the Divisional headquarters, North Ryde, N.S.W. The work of the headquarters laboratory also includes mice, *Drosophila*, *Paramecium*, microbial and viral genetics, molecular and developmental genetics and theoretical population genetics.

DUTIES: To cooperate with present staff in carrying out the current programme of research in the genetics of egg production and in the further development of that programme or of other relevant areas of the genetics of poultry production traits. The emphasis in the current programme is on selection for higher ovulation rate and on the genetics of variability of egg weight. The appointee will have adequate freedom to pursue personal interests, experimental or theoretical, within the overall programme.

QUALIFICATIONS: An appropriate Ph.D. degree or equivalent qualifications supported by satisfactory evidence of research ability. Satisfactory knowledge and experience of the statistical and computer methods applicable to analysis of population or quantitative genetics experiments are essential. Some experience of working with poultry would be an advantage.

SALARY: The appointment will be within the salary ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a.

TENURE: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications giving full personal and professional details, the names of at least two professional referees, and quoting Reference Number: 675/361 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64-78 Kingsway,
LONDON WC2B 6BD

by August 9, 1974.

Applications in U.S.A. and Canada should be sent to:

The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(247)

UNIVERSITY OF CAMBRIDGE**DEPARTMENT OF ZOOLOGY**

Research Assistant to work with Dr M. Burrows on neuronal mechanisms of insect flight. This three year post is supported by the Nuffield Foundation. Salary £1,467 to £2,388 p.a. Apply to Dr M. Burrows, Department of Zoology, Downing Street, Cambridge CB2 3EJ. (241)

UNIVERSITY OF BIRMINGHAM**DEPARTMENT OF PHYSICS****RESEARCH ASSOCIATESHIP**

Applications are invited for an appointment of up to three years under a Science Research Council grant for 'Experimental study of scattering of light by liquid and solid helium'. Candidates should have a Ph.D. in Physics. Experience in Cryogenics or high resolution Spectroscopy an advantage.

Salary on scale: £1,758 to £2,412 (exceptionally £2,931). Plus F.S.S.U.

Applications (3 copies), naming 3 referees should be submitted to the Assistant Registrar (S), P.O. Box 363, Birmingham B15 2TT by August 12, 1974. Please quote reference: NP2. (250)

CALL FOR PAPERS

Third International Conference on the Physics and Chemistry of Asbestos Minerals, August 17-21, 1975.

The Conference will be held at Laval University in Quebec City, Canada. Those interested in presenting a paper should communicate without delay with Dr C. A. Olivier, Mineral Research Center, Department of Natural Resources of Quebec, Canada CIV 4C7.

Sessions on the following topics are planned:

Physics—Surface properties; Mechanical properties; Electrical properties; Magnetic properties; Electron microscopy.

Chemistry—Composition; Spectroscopy; Crystal structure; Solid state reactions (dehydration, oxidation, reduction); Reactions with solutions.

Geochemistry—Formation; Synthesis; Phase rule studies; Trace elements.

Technology—Progress in applications of the properties of asbestos to industrial processes and products (e.g. asbestos-cement, reinforced plastics; insulation materials; textiles, etc.).

Biological Research in Asbestos—Biochemical and Biophysical effects of asbestos minerals. (255)

NURSING OPPORTUNITIES IN AUSTRALIA**NATHALIA DISTRICT HOSPITAL**

NATHALIA, VICTORIA •

**GENERAL AND MIDWIFERY
SISTERS**

are invited to apply for two vacancies at this 18-bed hospital. Salary is currently \$A130.90 per week plus penalties with an above award payment of \$156.00 paid half yearly.

The hospital is modern and the nurses' home comfortable. Nathalia is situated in central Victoria in the Murray Valley and has a mild and sunny climate. Regular bus service to Melbourne and other provincial cities. All social and recreational facilities are available locally.

Help will be given successful appointees in travelling to Australia.

Apply without obligation to Matron B. Millar, District Hospital, Nathalia 3638, Australia. (256)

**AMGUEDDFA GENEDLAETHOL
CYMRU****NATIONAL MUSEUM OF WALES****APPOINTMENT OF****ARCHAEOLOGICAL CONSERVATOR**

Applications are invited for the new post of ARCHAEOLOGICAL CONSERVATOR (Research Assistant I or II) in the Department of Archaeology. The successful candidate will be in charge of the existing departmental laboratory, in which the conservation staff of the Council of Museums in Wales also enjoy facilities, and will be responsible to the Keeper of Archaeology. Applicants should have appropriate qualifications and experience in Conservation and particular knowledge of casting techniques and pottery restoration would be an advantage.

The grading of the post, either Research Assistant I (Salary scale £2,332 to £2,997) or Research Assistant II (Salary scale £1,507 to £2,445), and the starting salary in the appropriate grade will depend upon age, qualifications and experience. Contributory superannuation. Generous leave.

Further particulars and application forms may be obtained from the Secretary, National Museum of Wales, Cardiff CF1 3NP to whom applications should be sent by Monday, August 19, 1974. (257)

**NATIONAL INSTITUTE FOR
MEDICAL RESEARCH
MILL HILL, LONDON
JUNIOR TECHNICAL OFFICER**

required to assist on the functional characterisation of the muscarinic cholinergic receptor. This work involves both biochemical and pharmacological techniques. Applicants should preferably have a degree in a relevant field and laboratory experience. Salary on scale to £2,199 p.a. (under review). Please write giving details of age, experience and qualifications to J. H. Woodcock, Personnel Officer, N.I.M.R., The Ridgeway, Mill Hill, NW7 1AA, Tel: 01-959 3666, quoting ref: JTO/MP (251)

**THE BRITISH COUNCIL
invites applications for the following post:
LECTURER IN STRUCTURAL
GEOLOGY
(THAILAND)
UNIVERSITY OF CHIENGMAI**

Men preferred between 30 and 50. Master's degree from a British university; at least 5 years' relevant experience.

Salary: £3,541 to £4,710 p.a.

Benefits: Free furnished accommodation, overseas and education allowances; terminal grant.

Two-year contract, usually renewable for a further period. Return fares are usually paid. Local contracts are guaranteed by the British Council.

Please write, quoting the reference number, for further details and an application form to The British Council (Appointments), 29 Bressenden Place, London SW1E 5DD. (258)

**UNIVERSITY OF ST ANDREWS
RESEARCH STUDENTSHIP**

Applications are invited from suitably qualified graduates to work with Dr C. R. Strong in the Department of Biochemistry, University of St Andrews. The 3-year project would involve studies on the control of lipid metabolism in mammary tissue, with particular reference to fatty acid synthesis and esterification.

The value of the award is £695 per annum plus approved fees. Applicants should contact Dr C. R. Strong, Department of Biochemistry, University of St Andrews, North Street, St Andrews, Fife, from whom further particulars can be obtained. (259)

**UNIVERSITY OF ST ANDREWS
DEPARTMENT OF CHEMISTRY**

Applications are invited for a post of Research Assistant to work with Dr C. Thomson on the computational aspects of ab-initio studies of the electronic structure of unstable intermediates. The appointment will be financed by the S.R.C. and will be for two years from October 1974 at a salary of £1,400 by £100. The successful candidate should possess an Honours degree or equivalent qualification, and have some background knowledge of computational chemistry, and experience in Fortran programming. Applications should be submitted by July 31, 1974 to Dr C. Thomson, Department of Chemistry, The University, North Haugh, St Andrews, Fife KY16 9ST, Scotland, from whom further details of the project may be obtained. (248)

**ZOOLOGICAL SOCIETY
OF LONDON
RECORDER**

for the *Zoological Record*, a bibliography of the world's zoological literature. Duties include scanning literature and indexing of relevant material.

Applicants should possess a degree in Zoology. Linguistic ability and previous experience in information work would be helpful but not essential. Posts based at units at the British Library Lending Division, Boston Spa and the British Museum (Natural History).

Salary on scale £1,526 to £2,096 plus £175 London Weighting if applicable (salary and weighting under review).

Applications, with brief details, in writing to Managing Recorder, *Zoological Record*, P.O. Box 9, Wetherby, Yorkshire LS23 7EG by July 31. (264)

**CLYDE RIVER
PURIFICATION BOARD**

**Senior Assistant
Marine Survey Officer**

Applications are invited for the above position in a multi-disciplinary team of highly qualified scientists engaged in the investigation and control of pollution on the West Coast of Scotland. A background in physical oceanography or mathematical modelling would be advantageous but applications from workers in other relevant fields will receive serious consideration. The post will involve a considerable commitment to the efficient organisation and management of the section and the appointee will deputise for the Marine Survey Officer. The work of the section currently includes major investigations related to the siting of marine outfalls, long-term biological and chemical monitoring of the marine environment and the study of circulation and dispersal of pollutants in the Clyde Sea Area. The Board has appropriate laboratory facilities and instrumentation and a replacement 54 foot survey vessel is nearing completion.

The post is superannuable. Salary will be within the range of £3,360 to £3,858. An essential user car allowance is provided.

Applications stating age, qualifications and experience, together with the names and addresses of two referees should be submitted to the Acting Director, Rivers House, Murray Road, East Kilbride, Glasgow G75 0LA, not later than July 31, 1974.

(254)

**NEW ZEALAND
Ministry of Agriculture & Fisheries**

Applications are invited for the undermentioned vacancy:

**Vacancy Scientist (Pasture
Agronomy)
Invermay Agricultural
Research Centre**

SALARY: According to qualifications and experience.

DUTIES: To assist with a large programme of varietal, fertiliser, cultivation and crop rotation studies. Opportunity exists for independent research. Good field and laboratory facilities are available on 500 hectare station.

QUALIFICATIONS DESIRED: Ph.D. or M.Agr.Sc.

PASSAGES: Fares for appointee and his wife and family, will be paid.

INCIDENTAL EXPENSES: Up to NZ\$120 for a single man and NZ\$800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, SW1Y 4TQ, with whom applications will close on 22 August 1974.

Please quote reference P/T 105 when enquiring.

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**British Museum (Natural History)
Department of Mineralogy**

Analytical Chemist

■ Undertake chemical analysis of rocks, meteorites and minerals using classical and modern instrumental techniques.

□ Degree, HNC or equivalent in Chemistry □ Age under 27 □ Appointment as Scientific Officer (£1900-£3000). □ Ref: SB/31/DK.

□ Application forms (for return by 6 August 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

**Ministry of Agriculture,
Fisheries and Food
Pest Infestation Control
Laboratory, Slough**

Information Scientist

■ To join Pesticides Survey Group, obtaining data on the uses of pesticides in food storage practice, animal husbandry and public health fields ■ Assist with design of surveys and draft questionnaires

■ Visit sample organisations ■ Process data and prepare reports.

□ Degree or equivalent in a biological or physical science □ 5 years' post-graduate experience (2 years' if good honours) □ Knowledge of Statistics and Computers, particularly in the data processing field, an advantage □ Age under 30 □ Appointment as Higher Scientific Officer (over £2550-around £3500) □ Ref: SB/9/AF.

Chemist

■ To work in the Pesticides Regulatory Section ■ Preparation of technical documents and reports on the chemistry, toxicology and related safety aspects of pesticides ■ Attend technical committees, and liaise with industry, universities and other government departments on new pest control techniques.

□ Degree or equivalent in chemistry or biochemistry □ Age under 27 □ Appointment as Scientific Officer (£1700-around £2800) □ Ref: SB/8/AF.

□ Application forms (for return by 2 August 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB or telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).



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FELLOWSHIPS AND STUDENTSHIPS

**UNIVERSITY OF MANCHESTER
DEPARTMENT OF CHEMISTRY
POSTGRADUATE STUDENTSHIP**

Applications are invited for an S.R.C.-C.A.S.E. studentship to work on the mechanism of chelation and demetallisation of metal complexes with Dr. J. A. Connor and in collaboration with Dr. R. Price of I.C.I. Organics Division. The project will involve both preparative organic and inorganic chemistry together with the application of physical methods of measurement in the elucidation of a problem which is of basic importance to transition metal chemistry and of significance to the dyestuffs and metallurgical industries. Applicants should write as soon as possible to Dr. J. A. Connor, Department of Chemistry, University of Manchester, Manchester M13 9PL. (54)

**ROYAL FREE HOSPITAL SCHOOL
OF MEDICINE
DEPARTMENT OF BIOLOGY
POSTDOCTORAL RESEARCH**

Applications are invited from graduates in pharmacology or related subjects for a three-year research post at the postdoctoral level, supported by The Nuffield Foundation. The project concerns the rôle of genetic variation in the ability to metabolise foreign substances in the mouse. Starting date October or November 1974. Technical help will be available.

Salary £2,091 to £2,220 to £2,385 p.a. Plus F.S.S.U. Applications, with curriculum vitae and two referees, to Dr. I. E. Lush, Department of Biology, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. (94)

**FELLOWSHIP IN SALMON
DISEASES**

Applications are invited by the Atlantic Salmon Research Trust for a Research Fellow to be based at the Farran Laboratory of the Salmon Research Trust of Ireland, Newport, Co. Mayo, Ireland, in association with the Unit of Aquatic Pathobiology of the University of Stirling, with whom the successful candidate will be expected to register for a Ph.D. Application forms and conditions of service are available from the Director, Atlantic Salmon Research Trust Ltd., 29 South Street, Farnham, Surrey. Completed application forms to be received by July 31. (90)

**INSTITUTE OF CHILD HEALTH
(UNIVERSITY OF LONDON)
RESEARCH STUDENTSHIP**

Applications are invited from Honours Graduates in Biochemistry for a 3-year research studentship at the Institute of Child Health. The work will be concerned with certain aspects of vitamin E transport in the small intestine both in the experimental animal and patients, and the successful candidate will be expected to register for a higher degree. Starting salary £1,506 p.a. Applicants should send their curriculum vitae and the names of 2 referees to Professor O. H. Wolff, Department of Child Health, Institute of Child Health, 30 Guilford Street, London WC1N 1EH. (166)

**Esro Fellowship in
Infrared Astronomy**

Applications are invited from experienced infra-red astronomers for Fellowships to work in the Astronomy Division, ESTEC, on Michelson instrumentation for measurements of the ionic fine structure lines in HII regions and on the heterodyne detection of molecular line emission at sub-millimeter wavelengths. Aircraft and balloon borne telescope will be used.

Candidates must hold a Ph.D. in a relevant field of research.

The posts are tenable for up to two years. Further information can be obtained from

Head of Personnel
European Space Research
and Technology Centre
Domeinweg
Noordwijk
Holland.

(157)

UNIVERSITY OF READING

Applications are invited for an S.R.C., C.A.S.E. Studentship in Colloid Science leading to the Ph.D. degree for work on the stability of emulsions. The work will be carried out jointly with I.C.I. Plastics Division, Welwyn. Applicants should possess a good honours degree in chemistry or equivalent. Apply to Dr T. M. Hardman, Department of Chemistry, The University, Whiteknights, Reading RG6 2AD. (Ref: MN 28). (178)

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY
AND CHEMISTRY**

Applications are invited from graduates with a 1st or upper 2nd class Honours degree in Biochemistry or other relevant subject, or from those graduating this year, for an S.R.C. Research Studentship tenable from October 1, 1974, leading to a higher degree in the University of London. Applicants should send a curriculum vitae and the names of two referees, as soon as possible, to Professor J. A. Lucy, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. (187)



AUSTRALIA

Queen Elizabeth II FELLOWSHIPS

in the Physical and Biological Sciences

To commemorate the Royal Visit to Australia in 1963 the Australian Government established the Queen Elizabeth II Fellowships Scheme. Under this scheme up to ten fellowships may be awarded each year for full-time research by young scientists of exceptional promise and proved capacity for original work. These are post-doctoral awards tenable in an Australian university or approved research institution normally for two years. Tenure of a Fellowship is expected to commence within nine months of the announcement of the award.

QUALIFICATIONS Queen Elizabeth II Fellows must be either Australian or United Kingdom citizens. They should have a Ph.D., or equivalent qualifications in one of the physical or biological sciences (which are deemed to include mathematics and the scientific aspects of statistics, engineering, metallurgy, agriculture and medicine).

Awards will in general be restricted to applicants who are not more than 30 years of age on the date when applications close.

STIPEND \$9,500 (Australian) per annum – increased to \$10,250 per annum at age 28 years.

ALLOWANCES are payable in respect of a Fellow's wife (\$500 p.a.), each dependent child (\$200), superannuation payments (up to 10 per cent of stipend), appropriate insurance coverage and necessary travel expenses. Host institutions are paid an allowance towards the cost of setting up the fellow and his research work.

APPLICATIONS Persons interested in applying for the above fellowships should obtain application forms and a statement of the conditions of award from the Secretary, Queen Elizabeth Fellowships Committee, Department of Science, P.O. Box 449, Woden, A.C.T., 2606, Australia; Education Liaison Officer, Canberra House, Strand, London, WC2R 3EH, England; or The Consul General, Australian Consulate General, 636 Fifth Avenue, New York 20, N.Y., 10020 U.S.A. Applications for the next round of awards which will be announced in December 1974 close at the Canberra address on 6th September 1974.

NEWCASTLE UPON TYNE POLYTECHNIC

Ellison Building, Ellison Place, Newcastle upon Tyne NE1 8ST

DEPARTMENT OF MATERIALS SCIENCE

RESEARCH FELLOW

This post is concerned with the development of plastics reinforced with carbon and other fibres for medical and dental applications. Candidates should have at least two years postgraduate research experience, preferably on engineering aspects of the use of materials.

RESEARCH ASSISTANTSHIPS AND STUDENTSHIPS

Several posts are available for work on the following topics:

- (1) Mechanical Properties of Composite Materials.
- (2) Spectroscopic Studies (I.R., N.M.R., Raman) on
 - (a) Structure and Properties of Polymers.
 - (b) Structure and Properties of Biological Materials.
- (3) Thermal Behaviour of Polymers Including the Effects of Fire on Structural Materials.
- (4) Studies on the Application of Polymer Films in Spectroscopy and Medicine.

Candidates should have or expect to have a good first degree or professional qualification in an appropriate science or technology, and must be eligible to register for a higher degree (M.Phil., Ph.D.).

RESEARCH TECHNICIAN

Candidates should have a good knowledge of electronics and instrumentation, and will be required to operate and maintain a wide range of equipment associated with the research laboratories.

All posts described above will be under the supervision of Dr T. R. Manley.
Salary Scales:

- Research Fellowship—£1,926 to £2,235.
- Research Assistantships—£1,200 to £1,500 (under review).
- Research Studentships—S.R.C. rates.
- Research Technician—£1,242 to £1,644.

For further details and application forms, returnable by August 7, 1974, send stamped addressed foolscap envelope to the Chief Administrative Officer. (185)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

SENIOR RESEARCH FELLOWSHIPS IN SYSTEMS STUDY OF INTENSIVE FOOD PRODUCTION PROCESSES

Applications are invited for two Fellowships that will appeal to graduates between 24 and 28 years of age, who, already having experience in their profession, would enjoy thinking in a broader perspective touching on futures research. No particular qualification is prescribed since the members of study group will be complementary, but appointees are likely to have experience in more than one of the following: microbiology, food science, food processing, chemical plant design or operation, thermodynamics, physical chemistry or system dynamics. An interest in economics and systems would be useful.

The Fellowships are tenable for two years from September 1974 at a salary up to £3,000 per annum with F.S.S.U.

Applications and enquiries (quoting R23/74) should be addressed to Dr M. Slesser, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, as soon as possible. (172)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF CHEMISTRY

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP for studies on chemical carcinogenesis. The appointee will join a team working on chemical and biochemical aspects of polycyclic aromatic hydrocarbon binding to DNA, ranging from synthetic and structural organic chemistry to tissue culture. Candidates should hold (or expect to gain) a Ph.D. in organic or biological chemistry and experience in nucleic acid chemistry is desirable but not essential. Tenable from October 1, 1974 or a date to be arranged until September 10, 1975 at a salary not less than £1,923 per annum plus F.S.S.U. Applications, including a curriculum vitae and names and addresses of two referees to: Dr G. M. Blackburn, Department of Chemistry, The University, Sheffield S3 7HF. Please quote reference R106/G. (156)

UNIVERSITY OF MANCHESTER

A Research Fellow and a graduate Research Assistant required to work on the immunology of bladder cancer. The Fellow should have previous experience of cellular immunology and tissue culture techniques and may have either medical or scientific qualifications. Postgraduate experience is not essential for the Research Assistant post, and the successful candidate may be able to register for a postgraduate degree. Both appointments are for 3 years. Salary p.a. according to age, experience and qualifications (maximum initial rate for the Fellowship, £3,000, for the Assistantship, £1,900). Applications, including a full curriculum vitae with names and addresses of two referees, as soon as possible to: Dr Geoffrey Taylor, Immunology Laboratory, University of Manchester, Stopford Building, Manchester M13 9PL. (188)

THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

A M.R.C. Postgraduate Studentship is available in the above Department for research in Labour Ward data handling. Minimum qualification required: good second class honours in science or engineering. The Labour Ward includes a comprehensive patient monitoring system based on equipment designed in department. PDP 8/E computer available, currently used to analyse fetal heart rate data and predict fetal outcome; proposed to extend this facility to include more data from mother and baby. Applications to Dr R. J. Parsons, Lecturer in Medical Physics, Jessop Hospital for Women, Sheffield S3 7RE, as soon as possible. Quote Ref. R.107/G. (170)

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

M.R.C. RESEARCH STUDENTSHIPS

available for candidates with good Honours Degrees in Physiology, Pharmacology or Biochemistry wishing to study for a Ph.D.

Apply immediately to Department of Neurology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF or telephone 01-703 5411 ext 136. (196)

AUSTRALIAN NATIONAL UNIVERSITY

JOHN CURTIN SCHOOL OF MEDICAL RESEARCH

RESEARCH FELLOW/SENIOR RESEARCH FELLOW IN PHARMACOLOGY

Applications are invited for appointment as Research Fellow or Senior Research Fellow in the Department of Pharmacology (Head: Professor D. R. Curtis, F.A.A., F.R.S.). Applicants will be expected to have research experience in either neuropharmacology using micro-electrophoretic techniques (Professor D. R. Curtis), neuro-chemistry or organic chemistry of centrally active compounds (Dr. G. A. R. Johnston).

Further information may be obtained from Professor Curtis (July-September c/o Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD) or Dr Johnston, in the University.

Closing date: August 1, 1974.

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH FELLOW

DEPARTMENT OF ENVIRONMENTAL BIOLOGY

The Department (Head: Professor R. O. Slatyer, F.A.A.) has an active programme of research in plant and animal ecology and related areas of plant physiology. A post is now available for an ecologist interested in aspects of community or population ecology. Present interests in these subjects include: (a) Effects of environmental factors on the structure and function of communities and of whole ecosystems (b) First-order interactions between species within an ecosystem, e.g. competition between species dependent on similar physical or biotic resources, interactions between herbivore and plant, between predator and prey; (c) Higher order effects in which the interaction between species are mediated by the activity of other biotic factors in the system.

Applicants should have a Ph.D. in ecology or related areas of biology and an interest in the general areas described above. The Department has excellent facilities and support for both laboratory and field work, plus a strong team of technical supporting staff.

Closing date: August 2, 1974.

SALARIES: Salary on appointment will be in accordance with qualifications and experience within the ranges Senior Research Fellow SA13,163 to SA15,548 p.a.; Research Fellow SA9,002 to SA12,269 p.a.

OTHER CONDITIONS: Tenure: Senior Research Fellow and Research Fellow normally for three years with the possibility of extension to a maximum of five years.

Reasonable travel expenses are paid and assistance with accommodation is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (231)

UNIVERSITY OF BATH
SCHOOL OF PHARMACY AND
PHARMACOLOGY
RESEARCH STUDENTSHIP

Applications are invited from good Honours students in Pharmacy, Pharmacology or Biochemistry for this industrially-sponsored award. The successful candidate will carry out a project concerned with the mode of action of a new anti-Parkinsonian drug, and which will involve both animal and clinical pharmacology. The award is for one year at the normal S.R.C. rate, and the appointee will be expected to register for an M.Sc. degree. Applications to Dr. P. H. Redfern, School of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY. (202)

UNIVERSITY OF BIRMINGHAM
CHEMISTRY DEPARTMENT
RESEARCH STUDENTSHIPS

A number of S.R.C. Research Studentships, C.A.S.E. Awards, Departmental Grants, etc., are available for suitably qualified Science graduates for work on a wide variety of topics in various branches of Chemistry. Excellent facilities are available for higher degree studies of chemical properties of carbohydrates, fluorocarbons, polymers, enzymes, various natural products, nucleic acids, etc. Please apply without delay to Professor M. Stacey, C.B.E., F.R.S., Chemistry Department, Birmingham University, P.O. Box 363, Edgbaston, Birmingham B15 2TT. Telephone No. 021-472 1301, Ext. 3100. (235)

UNIVERSITY OF LIVERPOOL

Department of Inorganic, Physical and Industrial Chemistry

RESEARCH STUDENTSHIPS

A number of S.R.C. studentships are available in the above department for research in various aspects of Inorganic and Physical Chemistry. Areas of current activity include: Chemistry of organoboron and organometallic compounds and transition metal complexes; combustion chemistry; radiation chemistry; radiochemistry; surface chemistry; diffusion processes in solids and in polymer films; spectroscopy (NMR and ESR); colloid science and crystal growth; polymer science; theoretical chemistry.

Studentships related to the S.R.C. Polymer Centre, Liverpool are also available. Present research interests include: synthesis of new monomers and polymers; mechanisms of polymerization processes; polymer morphology; physical properties of polymers in the solid state and in solution.

The studentships are tenable from 1st October 1974 and successful applicants will register for the degree of Ph.D. Applications, including academic qualifications, names of two referees and an indication of field of interest, should be sent to The Registrar, The University P.O. Box 147, LIVERPOOL L69 3BX. Quote ref. RV/N/276122. (204)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF PHYSICS

NUFFIELD RESEARCH FELLOWSHIP

Applications are invited for this post, tenable in the first instance for two years, from candidates with good research experience in any of the fields in which research facilities are currently available e.g. high energy physics, nuclear structure physics, applied nuclear science (neutron and reactor physics, and medical applications), solid state physics (low temperatures, crystallography, Mossbauer spectroscopy etc.), University physics teaching methods.

Salary scale: £2,118 to £2,412 plus F.S.S.U.

Further particulars may be obtained from the Assistant Registrar (S), P.O. Box 363, Birmingham B15 2TT, to whom applications (3 copies), naming 3 referees should be submitted by August 12, 1974. Please quote ref.: NPI. (249)

UNIVERSITY OF NEW ENGLAND

ARMIDALE, NEW SOUTH WALES

**TEACHING FELLOW IN
GEOGRAPHY**

The appointee will assist in the teaching programme of the Department, both in the laboratories and in the field, for internal and external students in Geography and will also carry out research towards a higher degree in Geography. Candidates should have specialist training and experience in physical or human geography and should have at least a good Honours degree in Geography or, preferably, a Master's Degree. While ability in quantitative techniques is expected, preference may be given to candidates with experience of tutorial, external, or audio-visual teaching, or fieldwork in geography.

Salary: \$A5,985 to \$A7,285. Conditions of employment provide assistance with travel and removal expenses.

Appointments with an academic fellowship component may be considered. Such appointments will normally be for an initial period of three years and will be subject to confirmation in the light of satisfactory service and progress towards a degree at the end of the first year of service. Appointments may thereafter be extended annually on the recommendation of the Head of Department. Full time assistantships will in the first instance be normally for a period of not more than one year. However, appointments may be extended on the recommendation of the Head of Department by a subsequent period of three years.

Further information may be obtained from the Staff Officer, University of New England, Armidale, New South Wales, 2351, Australia with whom applications, together with a recent photograph, and the names and addresses of three referees, close on **August 2, 1974**. Applicants in the United Kingdom and Europe should forward an additional copy, by the same date, to the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further information may also be obtained. (253)

ROTHMANS FELLOWSHIPS

Applications are invited for Rothmans Fellowships which are awarded under the Rothmans University Endowment Fund set up by Rothmans of Pall Mall (Australia) Limited to enable Fellows to undertake postgraduate work within an Australian University.

Rothmans Fellowships are of an annual value of \$A8,100 up to \$A12,400. A Fellow may be paid travelling expenses incurred in taking up the Fellowship and returning to his home.

In addition, an amount of \$A1,000 per annum towards fees and expenses including the purchase and maintenance of equipment may be paid to the University where the Fellow is working.

A Fellow shall take up a Fellowship before attaining the age of twenty-eight. The Fellowships are open to graduates of any University who have had at least three years' postgraduate experience in research. The Fellowships are not open to permanent members of academic staff or applicants proceeding on sabbatical, study or other leave (including leave without pay). The Fellowships must be held at an Australian University.

Application forms and further details may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **September 13, 1974** with the Secretary, Rothmans University Endowment Fund, c/o The University of Sydney, N.S.W., Australia 2006. (252)

**RESEARCH STUDENTSHIP IN
MICROBIOLOGY**

Applications are invited from suitably qualified graduates for a S.R.C. C.A.S.E. studentship to study "Stabilisation of enzyme systems in micro-organisms" at Queen Elizabeth College (University of London) and Shell Research.

Applications to Dr H. J. Somerville, Woodstock Laboratory, Shell Research Limited, Sittingbourne, Kent ME9 8AG. (263)

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LECTURES AND COURSES

BRITISH POSTGRADUATE MEDICAL FEDERATION
UNIVERSITY OF LONDON

Medical Rehabilitation

A two-week course in Medical Rehabilitation will be held from Monday, September 30 to Friday, October 11, 1974 in the Department of Rheumatology, The Middlesex Hospital, London W.1.

The course which will cover the organisation of medical, industrial and social services is primarily designed for consultants and senior registrars drawn from any specialty in the National Health Service and in particular geriatrics, neurology, orthopaedics and rheumatology, but applications will be considered from community physicians and other doctors with a special interest in the subject. The programme includes visits to a spinal injuries and limb fitting centre, medical rehabilitation centres, industrial rehabilitation units and skillcentres, the Royal National Orthopaedic Hospital and Nuffield Orthopaedic Centre, Oxford, the Disabled Living Foundation's Aid Centre and Queen Elizabeth's Foundation for the Disabled at Leatherhead.

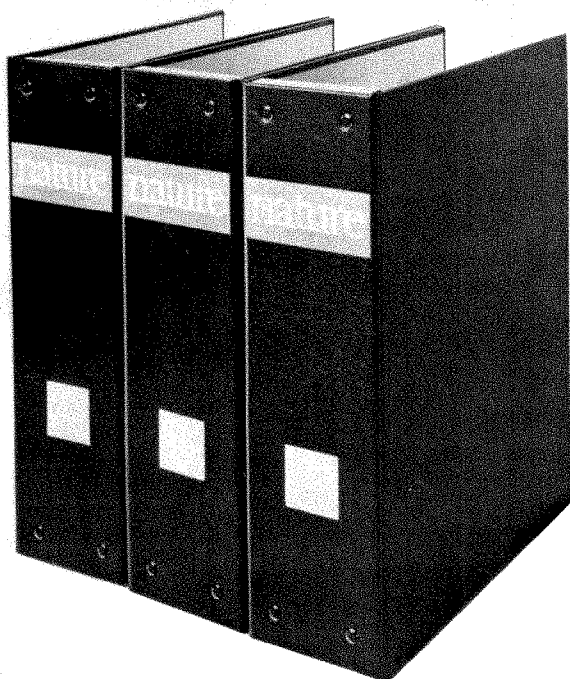
Course fee: £40. Approved under the D.H.S.S. Advanced Postgraduate Training Scheme. Application forms and further details from: The Secretary (Rehabilitation Course), B.P.M.F., 33 Millman Street, London WC1N 3EJ. Closing date for applications: August 31, 1974. (220)

UNIVERSITY OF SWANSEA
College of
Swansea

**RESEARCH STUDENTSHIP IN
GENETICS**

The Medical Research Council is prepared to offer a postgraduate studentship to a suitably qualified graduate, commencing October 1, 1974. Applicants should hold a first or upper second class degree in Biological Science. The work will involve an investigation of the possible genetic activity of environmental chemicals. An interest in the assessment of the hazards of environmental pollutants would be an advantage.

Applications giving details of qualifications and the names of two referees should be sent to the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea SA2 8PP, as soon as possible. (234)



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Molecular Biology comes of age

This special **Nature** Supplement has been reprinted as a separate publication. First published in the issue of 26th April 1974, '**Molecular Biology Comes of Age**' looks back on the circumstances surrounding the original appearance in **Nature** of Watson and Crick's revolutionary paper on the double helix structure of DNA in 1953. It discusses the development in Molecular Biology up to the present day as well as prospects for the future. Among the distinguished contributors are Crick, Pauling and Chargaff.

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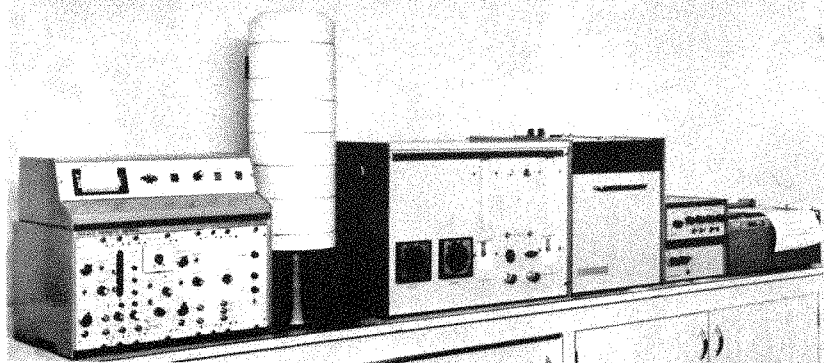
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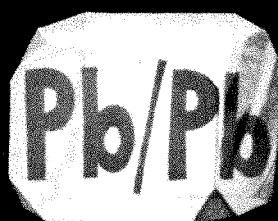
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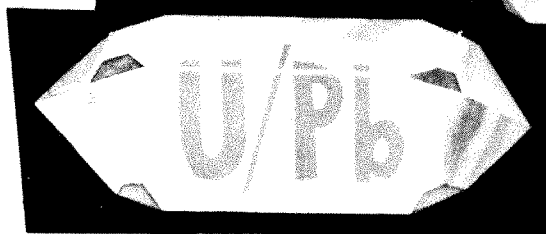
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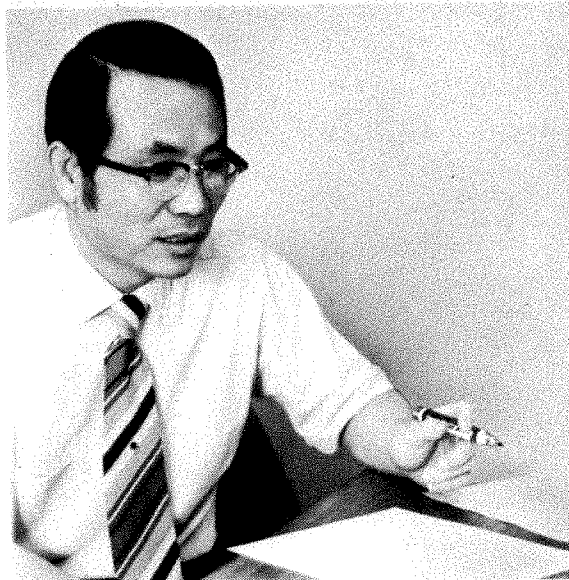
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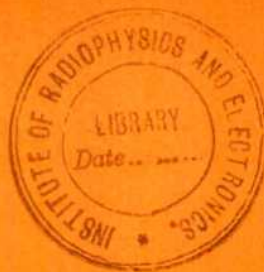
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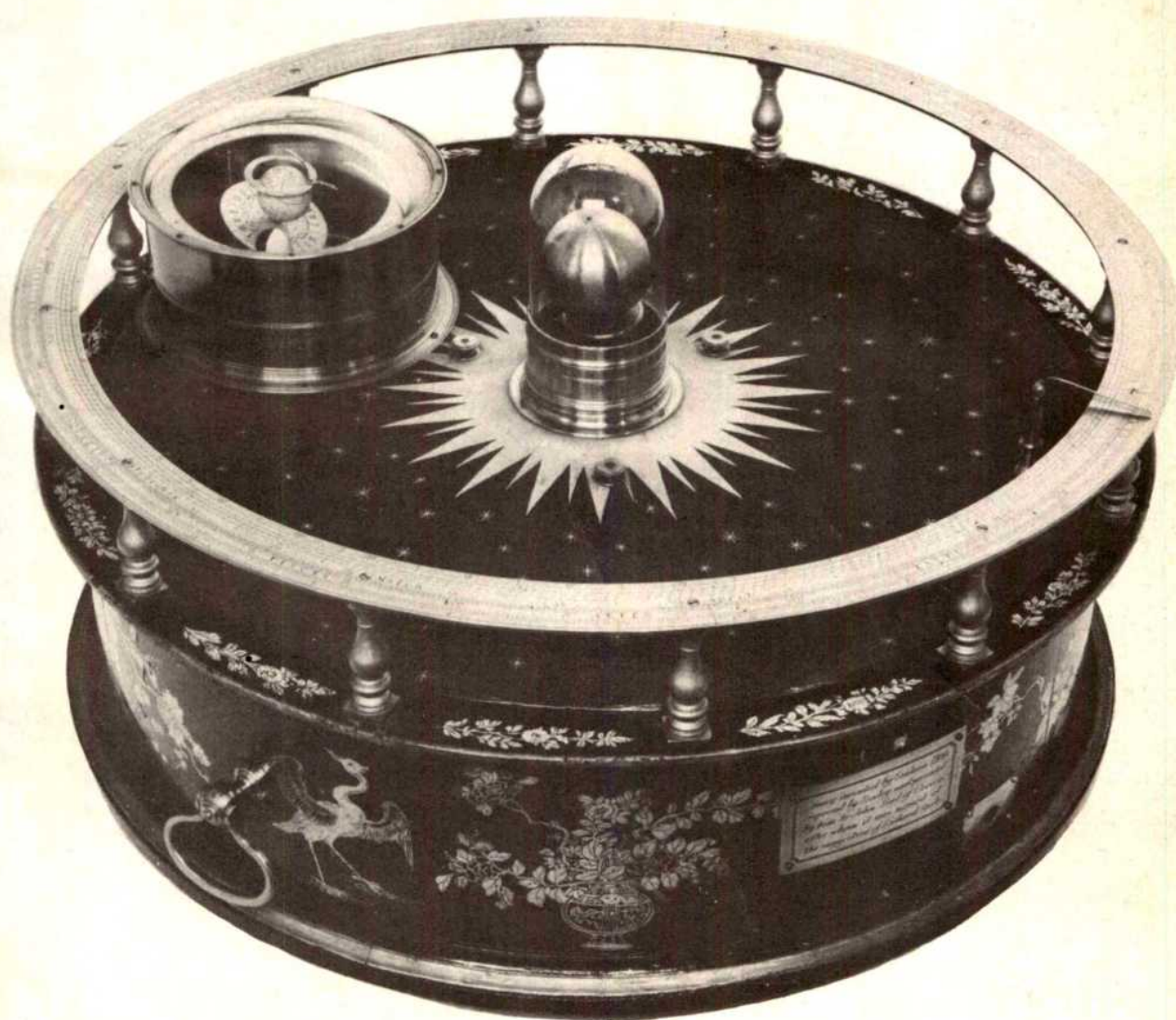
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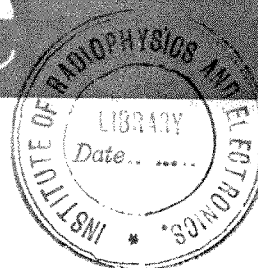
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Cover picture

The orrery, an instrument for showing the position of the Earth and Moon relative to the Sun at a given date and time. This fine example of the instrument maker's craft was made by Rowley in about 1716, based on an original invention by Graham. Rowley presented it to the Fourth Earl of Orrery, after whom it was named. This orrery, the oldest instrument of its kind, has recently been purchased for £29,000 by the Science Museum from the present Earl of Cork and Orrery. (Science Museum photo, Crown Copyright).

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nature

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July 19, 1974

Britain takes up the SGHWR

AFTER four years of debate, during which it often seemed that no decision would ever be made, the government has finally jumped off the fence, bowling over Sir Arnold Weinstock and the Central Electricity Generating Board (CEGB) in the process. For, like it or not, the CEGB is going to have to order a small number of steam generating heavy water reactors (SGHWRs) in the next few years rather than a considerably larger number of the American pressurised water reactors (PWRs) which it favours.

The remarkable thing about all this is that the decision could equally well have been made in 1972 when Mr John Davies, then Secretary of State for Trade and Industry, announced a programme of component development for the SGHWR. Other options for the short term could still have been kept open and indeed that is exactly what Mr Eric Varley, Secretary of State for Energy, has done nearly two years afterwards in saying that the Nuclear Installations Inspectorate should complete its examination of the safety of the American reactors.

Even if Mr Davies had also opted for a relatively modest scheme, Mr Varley might now be in a position to enlarge a programme to which the nuclear power industry and the CEGB would have had two years to adjust. As it is, Sir Arnold, who is head of GEC (a 50% stakeholder in the National Nuclear Corporation), is sulking and threatening to reduce considerably, or even eliminate, his company's share in the corporation. That would be embarrassing, as GEC is supposed to manage the building of the next generation of nuclear reactors in Britain and it will no doubt cause wry smiles among members of the Select Committee on Science and Technology which thinks that a shareholding of more than 30% by any commercial interest should not have been allowed in the first place. In fact it is difficult to see how the corporation could continue if Sir Arnold were to depart completely.

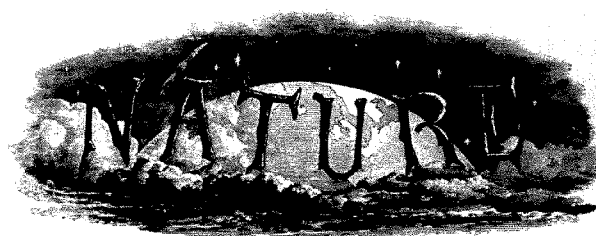
Ultimately, however, it is the CEGB which has to generate the electricity and its views deserve the most sympathetic consideration. It is that body, one has to bear in mind, which still has all five advanced gas cooled reactor stations waiting to be completed almost ten years after the first one was ordered. Basically the CEGB wants to order nuclear power stations in such numbers over the next decade or so that its reliance on fossil fuel is decreased, and it wants those nuclear power stations to work properly. Not surprisingly, therefore, it criticises the government's decision on the grounds that the SGHWR is untried in a commercial situation and that because of this the government is only risking a programme of some 4,000 MW to be ordered over the next four years and commissioned in the 1980s. Even the quite modest rate

of growth of electricity demand now envisaged by the Department of Energy requires 35,000 MW of new capacity by 1990, the lion's share of which will evidently be based on fossil fuels unless the SGHWR turns out to be such a success that more can be rapidly ordered after the first has become operational, perhaps in 1981.

The CEGB points out, and the government apparently agrees, that more development work will have to be done before the SGHWR can be built on even the scale that Mr Varley envisages (small units of 660 MW). The argument goes that the test reactor at Winfrith is only 100 MW and is based on technology that is already 10 years old. Parts will have to be redesigned, says the CEGB, and different materials will have to be used. But is all this really necessary? Does a new piece of functional equipment necessarily have to incorporate the most up-to-date technology wherever possible? Excessive zeal in this direction would seem to be ill advised, especially if an attempt to wring that extra bit of efficiency or cost out of the system were to cause more expense in the long run than could ever be saved. After all the suggestion that more Magnox stations should be built is only a nonstarter because of the high capital cost compared with more modern reactor designs.

What the CEGB seem to overlook is that the Canadian CANDU reactor, which is similar in many ways to the SGHWR, has been working successfully for several years and that technological cooperation between Britain and Canada is thought by both countries to be a mutually advantageous prospect. And there seems no reason why Britain should not now take advantage of the export opportunities which may still exist for the SGHWR.

100 years ago



WE rejoice to see from the tone of the replies to questions in the House of Commons on Monday by Mr. Disraeli and Lord Henry Lennox, that Government is conscious of how poorly housed some of our scientific collections are, and seems really disposed to take steps to remedy the evil. Mr. Disraeli said, in reply to a question concerning the Patent Museum, that it is not the only public institution which is suffering from want of space and of suitable accommodation. "That is now a crying grievance with respect to all our public buildings, collections, and offices. In regard to the Patent Museum, however, I am aware from a communication which I have received from my noble friend the First Commissioner of Works, that the matter is at present engaging attention." Lord Henry Lennox confirmed this by subsequently stating that he intended to propose to Her Majesty's Government a scheme which, if it were agreed to, would enable him to offer the Patent Museum suitable accommodation in the southern block of the International Exhibition buildings.

From *Nature*, 10, 232, July 23, 1874.

NATO and civil science

Peter J. Smith, who once accepted a NATO Fellowship because it was the only support available for work in a nonuniversity research institution in the United States, discusses NATO's involvement in civil science.

WHAT is the connection between stress and anxiety in modern society, the chemistry of insects, cosmological models, and the mooring and berthing of ships? The answer is that in common with more than 50 other science-based activities they were the subjects of well financed conferences during 1972. On the face of it, of course, there is nothing very strange about that; it is a common enough occurrence for scientists to congregate with their colleagues around the world to discuss new results and ideas, and some of them even find it useful. But there is something a little odd about it in this case, for all 56 meetings were sponsored and paid for by a single organisation which has nothing directly to do with academic science at all—the North Atlantic Treaty Organisation (NATO). The question is: why?

Scientists do pretty well out of NATO, and not just in the matter of conferences. The total budget of the NATO Science Committee in 1972 was \$4.83 million of which only \$0.93 million went on scientific meetings. Well over half was spent on the Fellowship Programme. Research grants then accounted for a further \$0.61 million, and a few specialised programmes (such as operational research, oceanography and human factors) and administration took the rest.

The mandate for all this involvement in basic science is Article 2 of the North Atlantic Treaty (1949) by which “the Parties will contribute toward the future development of peaceful and friendly international relations by strengthening their free institutions, by bringing about a better understanding of the principles upon which those institutions are founded, and by promoting conditions of stability and well-being”. For the first eight years of NATO's existence the emphasis was clearly on “defence”; but in 1957 Paul Henri Spaak, the second Secretary General, made a serious attempt to widen the field to include ostensibly nonmilitary collaboration. One result was the establishment in 1958 of the

NATO Science Committee to “strengthen the Alliance and its political substance”—a form of words whose deeper meaning has apparently never been in doubt, for NATO has never made any secret of the fact that it sees its support for science as contributing, however indirectly, to its military and political aims.

In view of the often violent reaction against the military sponsorship of university science in recent years, a basically military organisation which not only publicises its involvement in civil science but also extols the virtues of it in print may appear at first sight to be either remarkably honest or incredibly naive. In fact, NATO is neither. It is not genuinely honest to be completely open about a participation which would surely be discovered in any case; and the charge of naivety would only be substantiated if it could be shown that members of the NATO Science Committee were unaware of the strength of feeling already expressed on the issue of military sponsorship. Moreover, to the extent that the sort of science sponsored by NATO furthers military aims no more nor less than it would if sponsored by an overtly civil body, financial aid from NATO as such would seem to have little relevance to that organisation's stated aims. One can only conclude, therefore, that NATO's public stance is designed to conceal motives which are altogether more subtle.

Indeed, what NATO has really been after from its large scale support of basic science—and what it has to a large extent achieved—is a sort of social respectability and prestige among a community containing elements who are decidedly touchy over the relationship between science and the military. Since 1959 over 10,000 NATO Fellows have written tens of thousands of legitimate scientific papers, each of which records gratitude for NATO support; every moderately sized scientific library in the world now contains hundreds of volumes bearing witness on their title pages to NATO's generosity; and every conference announcement implicitly proclaims NATO's apparent concern for disinterested scientific scholarship and culture. In addition, the NATO Science Committee has always gone out of its way to attract to membership established and high-ranking scientists or government scientific advisers who are able to confer their own brand of legitimacy on NATO's activities.

As might be expected, some scientists have argued that none of this matters—that given that the relevant activity is essentially nonmilitary, who pays for it is of no concern. Unfortunately, however, there is evidence that NATO's scientific involvement is

not entirely beneficial to science itself, even if motives are ignored. For one thing, although the Science Committee is always quick to point out that up to 20% of the participants in its conferences come from non-NATO countries, including the communist nations of eastern Europe, it is less ready to publicise the fact that non-NATO delegates are forbidden to attend on NATO funds. And in spite of boasts about participants from the Soviet Union and elsewhere, it is doubtful whether scientists from, say, Cuba or North Vietnam would be able to attend a NATO conference in the United States. In fact, as far as I know (and certainly up to the end of 1972), no scientist from either of these countries has ever attended any NATO conference anywhere.

Thus although scientists pride themselves on their international outlook, they are not above doing less than justice to their ideals for the sake of a quick buck. And it is because of this sort of opportunism—not to mention the skilful casuistry displayed in rationalising it—that NATO science has been given little opposition.

Few would suggest that military sponsorship is the most important problem facing civil science today. At the same time, however, this is far from saying that it is of no concern at all; and there is clearly a significant minority which feels sincerely that NATO involvement in particular is a blot on the scientific landscape. The irony is that the whole NATO sponsorship scheme is totally unnecessary, except perhaps as perceived by NATO itself. The fact is that the Fellowship programme, which accounts for about 60 per cent of the NATO science budget, is administered not by NATO but by agencies in the constituent countries (the Science Research Council in Britain, the National Science Foundation in the United States). A large proportion of NATO science money is thus returned directly whence it came (and almost all of it is repatriated to the constituent nations in one form or another), NATO's sole contributions being the use of its name and, as one cynic has pointed out, an addition to bureaucratic overheads. Since most of the national agencies concerned now have parallel programmes of their own, there seems to be little point in perpetuating NATO's involvement any longer. Alternatively, if an overtly international programme of science support is felt to be desirable, it would be far preferable to make it truly international and hand over the whole NATO scheme to some clearly nonmilitary organisation such as UNESCO. Presumably this would keep everyone happy—including NATO, if it really means what it says.

international news

NAS ban on plasmid engineering

"RECENT advances in techniques for the isolation and rejoining of segments of DNA now permit construction of biologically active recombinant DNA molecules *in vitro*. For example, techniques employing DNA restriction endonucleases, which generate DNA fragments containing cohesive ends especially suitable for rejoining, have been used to create new types of biologically functional bacterial plasmids carrying antibiotic resistance markers (Cohen *et al.*, *Proc. natn. Acad. Sci.*, **70**, 3240; 1973; Chang *et al.*, *Proc. natn. Acad. Sci.*, **71**, 1030; 1974) and to link *X. laevis* rDNA to DNA from a bacterial plasmid. This latter recombinant plasmid has been shown to replicate stably in *E. coli* where it synthesises RNA complementary to *X. laevis* rDNA (Morrow *et al.*, *Proc. natn. Acad. Sci.*, in the press). Similarly, segments of *Drosophila* chromosomal DNA have been incorporated into both plasmid and bacteriophage DNAs to yield hybrid molecules that can infect and replicate in *E. coli* (Hogness, unpublished; Davis, unpublished; Boyer, unpublished).

"Several groups of scientists are now planning to use this technology to create recombinant DNAs from a variety of other viral animal and bacterial sources. Although such experiments are likely to facilitate the solution of important theoretical and practical biological problems, they would also result in creation of novel types of infectious DNA elements whose biological properties cannot be completely predicted in advance.

"There is serious concern that some of these artificial recombinant DNA molecules could prove biologically hazardous. One potential hazard in current experiments derives from the need to use a bacterium like *E. coli* to clone the recombinant DNA molecules and to amplify their number. *E. coli* strains commonly reside in the human intestinal tract, and they are capable of exchanging genetic information with other types of bacteria, some of which are pathogenic to man. Thus, new DNA elements introduced into *E. coli* might

possibly become widely disseminated among human, bacterial, plant or animal populations with unpredictable effects.

"Concern for these emerging capabilities was raised by scientists attending the 1973 Gordon Research Conference on nucleic acids (Singer and Soll, *Science*, **181**, 1114; 1973), who requested that the National Academy of Sciences give consideration to these matters. The undersigned members of a committee, acting on behalf of and with the endorsement of the Assembly of Life

In an unprecedented move, the National Academy of Sciences has called for a voluntary worldwide moratorium to be placed on an area of scientific research because of potential and unpredictable hazards to human health. A statement drawn up by a committee of eminent biomedical scientists and released by the academy this week calls for a temporary halt on two types of genetic engineering research because of the risk of infecting man with bacteria containing hybrid DNA molecules whose biological properties cannot be predicted in advance.

The academy is concerned about experiments which combine fragments of DNA from different sources to form a hybrid molecule which can then replicate in bacteria such as *E. coli*, which is normally present in the human intestine. The Committee on Recombinant DNA Molecules, whose members* have all agreed individually to renounce two types of experiments involving such techniques until the potential hazards have been evaluated, has called for a committee to be established to define the hazards and to develop guidelines under which such research should be conducted. Part of the statement is given here.

*PAUL BERG, Chairman; DAVID BALTIMORE, HERBERT W. BOYER, STANLEY N. COHEN, RONALD W. DAVIS, DAVID S. HOGNESS, DANIEL NATHANS, RICHARD ROBLIN, JAMES D. WATSON, SHERMAN WEISSMAN, NORTON D. ZINDER.

Sciences of the National Research Council on this matter, propose the following recommendations:

"First, and most important, that until the potential hazards of such recombinant DNA molecules have been better

evaluated or until adequate methods are developed for preventing their spread, scientists throughout the world join with the members of this committee in voluntarily deferring the following types of experiments:

"Type I: Autonomously replicating bacterial plasmids that might result in the introduction of genetic determinants for antibiotic resistance or bacterial toxin formation into bacterial strains that do not presently carry such determinants, or construction of new bacterial plasmids containing combinations of resistance to clinically useful antibiotics unless plasmids containing such combinations of antibiotic resistance determinants already exist in nature.

"Type II: Linkage of all or segments of DNA from oncogenic or other animal viruses to autonomically replicating DNA elements such as bacterial plasmids or other viral DNAs. Such recombinant DNA molecules might more easily be disseminated to bacterial populations in humans and other species, and thus possibly increase the incidence of cancer or other diseases.

"Second, plans to link fragments of animal DNAs to bacterial plasmid DNA or bacteriophage DNA should be carefully weighed in light of the fact that many types of animal cell DNAs contain sequences common to RNA tumour viruses. Since joining of any foreign DNA to a DNA replication system creates new recombinant DNA molecules whose biological properties cannot be predicted with certainty, such experiments should not be undertaken lightly.

"Third, the Director of the National Institutes of Health is requested to give immediate consideration to establishing an advisory committee charged with (a) overseeing an experimental program to evaluate the potential biological and ecological hazards of the above types of recombinant DNA molecules; (b) developing procedures which will minimise the spread of such molecules within human and other populations, and (c) devising guidelines to be followed by investigators working with potentially hazardous recombinant DNA molecules.

"Fourth, an international meeting of involved scientists from throughout the world should be convened early in the coming year to review scientific progress in this area and to further discuss appropriate ways to deal with the potential biohazards of recombinant DNA molecules."

Wind of change at WMO

by John Gribbin

THE World Meteorological Organisation (WMO) has acted promptly on the statement made by Dr Henry Kissinger to the sixth special session of the United Nations General Assembly calling attention to the possible implications of climatic change for global food and population policies. It has been decided to establish a "Panel of Experts on Climatic Change" with the terms of reference:

- To review present WMO activities relating to climatic change and to make recommendations on any additional steps which may be necessary to integrate these activities into a coherent programme.
- To act as the focal point for WMO activities on climatic changes, trends and fluctuations, and their implications on the natural environment of mankind and on world food production.
- To advise on any necessary measures for the coordination of these activities.
- To advise on the policy implications for WMO of decisions of other international organisations relating to the impact of climatic changes.

Members of the panel have not yet been appointed, with the exception of the chairman Dr E. Süssenger, who is a member of the Executive Committee of WMO and head of the German meteorological service.

The panel will meet as soon as possible and is requested to prepare a report in time for consideration at the Seventh Congress of WMO, next year.

At present, the WMO is active in several areas of climatic research, although these are not coordinated. Perhaps best known is the work on the physical basis of climate and climate modelling, which is being carried out jointly with the International Council of Scientific Unions (ICSU) within the framework of the Global Atmospheric Research Programme (GARP). There are several international conferences in the pipeline including one on "Long-Term Climatic Fluctuations" to be held at the University of East Anglia next August; a spokesman for the Climatic Research Unit there said last week that many participants have already accepted invitations to attend.

Other aspects of climatic research are at present being studied under the aegis of WMO by their Commission for Atmospheric Sciences (CAS), and there is also a Commission for Special Applications of Meteorology and Climatology (CoSAMC) working group looking into problems of "Climatic Fluctuation and Man". It is perhaps hardly surprising that the president of the Commission for Agricultural

Meteorology (CAGM) is about to appoint a Rapporteur on climatic fluctuations and food production, or that the WMO is preparing papers relating to climatic fluctuations for presentation at the World Food Conference in Rome in November 1974. But clearly the time is certainly ripe for one panel to coordinate all these activities, and also to relate observations by the World Weather Watch (WWW) to the WMO studies. The new panel will include experts nominated by CAS, CoSAMC, CAGM, and the Joint Organising Committee of GARP.

Among the possibilities considered by the WMO Executive Committee were to designate either the CAS Working Group on the Physics of Climatic Fluctuations or the CoSAMC Working Group on Climatic Fluctuations and Man as a panel of the Executive Committee overseeing the WMO's climatic work. But the committee decided "in view of the desirability of formulating proposals . . . for consideration at the Seventh Congress, and bearing in mind the number of constituent bodies involved . . . to establish a [new] panel of experts" with the terms of reference as outlined above.

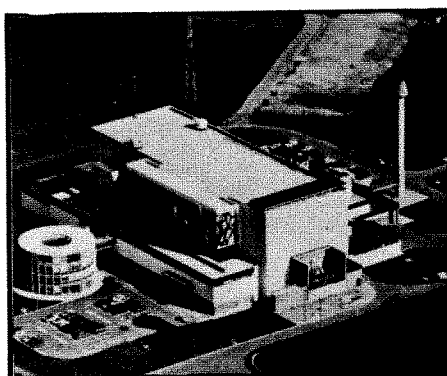
This panel will also be responsible for ensuring that WMO initiatives in this area are related to studies and work by other international bodies such as

UNESCO, the Food and Agricultural Organisation (FAO), the United Nations Environmental Programme (UNEP) and ICSU. A UNEP representative at the recent WMO Committee meeting confirmed the desire of UNEP to be associated with WMO in further studies of climatic change, and mentioned particularly the possibility of accelerating "those aspects of the WMO programme which would be of value for planning food production, especially in the developing regions". He suggested that statements of the statistical probabilities of successive years of unfavourable weather conditions would be particularly useful.

A spokesman for the British Meteorological Office welcomed this development, and said that the Met. Office accepts the WMO view that the future of our climate is an important topic for study. He pointed out that although climatic change has been receiving more attention lately, this has included publicity for some "shakily based views" ranging from claims that a new ice age is just around the corner to fears that the Earth is heading for a global desert situation. So it is certainly, in the view of the Met. Office, a good thing for an authoritative study of this kind to be carried out, and to put the various claims made about climatic change into perspective.

UK reactor choice

by Eleanor Lawrence



Winfrith SGHWR

At last the steam generating heavy water reactor (SGHWR) has been officially passed as the reactor choice for the next phase in the British nuclear power programme.

Announcing his decision in Parliament last week, Mr Eric Varley, Secretary of State for Energy, stressed five grounds on which he favoured the SGHWR.

- It will provide power reliably
- It can be ordered quickly
- The Nuclear Installations Inspec-

torate will give it safety clearance quickly

- It offers scope for British technology which should be exploited
- A 100-MW prototype at Winfrith has been operating successfully for six years and is designed to produce the operating conditions of a commercial unit.

Mr Varley plans to start with modest reactor units of around 600 MW and an initial ordering programme of not more than 4,000 MW over the next four years.

The government's decision runs counter to the strongly expressed wishes of the Central Electricity Generating Board (CEGB) in several respects. It wanted a large programme of a total of 36,000 MW using the American pressurised water reactors (PWRs).

Doubts on the safety of the PWR have been expressed both in the United States and in Britain, notably by Sir Alan Cottrell, former Chief Scientific Adviser to the Government. The Select Committee on Science and Technology was also opposed to the PWR. One of the main attractions of the SGHWR is undoubtedly its safety. Unlike the PWR there is no large pressure vessel, but a system of small-diameter pres-

sure tubes. These offer the 'leak-before-break' safety features which people feared might not occur with the pressure vessels of the PWR.

All the components of the SGHWR, except for the heavy water used as a moderator and perhaps the zirconium pressure tubes, can be made in Britain. Mr D. R. Smith, chief SGHWR engineer with the Nuclear Power Group, said that there would be few scaling-up problems except perhaps with the calandria tank surrounding the radioactive fuel elements. If the first orders were placed fairly quickly, as promised by Mr. Varley, building could start next year. Prospective sites include one at Torness in Scotland, for the South of Scotland Electricity Board (SSEB), and at Sizewell in Essex for the CEGB. Mr Smith thinks that the reactor could be completed.

The satisfactory Canadian experience with the similar CANDU reactor was probably a major factor in Mr Varley's decision. British experts who visited Canada recently were enthusiastic and close cooperation with the Canadians is expected. For the present, until the British programme is expanded, heavy water for the reactors will be imported from Canada. As soon as the SGHWR programme proves its worth, however, a British heavy water plant is envisaged, which would take advantage of the Canadian experience.

Money for old excavations

from Ian Caruana

THE Excavations Annual Reports, recently published by the British Department of the Environment (DOE) reveal that in at least one way archaeologists are failing to justify the additional cash they have been given in the past two years. In 1972 the annual government budget was in the region of £400,000; this year it has topped £1 million. One of archaeology's unceasing and justified complaints has been that the system of ministry grants for excavation never took account of the overriding need for publication, and financial austerity often made it a matter of necessity that an excavator spent almost all his time in the field just to make a living. It is, however, clear from the DOE's Reports that more money has not meant more publications. In fact while the number of digs financed directly by the DOE has risen steadily from under 80 in 1961 to over 200 in 1972 the number of reports published each year has remained more or less constant. The result is that there are now proportionally more excavations whose findings go unpublished than there were in 1961.

There is, however, one area above criticism—namely the permanent units

that are a developing feature of modern archaeology. (Their activities do not swell the DOE statistics because, although they receive a lot of government money, they also tap other sources and the DOE is quite happy to abdicate control in such cases.)

The Guildhall Museum's Department of Urban Archaeology in London is one such unit which, although set up only recently, has a good record. The results of two excavations from 1972, at Aldgate and Bush Lane House, were published last year. Four more reports are due out soon, one of which (dealing with work at the Customs House) took only five months to complete. Brian Hopley, the director, is responsible for a policy that ensures publication as quickly as possible. Each time a supervisor finishes a site he is immediately removed from the field and has to finish his site report before being given another site to run.

In addition the London unit is engaged in clearing the backlog of unpublished sites in the City. Through lack of resources, Peter Marsden (now Hopley's deputy) accumulated 70 sites which have so far yielded no published information about London's history. Now he faces a ten-year programme of publication—a feasible proposition inside a large unit with enough resources to back him up and to enable others to carry on with imminent excavation needs.

Salyut and after

from Verd Rich

ALTHOUGH the flight of Salyut 3 must inevitably be seen, in the short term, as a preparation for the joint Apollo-Soyuz programme of 1975, some of the experiments included seem directed at the longer term aim of establishing a permanent Earth-orbital station, as envisaged by Tsiolkovskii, and/or long term deep-space missions.

Accordingly, one of the major tasks of this mission is the testing of the life support systems, including the heat regulation system "in various regimes", and the regeneration of water from atmospheric condensation. The medical tests, effected with the multipurpose "Polinom-2M" apparatus, include the investigation of haemodynamics (the state of the circulation and the "rate of propagation of pulse waves along the arteries"), the ventilation of the lungs, and the collection of samples of exhaled air for subsequent laboratory analysis. It is intended to use the respiratory data to calculate the energy consumption in performing various tasks under weightless conditions—results which could be of considerable importance in planning the nutritional

requirements of a fully operational long term station or mission.

What these tasks are, either in the present mission or in future plans, is so far not entirely clear. The TASS reports, as ever, are tantalisingly brief, stating in this case, that the cosmonauts have "begun to test the possibility" of manufacturing hitherto unknown substances. A hint from the mission control station suggests that these may include steel "lighter than wood", and glass/metal alloys. In view of this brevity, speculation would seem fruitless at present.

Nevertheless, this type of experiment does suggest the Tsiolkovskii concept of a fully operational and self-maintaining orbital station, utilising the space "environment" for technology as well as research work. Although Tsiolkovskii's belief that the creation of such a station would be a necessary preliminary to any lunar or deep-space mission has been superseded by subsequent technology, Tsiolkovskii's name still exerts a considerable charismatic effect on the Soviet space planners. It is perhaps significant that, in a *Pravda* "background article" to the present flight, Candidate of Sciences N. Pisarenko of the Institute of Space Research of the Soviet Academy of

Sciences, discussing the radiation hazards of long term flights, states that primary cosmic radiation of galactic origin would, in the present state of the art, form a definite barrier to any manned Mars mission. He notes that "ways of overcoming this barrier" are being investigated, including "study of the self-shielding, restorative functions of the human organism and possibilities of intensifying them by the use of pharmacological preparations", as well as improvement of the shielding of the spaceship itself. For orbital stations, however, the hazard is considerably less, since according to estimates obtained from the data of Kosmos satellites, at the altitudes at which space stations operate (apogee of up to 500 km) cosmic radiation of solar origin is virtually absent and galactic primary cosmic radiation reduced by some three or four times in comparison with the deep-space value.

Both types of protection received an unexpectedly severe test when a solar flare occurred during the flight. According to TASS (July 15) "some physicists" wished to recall the cosmonauts. But it was decided that the Earth's magnetic field would protect them from the worst of the radiation, and they were instructed to use their antiradiation drugs.

MRC restructuring

by Peter Newmark

FROM September 1, 1974, substantial changes in the research boards that serve the Medical Research Council (MRC) will take effect. The Tropical Medicine Board is to be retained but both the Biological and Clinical Research Boards will be scrapped. In their place there will be three new ones—the Neurobiology and Mental Health Board, the Cell Biology and Disorders Board and the Physiological Systems and Disorders Board. It is the task of each board to advise the council on policy and to initiate and support research within its designated area of scientific objectives. In addition each board has the responsibility of maintaining research in and deciding the level of support needed for each of the disciplines or specialities that it encompasses.

As well as the four boards there is also to be an Environmental Medicine (Research Policy) Committee. This will cover all aspects of environmental, occupational and social medicine. The fact that these areas will include subjects (for example radiological protection) that are also of concern to the boards is recognised by ruling that the committee will allocate its funds as far as possible through the boards. Similarly each board will be able to use its funds for subjects covered by the committee.

The new boards seem to have a degree more power than previously. Whilst the council still carves the cake, it will be largely for the relevant board to allocate its own slice. One can hardly help noticing that the total number of board members has expanded much faster than the money available for them to share out (not forgetting that by 1975–76 about 25% of the MRC's grant-in-aid will be transferred to the Department of Health and Social Security and the Department of Employment to enable them to commission contract research to be carried out by the council).

The main objective behind the new MRC structure is clearly a step, or at least a nod, in the direction of Rothschild thinking. Instead of a horizontal structure dividing clinical from basic research each board is now vertically structured.

Erratum. In the article "Foetal research aborted in the United States" (*Nature*, July 12) the last sentence of paragraph 3 should have read "... research on fetuses *in vivo* has already produced some valuable conclusions and its banning is seen by some as shortsighted and ill-founded."

Lecturers lectured

FOUR lecturers at the Hebrew University in Jerusalem have been fired and another 12 transferred to new posts for poor teaching. Their achievements as teachers, as those of 220 colleagues, were evaluated on the basis of a survey carried out among several hundred science students at the Hebrew University by the Jerusalem-based Institute for Applied Social Research. The lecturers were graded on a five-point scale, with one point going to the worst and five to the best. Taken into account were student answers to questions such as: "Is the material usually presented in an interesting manner?"; "What attitude does the lecturer have towards relevant comments and questions?"; and "To what extent does the lecturer add to the information already available in textbooks?"

Analysis of the replies received has enabled the university to advise even relatively effective lecturers how they can overcome weak points. Professor Yitzhak Marcus, Chairman of the Curriculum Committee in the Natural Sciences Faculty of the Hebrew University, explains that the survey was not undertaken "to punish" lecturers.

New ways with fuels

by Allan Piper

IT was encouraging to hear original ideas given an airing recently in London where the Institute of Physics had arranged a meeting on "Novel physical methods of winning, burning and conserving fuels". Plants could be harnessed to produce hydrogen, said one participant, and another introduced the futuristic concept of mechanical miners. Most of the ideas are already well developed, but inevitably the 'establishment' found them 'uneconomical', and by implication not worth developing.

One topic was the potential use of solar energy through photosynthetic conversion to energy-rich plant material, which can then be converted by pyrolysis to alcohol, gas, oil and solid fuels. Dr O. D. Hall (Kings College, London), a botanist, pointed out that the amount of solar energy arriving at the Earth in three hours can meet the world's power demands for a year.

Photosynthesis in many economic crops is relatively inefficient. But in some other plants a more photosynthetic system, C₄, operates. Attempts to introduce the C₄ system into economic crops are in progress, together with a programme of plant breeding

aimed at developing plants which are more efficient and will produce specific desirable products. Hydrogen gas, a likely fuel for the future, can also be produced by photosynthesis, said Dr Hall. Under certain growing conditions algae can produce hydrogen, and model chloroplast-membrane systems have generated hydrogen in the laboratory.

If sunlight could be converted with an efficiency of 10% (compared with 0.2% naturally) only 1.5% of the land area of the United States would be needed to provide the total energy requirement of that country. Sunnier South Africa and Australia would need less still and even densely populated Britain would need to use only 9% of her land area.

Dr Hall stressed that an integrated approach using a variety of organisms, not only green plants, would be needed but that it would then be possible to harness all the sunlight between wavelengths of 400 and 900 nanometres.

Participants who found Dr Hall's ideas too revolutionary found little consolation in the next lecture. Professor M. W. Thring (Queen Mary College, London) said that much of the world's coal resources can never be tapped if we continue to use human miners. He introduced the concept of 'telechurics' hands at a distance. Already such automation has been used to service nuclear reactors and on the Moon. But the machines for mining coal would have to perform all the functions of a human miner. Professor Thring believes that expenditure of less than £100 million could develop such mechanical miners within 10 years; they would be operated by one worker from the safety of a control room.

But whatever we burn, we must learn to do it more efficiently. Professor F. Weinberg (Imperial College, London) explained that natural burning is a very inefficient process. Extremely low concentrations of combustible material in air—'lean mixtures'—cannot be burnt using conventional methods of combustion. But the efficiency of burning can be improved by preheating mixtures, said Professor Weinberg, thus allowing much leaner mixtures to be burnt. Useful supplies of energy could be extracted from waste gases, which at present are allowed to escape as pollutants into the atmosphere.

Professor Weinberg also discussed how electric fields could be used to regulate the behaviour of various pollutants within a flame, such as carbon or lead oxide smokes. Pollutants could then be induced to deposit in a particular position, on an electrode for instance, and particles could be manipulated, allowing control of their development and consumption in the combustion zone.

Business: multinationals in the news

by Roger Woodham

A COMMISSION on Multinational Corporations is called for in a recent report to the Economic and Social Council of the United Nations (UN) by a "group of eminent persons". The group was set up by the council to study the "impact of multinational corporations on development and on international relations", and during its deliberations it questioned some 46 witnesses including Mr. Ralph Nader, Mr. Altiero Spinelli (a member of the Commission of the European Communities) and Sir Val Duncan (Chairman of Rio Tinto Zinc).

The report makes it clear that the primary responsibility for the behaviour of multinationals rests with individual governments, which should be backed up by action at the international level to promote cooperation and harmonisation. The commission, the group envisages, will be made up of experts acting in their individual capacities and assisted by an information and research centre within the UN Secretariat. Some sort of general international agreement is the eventual goal (perhaps something like the General Agreement on Tariffs and Trade, GATT).

Particular concern is shown in the report about the effects of multinationals on developing countries, in many of which there is "widespread concern over foreign control of key sectors of the economy". The group thinks that host countries should clearly define the areas in which they are ready to accept foreign investment and that developing countries, especially, should be encouraged to retain ownership of their natural resources or control of the use of them. More

joint ventures between multinationals and individual governments may be the answer, with capital being made available by international financial institutions.

On the technological side, the report reiterates what others have said before, namely that the most up-to-date technology is not necessarily the most appropriate for a developing country. One of the group's suggestions is that host countries set up centralised negotiating machinery to deal with all aspects of proposals for foreign investment, the nature of the technology included.

The group thinks that the matter of multinational corporations needs to be discussed at least once a year by the Economic and Social Council. And in something of an understatement the report declares that "their activities are not *per se* geared to the goals of development".

● Hoechst UK, British subsidiary of the German chemical giant Farbwerke Hoechst, formally opened its new pharmaceutical laboratories at Milton Keynes last week. With a budget of £1 million a year the new laboratories are the latest in a series that Hoechst has been setting up throughout the world in the past six years—the others are in the United States, Japan and India. The emphasis at Milton Keynes is, however, to be on development, and the eventual full complement of 150 scientists will work on diagnostics and the development of possible new drugs rather than search for new chemical entities.

Hoechst has become particularly sensitive to the strong feelings that surround the use of animals for testing drugs because on two occasions last year a group calling itself the Band of Mercy attempted to burn down the laboratories as they were nearing com-

pletion. It failed, but the conspicuous presence of uniformed gentlemen from a private security company and the neatly rolled fire hoses near hydrants showed that the company is taking no further chances. Dr J. Coombes, head of Hoechst pharmaceutical research in Britain, made a special point of saying that "we would, of course, prefer to avoid the use of animals, with all their disadvantages, and we try to do this wherever we can, but in most cases it is just impossible." Safety tests on animals are required by law under the Medicines Act, he added.

First aid for the Aral Sea

from our Soviet Correspondent

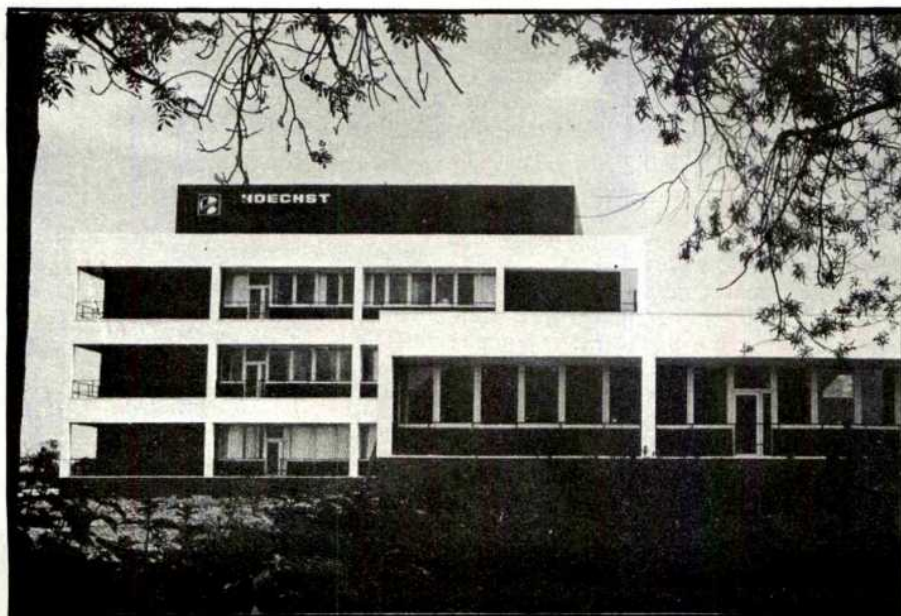
UNTIL the grand, often publicised schemes for diverting the North Siberian Rivers Ob' and Yenesei so that instead of discharging into the Arctic they will irrigate the steppes of Central Asia, can at last be brought into effect, first aid measures are needed to save the Aral Sea.

This small inland sea, fed by the waters of the Amu-Darya and Syr-Darya was first hydrographically surveyed by a military expedition in 1848-9 (as part of the drive towards India), and has in the subsequent century and a quarter fallen some 3 m in level, because of the use of the rivers for irrigation.

The Aral basin contains more than half of the irrigated lands of the Soviet Union, 90% of the cotton crop, 40% of the rice and considerable quantities of fruit and vegetables. It is not, however, a lack of water in absolute terms that is the trouble, but a piecemeal use of what there is. During the spring floods the irrigated settlements are in considerable danger from flooding, and the only remedy at present is to divert the floodwaters to go to waste in the deserts.

The solution proposed is simple—a system of 13 dams to be built on the two rivers to contain the floodwater, distributing it for irrigation as required with the remainder being discharged into the sea. The surplus irrigation water will be collected and reused for irrigation and for leaching salts out of the soil, a method already in use in many of the Soviet Central Asian cotton farms.

The methods seem standard enough, but the publicity given to it and to its initiator, a certain Konstantin Rakitin (meriting a page and a half of a Novosti press release), would suggest perhaps that the prospect of a spectacular future solution—the diversion of the great Siberian rivers—has until now led to a neglect of the necessity for short term conventional measures.



New Hoechst laboratories

correspondence

Newton and Kepler

SIR,—The exchange of letters between J. Herivel and D. T. Whiteside (*Nature*, April 19, 634) arising from Herivel's review of my *Introduction to Newton's Principia* (*Nature*, 247, 163-4), hinges primarily on my alleged interpretation of an article by Whiteside (1964) and only secondarily on the substantive issue: Isaac Newton's possible knowledge of Kepler's law of areas before, 1676 or so, or even later.

When Whiteside's article appeared in 1964, I had already been engaged for several years in wholly independent research on the development of planetary astronomy before the *Principia* and on the source of Newton's knowledge of Kepler's laws. One of the central topics of my investigation had been the use of one of three then-current variant substitutes for (or approximations to) the area law—associated with the names of Bullialdus, Ward and Mercator. As I read through Newton's early manuscripts and book annotations, I was struck by the fact (it would have been impossible not to have been!) of the obvious and conspicuous lack of any early documentary reference by Newton himself to the law of areas—there is none dating from the period up to the famous exchange of letters between Hooke and Newton in 1679-80.

When Whiteside's brilliant article appeared, I temporarily laid aside my own book-length manuscript, but the postscript (page 137) to Whiteside's article refers to this work of mine. Accordingly, Herivel's remarks as to whether my opinion is or is not simply a correct interpretation of Whiteside's article may be seen to be irrelevant.

The substantive point at issue is not whether Newton may possibly have encountered the law of areas before 1676 (at which time he may very well have read the statement of the law in Mercator's treatise). It is rather whether at any time earlier than the end of his exchange of letters on motion with Hooke, in 1678-79, Newton consciously gave to this law any serious consideration as a possible basis of physical principles, or as an accurate (or most accurate) descriptive statement of the variations in planetary orbital speeds, or even as a major or significant element in considering planetary motions of the same order of

importance, say, as the elliptical orbits or the harmonic law. On the basis of ordinary canons of historical evidence, and to give my view the most accurate expression possible, the footnote in my *Introduction*, which is the occasion for these letters may be (in part) expanded and rewritten more fully so as to read:

"There is no documentary evidence that Newton was consciously aware of Kepler's law of areas (much less that he considered this law in any significant manner) prior to 1676, when he might well have encountered it in Mercator's *Institutionum Astronomicae Libri Duo*. At the end of his correspondence on motion with Hooke in 1679-80, or possibly soon thereafter, but at least by 1684, he used this law to solve the central problem of elliptical planetary motion, shown to result from the action of a centrally directed inverse-square force on a body with a component of linear inertial motion."

Yours faithfully,

I. BERNARD COHEN

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Family planning

SIR,—Poor parents in poor countries do not readily adopt family planning methods even when these are available to them, because they do not want to have small families. They want large families for various reasons, many of which are economic; children provide free labour on family farms, security in old age and so on. Economic motivations can be changed by economic means. Under present circumstances a substantial reduction in the rate of population growth appears to depend on widespread economic improvement: but in many developing countries widespread economic improvement does not seem feasible unless there is a substantial reduction in the rate of population growth.

The problems are familiar, but they may not be insoluble. In many parts of the world the parents' desire for large families seems to represent to a great extent a desire for sons. If such parents could choose to have sons rather than daughters they would probably do so; if a cheap and simple method existed which increased the chances of having sons rather than

daughters, it would probably be readily adopted. The total number of children needed in order to obtain any given number of sons would be reduced. And in the long term, the shortage of women of child-bearing age would lead to a further reduction in the birth rate.

Human sperm bearing X and Y chromosomes can be separated *in vitro* on the basis of their differential motility. Y-sperm, which are male-determining, have a greater ability to penetrate an interface between a less viscous and a more viscous fluid and also out-distance X-sperm by swimming faster in a fluid of relatively high density and viscosity¹. It is not inconceivable that some method could be devised whereby a suitable viscous solution could be introduced (for example within a capsule) into the female genital tract in such a way that after sexual intercourse the X-sperm were selectively retarded and the chances of conceiving a male child were increased.

The idea that the ability of parents to choose to have sons rather than daughters could lead to a substantial reduction in the birth rate, especially in countries with high rates of population growth, has been proposed before²; the recent findings on differential sperm motility suggest that this idea should now be taken seriously.

If a suitable method could be developed and if it were adopted on a wide scale, the increased proportion of males in the population would undoubtedly create new problems, some of which are easy to imagine. But what are the imaginable alternatives? In India, for example, in spite of a well-established, government-sponsored family planning organisation and mass sterilisation programme, the population is now increasing by one million every twenty-five days.

Yours faithfully,

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¹ Ericsson, R. J., Langerin, C. N., and Nishino, M., *Nature*, 246, 421-424 (1973).

² Postgate, J., *New Scientist*, 12-16 (April 5, 1973).

news and views

What does BL Lac?

MANY extragalactic objects—the nuclei of some galaxies, quasistellar objects, N systems and objects of the BL Lacerta type—contain very small components which vary in optical light, and in radio flux. The variations take place on time scales which vary from days or weeks up to many years. Objects with a wide range of redshifts are involved, and of course until recently no lines had been identified in objects of the BL Lac type.

Radio astronomers using very long baseline interferometers have measured very small angular sizes 10^{-3} – 10^{-4} arc second in some sources, and apparent changes in angular structure have been seen in several of them. The time variations which occur either in flux or in angular size, when interpreted using distances obtained from redshifts (that is on the cosmological assumption), have led to various apparent difficulties in interpretation.

The very small sizes set by the light travel time and the very large optical fluxes set by the large redshifts lead to immense photon fluxes, and apparent contradictions involved in the interaction of the photons with the electrons which must generate them. This problem, originally raised by Hoyle, Burbidge and Sargent in 1966, has been argued about, skirted around and sometimes denied.

In cases where one has angular size measurements and radio spectra which turn over, suggesting synchrotron self-absorption, detailed calculations can be made (see for example Jones, O'Dell, Stein and Burbidge *Astrophys. J.*, **188**, 353; 1974). If flux variations in such radio sources are explained in terms of a simple expanding cloud model, it is found that for all sources with large cosmological redshifts and rapid variations the expansion must be highly relativistic. This means that enormous energies of the order 10^{50} erg or greater are released in frequent outbursts, part of the energy being associated with relativistic expansion against the surrounding medium. Because of the short time scales the cumulative energy problem is very severe.

In sources in which angular sizes seem to have varied, the observations were first thought to indicate relativistic motion of components—separations of doubles, and so on—at relativistic speed. Thus, there was talk of apparent superlight velocities. By now the difficulty and ambiguities of interpretation of the radio data have led some people to discount models of this type, and models in which it is supposed that several small spatially separated components are varying independently are now in vogue. These too have their difficulties.

All of these apparent problems raised by such sources stem, however, from the great distances implied by the redshifts. For comparatively close objects, such as the radio galaxy NGC1275, low and entirely reasonable optical and infrared radiation densities, non-relativistic motions and lower energies are required. Thus, for some time, either the unpopular view that the redshifts are not measures of distance, or the notion that nobody really understands the physics of the sources has been the basis of the two approaches taken by students of the subject.

Until recently the BL Lac objects which show very rapid flux variations and all of the other continuum energy properties of, for example, QSOs, could not be brought into the debate because no redshifts had been measured.

Now within the space of three months redshifts for two of them, BL Lac itself (Oke and Gunn, *Astrophys. J. Lett.*, **189**, L5; 1974) and PKS0735+178 (Carswell, Strittmatter, Williams, Kinman and Serkowski, *ibid.*, **190**, L101; 1974) have been published. What is one to make of them? The strongest claims have been made for BL Lac. Full publicity has been accorded to the view of Oke and Gunn that BL Lac is a QSO embedded in an elliptical galaxy with a redshift $z=0.07$. Discounting for a moment the fact that the paper and the news releases (which many scientists have read more carefully than the paper itself) appeared on April Fools' Day, how seriously should one take this claim? Earlier arguments of different kinds by Adams (*Astrophys. J.*, **188**, 463; 1974) and by Wlérick, Michet and Lelièvre (*C.r. hebdomadaire Acad. Sci., Paris*, **278**, 245; 1974) in which it has tacitly been assumed that a normal galaxy does underly the nonthermal object, have led to distance estimates which range from 130 Mpc ($z=0.022$) to 420 Mpc ($z=0.07$). The Oke-Gunn result rests on scanner observations of the outer parts of the object and it is claimed that weak absorption features, not all of which are identified, are those expected from stars in a normal elliptical galaxy. The problem is that the observation is very difficult, the features are weak and the reality of some, and perhaps all of the absorption lines, is in doubt.

What can be learnt from the other BL Lac object PKS0735+178? In this investigation the authors have made no claim to have discovered a stellar component. The redshift rests on two comparatively sharp absorption features which they attribute to Mg II, based on the separation between the two components of the doublet. These are not likely to be due to stars, otherwise the two components would be smeared into one broader line. The absorptions are more like those arising in gas clouds which are often seen in high-redshift QSOs. The object is variable in optical light and in polarisation on time scales less than a month.

Thus, on the face of it, these first two BL Lac objects in which spectroscopic features have been found seem to be different. One may have its source in a normal galaxy with a lineless QSO (a contradiction in terms of the original definition) embedded in it. The other, as far as is known, is a nonthermal continuum object with QSO-like absorption in it.

Should these interpretations be taken seriously? The answer is that one should be exceedingly cautious at this point. The most important thing is to check the BL Lac observations using independent and, if possible, superior equipment. It would not be at all surprising, despite the ballyhoo and the claims, if they did not survive. It is interesting to see how easily theoreticians tend to accept this result at face value, when compared with the way they looked sceptically at the original gravitational wave results.

What bearing do these results have on the problems mentioned earlier? If BL Lac is at a distance of 420 Mpc, then as Oke and Gunn point out, the luminosity and rapid variations of the central object give rise to all of the apparent difficulties associated with understanding the radiation properties of the large-redshift QSOs. The same can be said for PKS0735+178 which has an even larger redshift ($z=0.424$). At the same time the results do not provide direct evidence against the possibility of noncosmological redshifts. If it is supposed that a QSO is embedded in a galaxy, it must be proved that each component has the

same redshift if the noncosmological hypothesis is to be disproved. In BL Lac only the redshift of the outer part has been observed. The centre is excluded. This argument is the converse of that used by Kristian (*Astrophys. J. Lett.*,

179, L16; 1973) who, having observed QSOs whose redshifts had been measured and which have fuzz around their images, assumed, without proof, that these were galaxies.
GEOFFREY BURBIDGE

Three abundance classes of messenger RNA

GENE expression has both a quantitative and a qualitative component. It is important to know not only which genes are expressed but also how many transcripts of each are present. On page 199 of this issue of *Nature* Bishop, Morton, Rosbash and Richardson show by two independent methods that in HeLa cells approximately 1% of the single copy DNA, enough to code for about 35,000 different average-sized proteins, is transcribed into cytoplasmic messenger RNA which may be isolated by affinity chromatography on oligo (dT) cellulose. These mRNA molecules are present in three distinct frequency classes. About one fifth of them are transcribed from a very few (about 17) sequences but are present in great abundance (about 10^4 copies each per cell). One half of the mRNA is derived from a very large number (about 35,000) of different genes but individual mRNA sequences are present at low concentration (about 10 copies per cell). The remainder of the message population derives from an intermediate number of genes (about 350) and each is represented by approximately 450 copies per cell.

The kinds of experiment from which these numbers are derived involve purification of cytoplasmic mRNA by affinity chromatography on oligo (dT) cellulose. Although this method does not give a total message preparation (for instance histone mRNA will not be present), it seems likely that it will contain the bulk of the mRNA. Highly radioactive DNA complementary to the purified mRNA (cDNA) is synthesised by viral reverse transcriptase for use as a probe in nucleic acid hybridisation reactions. Bishop *et al.* performed two types of hybridisation reaction with their antimesage cDNA—one using total DNA in excess and one using purified mRNA in excess. The DNA-driven reaction shows that cDNA prepared from total cytoplasmic message hybridises to both repetitive (about 10%) and non-repetitive (about 70%) components. A control using purified radioactive HeLa single copy DNA instead of cDNA defined a rate constant for the renaturation of HeLa unique sequence DNA which is nearly identical to the rate constant determined for the second transition of the cDNA reaction, providing convincing evidence that cDNA is reacting with unique sequences. These results suggest that most of HeLa cytoplasmic message is transcribed from unique DNA but a small fraction (about 15%) derives from a class of intermediate repetitive sequences.

The rate of the RNA-driven hybridisation reaction between cDNA and mRNA template in excess is determined by the relative concentration as well as by the absolute number of different mRNAs in the total preparation. If all mRNAs are at equal concentrations then the reaction will have a single transition with a rate constant determined by the number of reacting species. But if some RNAs are present in greater amounts than others they will react faster and show up as an early transition or step in the progress curve of the reaction. The actual curve relating extent of cDNA hybridised to the R_{ot} (product of initial mRNA concentration and time) shows three such transitions suggesting at least three major frequency classes in the mRNA population. In order to interpret these curves, a kinetic standard of known sequence reiteration frequency and molecular weight is required. Reaction between excess rabbit haemoglobin α chain mRNA or a mixture of α and β

chain mRNA and homologous cDNA provides such a standard. A further requirement for estimating numbers of gene transcripts is a measurement of the number-average molecular weight of HeLa mRNA which was determined at 600,000 daltons—about three times the molecular weight of haemoglobin α chain mRNA. Dividing the observed $R_{ot/2}$ for each transition by the $R_{ot/2}$ of the kinetic standard corrected for the difference in molecular weights gives an estimate of the number of gene sequences contributing transcripts to that frequency class.

These calculations are necessarily approximate since they rely on unverified assumptions such as the general applicability of a number-average molecular weight determined for the total mRNA population to different subsets of that population and uncertainty about the contribution of repetitive gene transcripts to each of the three frequency classes. Furthermore, it is not clear at present whether all poly(A)-containing RNA is transcribed by reverse transcriptase with equal efficiency. In the worst case an entire frequency class might not be transcribed at all and clearly would not be detected by hybridisation. If on the other hand there were only slight variations in transcription efficiency this would not matter particularly since the rate of hybridisation depends on the concentration of cold driver, either RNA or DNA, and not on the concentration of cDNA. What might be mistaken though would be the relative amount each frequency class contributes to the total population since these are determined by the plateau values and not by the reaction rate.

A third type of experiment using purified ^3H -labelled single copy HeLa DNA instead of cDNA as the probe in an mRNA excess reaction allowed a totally independent estimate of the proportion of the genome transcribed to give cytoplasmic message. Both the cDNA and the purified single copy reactions led to the conclusion that about 1% of the unique sequence is represented in mRNA and allow some weight to be attached to this estimate.

This work represents a significant advance in the understanding of both gene expression and in the likely numbers of different sequences involved. But many intriguing questions remain. Do HeLa cells really synthesise 35,000 different proteins or do some unique sequence transcripts not code for proteins? Are HeLa cells expressing their maximum genetic potential or is even this large amount of information only a small part of their true capacity. In this regard it is noteworthy that a *Drosophila* cell line seems to express an order of magnitude fewer sequences (4,000) in cytoplasmic mRNA and it may well turn out that HeLa cells are atypical in the amount of information found in cytoplasmic message. The existence of three frequency classes of mRNA, most of which derive from single copy DNA sequences, clearly focuses attention on the differential control of mRNA synthesis and degradation. It may be that differential rates of synthesis are responsible for variation in the steady state level of particular mRNAs but equally likely at present are differential rates of degradation, for which there is already evidence in HeLa cells. Finally, it is clear that a cell contains different abundance classes of protein and it would be interesting to know to what extent this reflects the frequency classes in mRNA.

PETER J. FORD

DNA of hepatitis B virus

SIGNIFICANT advances in research on hepatitis B virus have been made recently by groups at several centres in the United States. Experiments by Hirschman and his colleagues (*Lancet*, i, 1099; 1971) first indicated the presence of a DNA polymerase activity in association with hepatitis B antigen. Crude pellets of antigen, obtained by high speed centrifugation of sera from a few patients with clinical and histological evidence of hepatitis, were found by electron microscopy to consist largely of the small pleomorphic 16–25 nm spherical particles of hepatitis B antigen, although the larger 42 nm spherical particles and tubular forms of varying lengths were also found. These preparations were found to stimulate the incorporation of tritiated dTTP into an acid-insoluble product in the presence of all four deoxynucleoside triphosphates, albeit at a low level. The reaction was considered to be dependent on an endogenous RNA template or primer since pretreatment of the samples with RNase abolished their activity. The reaction was, however, stimulated by the addition of poly (dAT) and not by poly (rA)-oligo (dT), as are the known RNA-dependent DNA polymerases.

Kaplan *et al.*, (*J. Virol.*, 12, 995; 1973) have reported the finding of a DNA polymerase activity in plasmas positive for hepatitis B antigen, selected because of their relative rich content of the 42 nm double-shelled spheroidal form of the antigen. DNA polymerase activity was associated with the core component of the 42 nm particle after removal of the outer surface antigen coat by treatment with the non-ionic detergent Nonidet-P40 (NP 40). Surprisingly the enzyme seems to function in the absence of any exogenous template. The incorporation of tritiated dTTP was dependent on the presence of all four deoxynucleoside triphosphates and magnesium ions, suggesting that a DNA or RNA template within the core probably directs the synthesis of DNA. The properties of the enzyme product were further assessed using ³H-dTTP as the trace label. Digestion with pronase followed by phenol extraction resulted in the appearance of about 20% of the acid-precipitable label in the aqueous phase. This extract possessed a buoyant density of 1.71 g cm⁻³ and a sedimentation coefficient of 110S, which was reduced to 15S after incubation with sodium dodecyl sulphate. Rate zonal centrifugation confirmed the close association of the polymerase with a proportion of the 42 nm antigen particles, within which the template molecule lies in a protected state. No enzymatic activity was found in highly purified small (16–25 nm) hepatitis B surface antigen particles. Kaplan *et al.* concluded that if the DNA polymerase is a virion enzyme and if the reaction is DNA dependent (as was suggested by inhibition of the reaction by actinomycin D and daunomycin), then the enzyme would be unique because similar virion enzymes have not been described so far.

Kaplan and his colleagues (*Nature*, 249, 762; 1974) went on to study hepatitis B DNA polymerase activity during the course of post-transfusion hepatitis. The polymerase activity was detected later than hepatitis B surface antigen but before serum transaminase levels were elevated so it was concluded that polymerase activity was related to viraemia and not to virus-induced liver damage. Krugman *et al.* (*New Engl. J. Med.*, 290, 1331; 1974) also concluded, after the examination of serial samples of serum from volunteers exposed to the MS-2 strain of hepatitis B virus, that DNA polymerase activity seems to identify the period of peak replication of hepatitis B virus.

Further observations on the nature of the polymerase and the product of its reaction were reported recently by Robinson of Stanford University School of Medicine at a seminar held at the London School of Hygiene and

Tropical Medicine (full details will be published in the *Journal of Virology*). Hepatitis B surface antibody removed the enzyme from the reaction mixture in the absence of the detergent NP 40, but in the presence of NP 40, which exposes the core, the enzymatic activity was found in the supernatant and could only be precipitated by the core antibody and not by the surface antibody. As expected from these results, the addition of purified 16–25 nm surface antigen particles effectively competed with the surface antibody and blocked the precipitation of the enzyme-containing particles in the absence of the detergent, but after treatment with NP 40 the purified small particles did not block the precipitation of the core by the core antibody. The DNA polymerase activity, after treatment of the antigen preparations with the detergent, was found at a closely similar position in a sucrose gradient as the 28 nm core particles. The 110S structure was found to be associated with the core by immunoprecipitation with hepatitis B core antibody. Advantage was taken of these two latter observations to provide a sensitive test for core antibody in sera of patients and carriers by using the DNA polymerase and the radioisotope-labelled DNA reaction product as markers for the core.

The initial finding that the DNA polymerase activity, associated with the core particle, does not require added DNA or RNA implies that the core contains nucleic acid which served as a primer template for the reaction. A major advance with this work has been the observation by electron microscopy of circular DNA molecules by shadow casting. Briefly, the procedure involved concentration of the 42 nm hepatitis B antigen particle by centrifugation followed by purification by repeated equilibrium centrifugation in sucrose density gradients. The preparations were exposed to DNase in order to eliminate free DNA. After treatment with the detergent NP 40 the cores were isolated by rate zonal sedimentation and treated again with DNase. After further centrifugation the core pellets were disrupted with sodium dodecyl sulphate to release any nucleic acid. Incubation with DNase I before electron microscopy removed all of the circular molecules, whereas RNase had little effect. The smooth, open configuration of the molecules suggests that they are double stranded and additional molecules of single stranded nucleic acid were not found after spreading in formamide.

In other experiments, dissociation of the core particles with lithium thiocyanate again revealed small circular nucleic acid molecules. The circular structures were not supercoiled and Robinson suggests that this appearance may be due to an open circular conformation with a nick in one of the two strands. Size distribution analysis give a mean length of the circular molecule of 0.78 μ m corresponding to an estimated molecular weight of the DNA of about 1.6×10^6 . This is smaller than double stranded DNA found in any known 'complete' virus and it is similar in molecular weight to adeno-associated virus. The thermal denaturation kinetics of the isolated DNA confirmed its double stranded nature and gave a result consistent with a G:C content of 48 to 49%.

This is a somewhat unexpected finding in view of the similarity to the base composition of mammalian DNA, and if this is representative of part or the whole of hepatitis B virus genome it is more consistent with those viruses possessing the ability to integrate their own genome into that of their host. Furthermore, it is difficult to envisage that this molecule is capable of coding for more than five or six proteins and would therefore almost certainly not contain sufficient information to satisfy the requirements both for active virus replication and the apparent complexity of the surface determinants on the particles associated with hepatitis B antigen. It is also difficult to imagine the active association of the enzyme and template within the confines of the core of the 42 nm particle. The

possibility of their segregation as components of different morphological forms, in a manner perhaps similar to the Fraenkel-Conrat covirus model for some plant viruses which require two or more particles to produce infection (a concept which was extrapolated to human hepatitis type B (Zuckerman, *Vox Sang.*, **19**, 304; 1970)) cannot yet be ruled out. There is also the possibility that the circular DNA is derived from a defective virus, and infectious particles with larger DNA molecules, which have not yet been detected, might be present. Alternatively, a helper virus might be required for replication (Kassanis, *J. gen. Microbiol.*, **27**, 477; 1962) and, although a second or helper virus has not yet been identified, such models for hepatitis have been proposed (Zuckerman *et al.*, *Nature*, **214**, 606; 1967).

The findings just reported by Robinson clearly represent one of the most significant steps forward in hepatitis research, but further work is required to establish conclusively the nature of the DNA polymerase template, to identify the enzyme as viral and not host specific and to clarify some of the other puzzling characteristics of the intriguing hepatitis B virus.

ARIE J. ZUCKERMAN
COLIN R. HOWARD

r and *K* rodents in Costa Rica

from our Animal Ecology Correspondent

THE demographic strategy employed by a species population is a function of, and is dependent on, the ecological complexion of its environment. This affirmation of the status of the population led MacArthur and Wilson (*The Theory of Island Biogeography*, Princeton University Press, 1967) to underscore and draw attention to the significance of *r* and *K* selection. In seasonally fluctuating environments it is argued that populations will seldom achieve a density corresponding to the carrying capacity (*K*) and so intra-specific competition for food and other resources will be low. Natural selection will favour those genotypes that produce large litters of early maturing young. In environments that lack a marked seasonal boost in productivity, that is, with *K* remaining constant, intraspecific competition will always be strong. Selection under these conditions (*r*) favours species that produce small litters of highly competitive young. Additionally one might expect that individual longevity is increased in non-seasonal environments along with other factors which serve to heighten survivorship such as deferment of sexual maturity. Thus *r* selection represents selection for a rapid energetic turnover

and *K* selection represents selection for efficiency of exploitation (Pianka, *Am. Nat.*, **104**, 592; 1970).

In general the concept is important for ecologists concerned with the artificial control of rodent populations. Microtines from the temperate regions are typified by high reproductive output (5 litters per year; 5-8 young per litter), low survival (2-4 months) and high population densities (up to 1,000 per hectare). Heteromyids from the arid regions of the New World have low reproductive outputs (2-3 litters per year; about 2.5 young per litter), high survival (up to 5 years in the field) and low population densities (about 0.5-5.0 per hectare). At this level the concept seems to hold up well. What demographic strategies are adopted by two closely related species of rodent which occupy seasonal and nonseasonal environments respectively within the same biogeographical region? A recent study by Fleming may help to answer this question (*Ecology*, **55**, 493; 1974).

Two study sites in Costa Rica, one a dry, seasonal tropical forest, the other a wet nonseasonal tropical forest were chosen. They were separated by about 130 km. The terms seasonal and non-seasonal were used after sufficient vegetational and climatic studies had shown there to be a distinct presence and absence respectively of an annual boost in primary productivity. Fleming chose to study the population dynamics of two closely related species of heteromyid rodents, *Heteromys desmarestianus* and *Liomys salvini*. In Costa Rica *Liomys* occurs in the seasonal dry forests and *Heteromys* in the nonseasonal wet forests. Although Fleming admits that a single comparison between two species can, at best, give only weak support to a general concept, the paucity of field observations demands that a second look is taken at what data there are. *Liomys* matures earlier than *Heteromys* (3-4 months as against 8 months) and has slightly larger litters (3.8 as against 3.1). Juvenile survival is higher and mean longevity slightly greater in *Heteromys*. *Liomys* juveniles and adults weigh significantly less than comparably aged *Heteromys*.

The field study lasted only 1 year with an additional month's study almost a year later. These observations must therefore be treated with caution. The effects of other ecological factors, such as predation pressure, while their existence was noted, were not measured. Nevertheless it seems that Fleming has provided a *prima facie* field example in support of the concept made all the better by it involving two closely related species (normally regarded as *K*-selected species) in adjacent, but ecologically distinct, habitats.

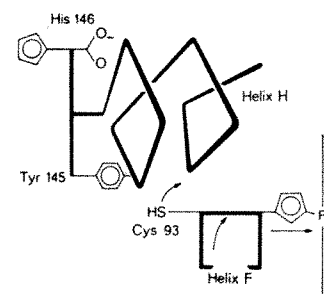
R to T with IHP

from a Correspondent

FROM a large number of interesting papers on structural and functional aspects of haemoglobin, a series of three long reports on the "Influence of Globin Structure on the State of the Heme" (*Biochemistry*, **13**, 2163, 2174, 2187; 1974) commends itself for attention. The series is noteworthy in carrying the names of ten authors from six different laboratories in the United Kingdom, the United States and Nigeria, and makes simultaneous use of a wide range of techniques.

In part 1 on human deoxy-Hb, Perutz, Ladner, Simon and Ho have used two modified haemoglobins and a mutant, all of which can be made to remain in the R (oxy) quaternary state when fully deoxygenated, and switch to the T (deoxy) state by addition of inositol hexaphosphate (IHP). By using such haemoglobin species, the effect of the R → T transition on the 5-coordinated ferrous haems can be studied in the absence of any dissociation of ligands. In the fully deoxygenated but R state, des-Arg(β -141)-Hb, NES-des-Arg(α -141)-Hb and Hb-Kempsey (β -99 Asp → Asn) all have electronic absorption spectra, ultraviolet circular dichroism and paramagnetically shifted proton NMR (nuclear magnetic resonance) spectra which differ markedly from those of normal deoxy Hb-A and resemble those of summed free deoxy α and β subunits. Addition of IHP switches them to the T state with electronic and NMR spectra like those of deoxy Hb-A. The switch also gives rise to small absorption and CD spectral changes that are most probably due to structural changes at the $\alpha_1\beta_2$ contact area affecting the trp (β -137) and tyr (α -142) residues.

Part 2, by Perutz, Fersht, Simon and Roberts, on allosteric transitions in methaemoglobin, makes use of optical, kinetic and NMR procedures to demonstrate that binding of IHP shifts the



Movement of SH on transition of haem to lower spin

Effect of changes in spin state of the haem on the F helix and on the freedom of the sulphhydryl group of cysteine-93 β to react with ligands. Quaternary R structure (from *Biochemistry*, **13**, 2182; 1974).

allosteric equilibrium of high-spin methaemoglobin derivatives (aquo-, fluoro-) in favour of the T (deoxy) state. The reduced reactivity of the β -93 sulphhydryl groups is also comparable with that observed for deoxy as compared with oxy-haemoglobin. IHP seems to bind to the same site in met- as in deoxy-haemoglobin, between the terminal amino groups of the β chains. Smaller changes in the UV spectra and SH group reactivities are induced in low-spin methaemoglobin derivatives (azido-, cyano-) by IHP without the sign reversal in the CD spectrum at 287 nm associated with the R \rightarrow T transition of deoxy-haemoglobin. Similarly, the NMR studies show that for the high-spin derivatives IHP causes larger changes in the ring-current-shifted resonances of some aromatic residues than it does for the low-spin derivatives. Taken as a whole, these observations indicate that solutions of high-spin methaemoglobin derivatives contain both R and T forms in equilibrium, with the R form favoured by high pH, and the T form by low pH and the binding of organic phosphates. This scheme can account qualitatively for the pH dependence of the redox equilibrium of haemoglobin and its sensitivity to chemical modification, and there is an interesting interpretation of the redox data in allosteric terms.

Part 3, by Perutz, Heidner, Ladner, Beetlestone and Slade, considers changes in the visible and near-IR absorption spectra, paramagnetic susceptibility, paramagnetically-shifted proton-NMR and electron spin resonances induced in high- and low-spin methaemoglobin derivatives by IHP, and relates them ultimately to changes in the distances between the iron atom and its ligands. The increased Fe-N bond distances observed in the T structure implies that the globin exercises tension on the haem which pulls the iron atoms further from the porphyrin ring plane, opposing the transition to the low-spin state needed for combination with oxygen and hence reducing the oxygen affinity. Finally, since haem-haem interaction is observed only when a change in quaternary structure accompanies reaction with ligands, the present findings imply that it is coupled to a change in tension at the haem, transmitted by a change in quaternary structure of the globin. This brings the discussion back to globin, which is the starting point of the series. It should perhaps be added that comprehension of the large amount of diverse experimental data is greatly helped by some summary tables and figures *en route* which summarise particular conclusions and implications reached during the course of the tightly argued discussion.

Mitotic spindle assembly *in vitro*

from a Correspondent

Two main approaches have been taken in studies of the mitotic spindle. Measurements of the birefringence of the spindle in intact cells by Inoue and others have suggested that the fibrous components of the spindle are in dynamic equilibrium with subunits. The birefringence of the spindle is lost when living cells are exposed to cold, high hydrostatic pressures and to colchicine and other antimitotic alkaloids. These agents are thought to depolymerise reversibly the microtubules of the spindle fibres and so prevent chromosome movement in intact cells. The other approach has been to isolate the intact mitotic apparatus, usually in the presence of stabilising agents such as glycols. This procedure has provided useful information about the chemistry of the mitotic apparatus but not how it works, because reversible assembly and disassembly have not been achieved with spindles so prepared.

Three groups of investigators have now reported the preparation of mitotic spindles which can be made to increase or decrease in size by appropriate treatment of the subcellular materials. The spindles are isolated in the presence of added rat brain tubulin and agents which chelate calcium, thereby preventing the disassembly of spindle microtubules. Cande, Snyder, Smith, Summers and McIntosh (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1559-1563; 1974) lysed rat kangaroo cells with the detergent Triton X-100 into a solution of polymerisable rat brain tubulin and obtained spindles which lose and gain birefringence when cooled and warmed; the spindles can move anaphase chromosomes to the opposite ends of cell preparations. Early anaphase cells lysed into buffers containing polyethylene glycol and nucleotide triphosphates showed spindle elongation and chromosome movement in the absence of added tubulin subunits. The authors conclude that although spindle growth requires microtubule polymerisation, anaphase motions do not.

Rebhun, Rosenbaum, Lefebvre and Smith (*Nature*, **249**, 113-115; 1974) report that spindles isolated from eggs of the surf clam (*Spisula*) cooled and then warmed with chick brain tubulin showed increased birefringence in the form of fibres similar in distribution to the mitotic apparatus of living cells. Sometimes such artificially polymerised spindles were larger than normal spindles. The isolated spindles increased in length but did not shorten and were stable to dilution of tubulin. It seems that they can assemble tubulin more

readily than they can break down the assembled units.

Inoue, Borisy and Kiehart (*J. Cell Biol.*, **62**, 175-184; 1974) found that tubulin purified from porcine brain augmented the birefringence of *Chaetopterus* spindles. The cells were lysed in a hypotonic calcium-chelating solution, and in the presence of tubulin, spindles and asters grew considerably larger than those in intact cells. The isolated augmented spindles depolymerised rapidly at 6° C, and birefringence was slowly recovered upon return to 23° C. The birefringence of the spindles also decreased in the presence of 2.5 mM calcium but not on dilution of tubulin or in the presence of colchicine or colcemid.

The results represent a useful advance in knowledge of the mitotic apparatus. They show that the conditions required for polymerisation of isolated spindle microtubules are similar to those defined by Weisenberg and others for tubulin from various sources, including surf clam eggs. The response of the isolated spindles to temperature reflect those observed in intact cells, but other responses, such as those to colchicine do not. Disassembly does not follow dilution of tubulins, so that a simple equilibrium between the protein subunits and spindle microtubules is unlikely to explain fully the behaviour of the spindle. Perhaps improvements in isolation technique will produce spindles that perform more like those in intact cells. Since exogenous enzymes, cofactors and inhibitors can gain access to the isolated spindles, it should be possible to analyse their role in spindle formation and disassembly. The spindle, however, is itself likely to contain a complex mixture of enzymes and other constituents, so that the analysis will not be straightforward.

Gibbons and others have found how useful sperm tail preparations made accessible to exogenous agents by detergents can be in analysing flagellar movement, and similar analyses should be possible with isolated spindles. It would be of great interest to know whether spindle assembly or disassembly depends on reversible polymerisation of tubulin and whether the presence of dynein, actin or some other contractile protein is required for chromosome movement. Apparently low calcium is necessary for assembly, as well as phosphorylation of GDP tightly bound to tubulin. Jacobs at King's College London, has recently been studying a transphosphorylase associated with tubulin, but separable from it, which seems to be involved in the assembly process. Among the many possible systems involved in the control of spindle assembly and action are the concentration of calcium and level of transphosphorylase activity.

The commonplace and unexpected from high energy physicists

from David J. Miller

LAST year's wonder has become this year's commonplace. On July 1, the opening day of the International Conference on High Energy Physics at Imperial College, London, five separate new pieces of evidence for neutral weak currents were reported. Moreover, the two groups who had published previously reported greatly improved statistics (see *Nature*, **245**, 119; 1973 and **249**, 211; 1974 for discussions of the first neutral-current result, from the Gargamelle bubble chamber at CERN, Geneva and of the early Harvard/Fermi Laboratory results).

The most striking of the new pieces of data comes from a group at the California Institute of Technology, which used an array of counters, spark chambers and magnets in a neutrino beam at the Fermi National Accelerator Laboratory (FNAL). The momentum of their neutrino beam is known better than in the first Harvard-FNAL experiment, and they have a clearer technique for identifying events without fast muons. Their ratio for muonless neutrino events (neutral-current mode) to events with a muon (charged-current mode) is 0.22 to 1. With an antineutrino beam the equivalent ratio is 0.33 to 1. Although their neutrino beam momenta are around 45 GeV/c or 120 GeV/c, compared with around 1 to 10 GeV/c in Gargamelle, this neutrino ratio is very close to the latest Gargamelle figure, and the antineutrino ratio is not alarmingly different, considering the greater uncertainties involved in small antineutrino statistics. A collaboration of 'East Coast' university groups, working at the 29 GeV/c Brookhaven accelerator near New York, has also seen a clear neutral-current signal in a spark chamber experiment.

Two groups reported the observation of neutral-current events with all the final-state particles identified. In Gargamelle and in the big spark chamber arrays, this has been difficult, since the neutrinos interact with the protons or neutrons of a heavy nucleus. At the Argonne Laboratory, near Chicago, a group from Argonne, Concordia College and Purdue University has used the 12-foot bubble chamber, filled with liquid hydrogen or deuterium, to identify about 14 events in which a neutrino struck a single proton (or a neutron in deuterium). The final state in each of these events contained a positive or a neutral pion, plus a recoil neutron or proton (and also a visible 'spectator' proton, in deuterium). As

with all neutral-current experiments, the final-state neutrino in each event was not observed. The numbers are too small, to date, to do more than to establish the existence of these processes. Experimenters from CERN obtained similar results by studying neutrino interaction on the free protons in propane, using film which was taken 7 or 8 years ago, before the existence of neutral currents was even suggested. It demonstrates that there should be a great deal of useful data to be obtained from the next Gargamelle run, with a propane filling and a more intense neutrino beam.

There can now be no doubt that neutrinos and antineutrinos interact with nucleons (that is, protons and neutrons) in two distinct ways. The charged-current mode is more copious, but the neutral-current mode, in which another neutrino goes off afterwards rather than a charged muon, is well established. It is even becoming possible to put realistic limits on the 'Weinberg angle', a parameter which governs the relative strength of the charged and neutral currents in the simplest unified theory of weak and electromagnetic interactions.

One interesting question still awaits a definite answer; that is, how do neutrinos and antineutrinos interact with electrons? The Gargamelle collaboration reported the observation of two events in which an electron appears to have been produced by an antineutrino interaction. Although the calculated background is small, there can be no certainty in the observation of such a small signal. But the rate is roughly what would be expected within the framework of the simplest theory, using a value for the Weinberg angle which is consistent with the neutrino-nucleon experiments. This is the first experimental evidence that the neutral neutrino current might interact with electrons as well as with protons.

Muon puzzles

There was speculation at the meeting about both new elementary particles and new interactions. One new effect is the direct production of charged leptons from hadron collisions. The leptons—electron, muon and neutrino—have never been observed to take part in strong interactions. The hadrons—the proton and neutron, the hyperons, the mesons and all of the resonant states—are the only known strongly interacting particles. They were thought to produce

leptons only in weak or electromagnetic decays, not in their strong collisions.

A group from CERN, Columbia and Rockefeller Universities in New York, and from Saclay in France, working at the CERN Intersecting Storage Rings in Geneva, has now found convincing evidence that electrons are directly produced in proton-proton collisions at the very highest observed energies. Another group from Columbia, working at the Fermi National Laboratory near Chicago, has seen the same sort of effect, with both electrons and muons coming from proton-proton collisions at somewhat lower energies. A Chicago, Harvard, Pennsylvania and Wisconsin group also has seen direct muon production at the Fermi Laboratory, and direct muon production has been reported from the even lower energy accelerators at Serpukhov in the Soviet Union and at Brookhaven, New York. All of these data have, of course, been corrected for indirect muon production by means of the weak decays of mesons. In every case the ratio of direct lepton production to pion production is about 1 to 10,000. The ratio does not vary much over the large energy span of these different machines. It also seems roughly constant as the transverse component of the lepton momentum, perpendicular to the incoming protons, is varied from 2 to 5 GeV/c.

Two sorts of explanation have been suggested for direct lepton production; the revolutionary or the merely surprising. One revolutionary explanation requires that 'charmed' particles are being produced, and decaying rapidly to normal particles including muons or electrons. 'Charm' is a postulated new quantum number, similar to the old-established 'strangeness' quantum number. It is needed to explain, among other things, the absence of strange-particle production in neutral-current weak interactions. The merely surprising explanation requires that most of the pion production in high energy proton-proton collisions actually comes from the production of massive resonances which decay to pions by the strong interaction. Some of these resonances, in particular the 'rho', the 'omega' and the 'phi' known as the 'vector mesons', also decay occasionally by means of the electromagnetic interaction to a pair of electrons or a pair of muons. Preliminary calculations, using vector meson decays, give a lepton to pion ratio of about 1 to 100,000. Nobody knows yet whether the extra factor of 10 can be found to make this calculation match the observed rate. But even if the factor can be found, and a revolutionary explanation is not needed, ideas of particular production at high energies will have been radically changed.

The Fermi Laboratory and Harvard

neutrino experiment has revealed two puzzling muon events of quite a different kind. Each seems to be the result of the interaction of a 150-GeV neutrino in their apparatus, producing two muons and a shower of hadrons. Ordinary 'charged-current' neutrino interactions contain one final-state muon, and neutral-current events have no muons. As a theorist remarked at the conference: "taking preliminary results seriously is a well known way of making a fool of yourself". Nevertheless, there is much speculation that these dimuons could be due to the decay of a totally new kind of particle. Some say 'charmed' particles again; some say it may be the 'intermediate boson' that carries the weak force, a sort of heavy photon; some say it is a heavy lepton of the kind needed in the unified weak and electromagnetic theory of Georgi and Glashow. Certainly, if this observation is correct, then no 'merely surprising' explanation will do.

Prospecting for a dead slab

from Peter J. Smith
Geomagnetism Correspondent

LITHOSPHERIC slabs descending beneath trench systems are known to have seismic velocities which are measurably higher than those in the surrounding mantle. Because such downthrusting slabs can be recognised in simpler and more convenient ways, not least by their deep seismicity, there may be little need to use the seismic velocity property to detect Benioff zones which are still active. But what about zones in which subduction has now ceased and which are seismically quiet or dead? When subduction ceases, the relevant slab, initially cooler than the mantle into which it was previously descending, will slowly warm until the thermal and compositional contrast between it and the surrounding mantle disappears; but until this process is complete the 'dead' slab may retain enough of its identity to maintain the velocity contrast. So can this contrast be used to detect the slab's presence?

That there are likely to be recently dead slabs to detect may be inferred by extrapolating known plate tectonic processes backward in time. The evolution on the western margin of North America is a good case in point and probably the most-studied example. Magnetic anomalies in the north-east Pacific suggest that a Farallon plate (sometimes called the Juan de Fuca plate or the Gorda plate) descended along the boundary between it and the North American plate until about 30 million years ago, at which time a spreading ridge between the Farallon and Pacific plates began to collide with

the western North America trench and strike-slip motion commenced along the San Andreas fault. In this way it is thought, the plate boundary along western North America began to change from a subduction zone to a transform fault. There is evidence from seismic reflection studies of the continental margin, from the occurrence of subcrustal earthquakes, from andesitic volcanism in the Cascades and from marine magnetic anomalies that north of the Mendocino fracture zone subduction may still be occurring very slowly. South of Cape Mendocino, however, subduction has ceased, although Atwater (*Bull. Geol. Soc. Am.*, **81**, 3513; 1970) has concluded that the cessation may have occurred no more than a few million years ago.

The implication here is that the remains of the Farallon slab may still lie beneath California and may still have sufficient of its original identity to produce a measurable velocity contrast. To test whether this is indeed so, Solomon and Butler (*Earth planet. Sci. Lett.*, **21**, 421; 1974) have attempted to detect such a contrast by analysing the travel-time delays of teleseismic P waves in the region. The method adopted was derived from that used by Davies and McKenzie (*Geophys. J.*, **18**, 51; 1969) to show that shallow earthquakes and explosions above a descending slab produce distinctive patterns of travel-time residuals for teleseismic P waves as a result of the velocity anomaly within the slab. The basic residuals obtained by Solomon and Butler were the travel-time delays at each station concerned for P waves from many different earthquakes and explosions. But to remove uncertainties in source locations and origin times and to reduce near-source contributions to the total travel-time residuals, the residuals actually plotted were the differences between the travel-time delays at each station and the corresponding delays at a reference station overlying what is presumed to be more uniform mantle. The sources were mid-plate earthquakes and explosions, deep earthquakes, and "carefully screened" (to avoid obvious near-source heterogeneities) earthquakes on spreading centres and transform faults.

In the absence of any detailed knowledge of the position and extent of the supposed dead slab, the stations chosen were those between latitudes 37°N and 50°N and between longitudes 116°W and 125°W at which P wave arrivals had been regularly reported during the period 1964–1970 (a selection process which eliminated all but seven stations, three of which were regarded as reference stations). The residuals obtained from various station pairs were plotted on residual-spheres, the resulting plots being a measure of the variation of travel-time delay with direction of wave

propagation in the upper mantle beneath the relevant station.

The residual-sphere for station LON in the Cascades in Washington (referred to BMO, Blue Mountain Observatory to the east) failed to indicate any systematic trends in residuals. If a high velocity slab lies in the upper mantle beneath western Washington and if its upward projection cut the Earth's surface near LON, an eastward-dipping band of negative residuals (early arrivals) might be expected. No such band was observed. But for station pairs based on the three primary stations in California (MIN in the southern Cascades, ORV and JAS along the western edges of the Sierra Nevada), a group of consistently negative residuals was observed towards the east in each case. Moreover, the average travel-time advance in the eastern quadrants of the residual-spheres (calculated in the same way for each sphere) decreased southwards from 1.2 s for MIN, through 0.8 s for ORV, to 0.5 s for JAS.

These patterns of travel-time residuals (irrespective of magnitude) are consistent with the presence of a dead slab dipping eastwards beneath California. On the other hand, they cannot be said to prove the point absolutely because the same data could in principle be explained using a model with undulations in the depth to the top or bottom of the low velocity zone. Moreover, the data are not as complete as they might be because of a paucity of seismic sources in eastern North America. But seen in the light of other evidence the case for a dead slab is more convincing. In addition to the plate tectonic inferences already quoted, support may be adduced from heat flow studies, for example. According to Roy *et al.* (in *The Nature of the Solid Earth*, McGraw-Hill, 1972), the low heat flow in the Sierra Nevada apparently requires a heat sink in the shallow (~50 km) mantle—a role that could easily be filled by the postulated slab. If this interpretation is accepted, the implication then is that between the Pacific plate and the Sierra Nevada the Farallon plate dips at an angle of perhaps no more than 10°–15°. A more detailed analysis of the travel-time data, on the other hand, suggests that east of the Sierra Nevada the dip is much more steep (possibly 40°–50°).

The southward decrease in the magnitude on the travel-time advance could result from differences in the positions of the stations with respect to the position on the Earth's surface of the upward projection of the supposed slab, or it could be due to a real southward decrease of the P wave velocity in the slab. Solomon and Butler favour the latter explanation on the grounds that the three stations are similarly

located very close to the western margin of the Sierra Nevada or the California Cascades, but admit that the evidence is not sufficient to rule out the other possibility. On the other hand, from Atwater's models, they deduce that subduction ceased beneath station MIN about 4 Myr ago, beneath ORV about 6 Myr ago and beneath JAS about 12 Myr ago. In other words, the dead slab seems to age towards the south.

This theory would offer a natural explanation for a southward decrease in P wave velocity, for the older the slab the more it will be thermally assimilated with the surrounding mantle and thus the lower will be the mantle-slab velocity contrast.

Eyeglow in butterflies

from our *Insect Physiology Correspondent*

It is a familiar observation that the dark-adapted eyes of nocturnal moths produce a conspicuous glow when viewed in a beam of light. The 'tapetum' responsible for this reflection is believed to be the rich network of air-filled tracheoles which invest the deepest levels of the retina. As Horridge (*Proc. R. Soc. Lond.*, **B179**, 98; 1973) pointed out, if the light entering one facet is confined to a single rhabdom (as in the light-adapted eye) its reflection will not emerge through another facet. On the other hand, whatever the precise mechanism of eyeshine may be, in the dark-adapted eye the scattered light may follow many possible routes within the eye and emerge through other facets.

Particular interest has been taken in the eye glow of butterflies, which can be very brilliant, and of diverse colours, in the dark-adapted state; but which disappears very rapidly on illumination. Miller and Bernard (1968) attributed this light to the flange-like taenidia of the tracheoles running vertically between the bases of the rhabdoms, which they suggested were functioning as a quarter-wave stack made up of alternating layers of air and cuticle with refractive indexes of 1.0 and 1.4 respectively. Swihart *et al.* (*J. insect Physiol.*, **20**, 359; 1974) now question this 'basal reflection theory' for the origin of eye glow in butterflies.

These authors concentrated on butterflies of the genus *Vanessa*, which show a uniform orange reflection over almost the entire eye. The band of wavelengths reflected is far narrower than was to be expected from a quarter-wave stack. The time required for the extinction of the eye glow is constant at some 5–10 s for a given eye; the cycle of extinction (and reappearance in the dark) can be repeated hundreds of times with little

variation. No detectable changes in pigment distribution can be detected. As Exner had shown, the response can be completely localised to a few ommatidia. The reflections are more intense than would be expected from a basal reflection—perhaps 20 to 50% of the incident light—and they can be upset by the slightest mechanical contact with the cornea.

Furthermore, the authors show that during light adaptation there is a photo-mechanical contraction of two specialised retinula cells; this causes the rhabdom to fold and the crystalline cone to be withdrawn away from the cornea. The elastic 'corneal process' between the cornea and the crystalline cone is stretched and its optical properties are altered. The corneal process is believed to be the source of the eyeglow reflections, the spectral properties of which will be largely determined by the overlying cornea. When the corneal process is stretched the reflections are extinguished. This 'distal reflection theory', according to the authors, has no physiological implications for vision in butterflies: these are diurnal creatures; at the levels of illumination they normally encounter the eyes are in the light-adapted state and the corneal process does not reflect—it is simply a portion of the dioptric apparatus, serving to conduct light from the cornea to the lens.

How swamp plants react to thermal pollution

from Peter D. Moore
Plant Ecology Correspondent

THERMAL pollution is a problem of the future; as nuclear reactors proliferate, so larger quantities of waste heat will be pumped into the environment causing problems of thermal tolerance to the organisms living in their vicinity. To date little is known about the effects on wetland plant communities of artificially raising the temperature, although some information exists regarding aquatic organisms.

Sharitz (*Oikos*, **25**, 7; 1974) has been able to study the effects of reactor effluent in a situation where adequate controls have been possible—in the swamp forests of the south-eastern United States. Many of the lower stretches of rivers in this area are surrounded by swamp forests which, as the term implies, are dominated by trees, such as *Acer rubrum*, *Fraxinus pennsylvanica*, *F. caroliniana* and *Planera aquatica*. In these swamps, 48% of the plant species are trees and a further 14% are shrubs and woody vines. Swamp forests experience considerable

seasonal fluctuation in water table and most of the tree species are intolerant of prolonged flooding. Even *Taxodium distichum* and *Nyssa aquatica*, which are the most tolerant to floods, are killed by lengthy exposure to a high water table.

Part of the Savannah River catchment in South Carolina is occupied by a nuclear reactor of the US Atomic Energy Commission, which pumps hot water into some of the tributary streams. One stream in the area is unaffected (providing a convenient control), two others are thermally polluted and a fourth has been recovering for more than 4 years following 14 years of thermal stress. The area therefore provides a useful setting for the study of the effect of nuclear plant discharge on swamp vegetation and the way in which the flora recovers after the activity is stopped.

The area currently polluted has water temperatures which frequently exceed 45° C and the vegetation of the area has been profoundly altered. All trees, shrubs and lianes have been destroyed and the only habitats suitable for plant growth are the islands formed from collapsed, dead timber; here some herbaceous plants, such as *Ludwigia leptocarpa* have become established. In fact the diversity (Shannon-Weaver index) of the ground flora is fractionally increased, resulting from the new microhabitats created.

The recovery area has similar stumps and logs, together with emergent, floodplain sediments which have been colonised by adventive herbs, such as *Polygonum punctatum*; a large proportion (35%) of the plants of this area are annuals. Some woody plants are recorded, including *Pinus taeda* and *Salix nigra*, but these are not species typical of the mature swamp forest, so that it is difficult to judge how long the area will take to return to the typical swamp forest if, indeed, it ever does.

The loss of woody species during flooding could be of particular concern if it resulted in local erosion and consequent silting of the Savannah River. It is unfortunate, however, that it has not been possible to distinguish between the truly thermal effects of disturbance and those resulting from a permanent raising of the water table. It is probable that the loss of trees and shrubs is due to flood intolerance rather than sensitivity to high water temperatures. Such a distinction, were it possible, would indicate whether the destruction of the swamp forest could be avoided by providing drainage conduits direct to the Savannah River. Although such action would stabilise the forest, it would not, of course, reduce the effects of thermal pollution on aquatic organisms in the river.

Photochemical war on the atmosphere

John Hampson

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Professor Hampson believes that many scientists have not recognised the potential danger of thermonuclear warfare. He outlines some of the changes in the photochemical regime of the atmosphere, which may be wrought by nuclear explosions, and suggests that these effects must be considered in future talks on arms limitation.

A FIVE per cent increase in the atmospheric content of ozone in recent years has been attributed to the recovery of the atmosphere following the injection of NO_x by nuclear tests during the winter of 1961–62¹. Estimates of the theoretical quantity of NO_x generated have been presented by several investigators^{2,3}. If the NO_x is not swept out of the stratosphere by the change of heating and transport induced by the new ozone structure, then, should the amount of nuclear discharge into the atmosphere increase by one order of magnitude, the total ozone content may be halved. At first sight it seems that the variation should follow a square root law. If the decreased screening by ozone, and the consequent increase in NO production is allowed for, however, then for particular values of the reaction rate coefficients and circulation parameters a linear dependence is a closer estimate of the maximum possible effect (within the possible range of errors). A further increase of one order of magnitude could reduce the total ozone in the atmosphere to a fraction of its present value. It is appropriate to enquire into the effect of such radical changes in the ozone content and the circumstances in which they might occur.

The photochemical behaviour of the stratosphere is far from fully understood, and the chemical effects of high yield, high altitude nuclear explosions have never been measured and reported. It is probable that there are changes besides those which are predicted by the simple NO_x theory. That is, however, no reason to reject the theory, for it forms a solid base for future work. It is difficult to believe that mankind can put into the chemosphere an energy comparable to the chemical energy of the ozone layer (~3,000 Mton) in less time than it took to create the layer, and still expect the chemosphere to remain intact.

It has been assumed⁴, and no alternative view has been presented, that the evolution of life on the exposed surface of the planet began with the formation of the ozone screen. The removal of this screen would mean that conditions of ultraviolet illumination at the surface would become similar to those of Precambrian days, and would presumably eliminate all surface life. The biosphere involves such a complex pattern of interacting influences that it is impossible to assess the effects of drastic reduction in ozone. 'Reasonable' approximations must be used. The ozone content in the path from the Sun to the summer pole is double that in the shortest Sun-surface path at the equator. Halving the ozone abundance would create a situation where the ultraviolet input is everywhere greater than it was previously. For want of a better yardstick I shall assume that this is the degree of ozone reduction which

could destroy the present biosphere. The argument here is simply that the biosphere has adapted to the available ultraviolet illumination, and a critical condition exists. Following a halving of the ozone content no living organism would be able to find a place on the surface of the Earth where the ultraviolet input was equal to or less than that previously available anywhere.

It is instructive to consider the circumstances in which a thermonuclear war could occur, halving the effectiveness of the ozone screen. The structure, deployment and potential use of nuclear weapons are dictated by an analysis of the probable damage and potential injury which would be inflicted on the 'opponent'. It seems reasonable to assume that it will occur whenever the predicted outcome would be more favourable to the instigator than any alternative action at his disposal. There is no evidence that any of the nuclear powers, or any of the nations who are allegedly attempting to forestall nuclear proliferation through the United Nations, have made any attempt to consider the photochemical problem in their deliberations. It must therefore be assumed that the decisions on thermonuclear war are based on a hypothetical body count of potential victims. That is the way the issue is posed in the published studies of nuclear war, particularly exemplified in the account by Kahn⁵.

To knock out one of the major powers, the United States, the USSR or China, 5,000 Mton must be accurately delivered, somewhat less would be required to destroy Europe. If the concepts and calculations of Johnston¹ are correct the use of the minimal deterrent would halve the ozone content. In these circumstances, the deterrent as such is a myth and becomes one of Kahn's Doomsday Machines⁶.

If NO_x is the critical reactant which is destroying the ozone then the effects of thermonuclear weapons may be greater than the estimates I have given. These estimates are based on low level bursts in which the NO_x concentration is calculated from consideration of the thermodynamic equilibrium in the fireball at a temperature of 2,000 K. A high proportion of the nitrogen atoms produced before and during that stage, during the development of the fireball, are recombined as N_2 , and the NO_x production represents a small proportion of the total energy of the nuclear burst. This is not the case for an explosion at high altitude, when the energy density of radiation below the burst point, at the altitude of maximum NO production, is sufficiently small that recombination through $\text{NO} + \text{N} \rightarrow \text{N}_2 + \text{O}$ plays a proportionally smaller role. If the concentration of N is small compared with that of O_2 , then the bulk of the NO produced by $\text{N} + \text{O}_2 \rightarrow \text{NO} + \text{O}$ may not be removed subsequently in the competing reaction between N and NO. High altitude explosions are much more efficient at producing NO than are ground level explosions. It is fortunate that the tests in 1962–63 were conducted in the lower atmosphere and not at high altitudes. Otherwise there may have been such a large production of NO that any subsequent debate on atmospheric chemistry would have been both impossible and superfluous.

Each of the two major nuclear powers seems to have

a stockpile in excess of the critical value, 5,000 Mton, and to be continually jockeying for a uniquely unassailable position. The only way this can be achieved is by using ballistic missile defence, and the nations concerned can be expected to make this a priority item of defence research. That constitutes a somewhat greater danger to the ozone screen than do the offensive weapons. It not only increases the required number of offensive weapons but also creates more NO per unit of energy released into the atmosphere, because interceptions must be made at high altitudes. Some years ago there was a far fetched, fanciful concept that the release of 1,000 Mton or so in the equatorial E region might have produced an artificial Van Allen belt of sufficient intensity and duration to clean up a great deal of the debris and decoys which clutter and confuse the radar return from a missile warhead. It would not have worked, but it is disturbing to discover that such concepts are advanced with absolutely no consideration of the potential effects on the Earth's photochemical system. Even the 'orthodox' defence systems agreed to in the Strategic Arms Limitation Talks (SALT) are themselves a major threat to the photochemical environment. Several hundred interceptors are involved, each with warheads in the Mton range. The actual yields of the interceptor warheads have not been published. Assuming that they may now, or eventually, be set at 10 Mton, which would give the maximum capacity for damaging the re-entry warhead and cleaning up the accompanying assorted objects, then the defence systems defined by SALT, which themselves give only a token and limited defence, would be capable of injecting 2,000 Mton into the atmosphere. They would do this at high altitudes and therefore have a much greater potential for modifying the NO_x environment than the low altitude bursts.

The threat to the ozone layer posed by thermonuclear discharges is much more serious than that posed by contamination from supersonic transport (SST) flights. We have time to analyse the photochemical environment and assess the potential effects long before widespread SST flights add an appreciable amount of NO_x to the environment. The bigger issue is whether the nuclear deterrent is a Doomsday Machine. It is astonishing that none of the deliberations on reduction of nuclear arms pay the slightest attention to this photochemical parameter. Surely there is a need to establish whether there is an absolute limit to nuclear war, involving energies considerably smaller than those at present used as a deterrent. Such information could be used as a lever to cajole the nuclear powers to move towards a more positive view of the need for arms control. Johnston¹ has performed a considerable service in drawing attention to the potential fragility of the ozone screen. It is to be hoped that increased attention will be paid to the effects of thermonuclear weapons and that this may provide a rationale for the reduction and control of nuclear armaments.

A *prima facie* case for NO as the agent controlling ozone having been clearly established, it should be noted that there are many factors of the chemistry and circulation of the ozone layer, and of the interrelation between the layer and the rest of the Earth-Sun system which have to be researched before the case is proven. This should be no excuse for disregarding the potential danger. It does, however, imply that we may find that nuclear weapons could exert dangerous effects in other, more subtle, ways.

A single, 10 Mton weapon exploded in space, less than an Earth's radius away, throws out about as much energy at the Earth as does the solar wind during the passage of the interplanetary magnetic field sector boundary. Can it be assumed that this could cause effects commensurate with those deduced by Roberts *et al.*?² If a 10% change in vorticity could occur with a single weapon, what would a space war do? We need to examine whether this effect

stems from a chemical source (see ref. 6).

There seems to be a disparity between the hydrogen escape deduced from observations at the exobase, and models of H transfer from the lower atmosphere to the exobase. In view of this can we neglect the possibility that a nuclear war in space would lower the hydrogen concentration above 40 km, and through an increase in the upper atmospheric ozone content reduce the formation of NO and increase the total ozone content?

We simply do not know the answers to many important questions. Granted an adequate programme of measurements, far more ambitious than that envisaged today, and sufficient clairvoyance (omniscience might be a better term) the minimum time necessary to assess natural variations resulting from solar activity in the chemosphere will be one solar magnetic cycle of 20 years. There can be little prospect of accurately defining how contamination can compare with or can couple into the natural variations in a shorter period.

We might as well accept both of the apparently contradictory propositions that contamination by NO_x can change the ozone abundance and that the observed increase in ozone is real but is not related to changes in the NO_x content.

From Johnston's estimates¹ it seems reasonable to infer that a fleet of 100 Concorde might change the ozone content over a period of 5 years to an extent comparable with the observed rate of increase of ozone during the decade 1960-70. This fleet seems to correspond to the maximum scale of production and deployment to be expected between now and 1980. There seems to be adequate time between now and 1980 to refine the observations and theoretical analyses of the chemosphere to the point at which we can estimate a 'limiting, scale of operations for supersonic aircraft and other potential sources of stratospheric contaminants. This period of grace should be used to ascertain whether the observed increase in ozone during 1960-70 continues into the next decade and is therefore not related to recovery from the nuclear tests.

Contamination by CO₂ is recognised, but can hardly be averted at the moment. Studies of the effect of changes in the CO₂ content have been limited to evaluations of the greenhouse effect in the lower troposphere. No attention has been paid to possible alterations of temperature and vertical stability above the tropopause. These can modify the transport of reactive minor constituents, and readjust the photochemical balance. There is insufficient information on the details of the nitrogen cycle. We cannot be absolutely sure that the scale of human modification of the critical elements in the cycle will have no effect.

The effects of nuclear weapons are another matter. The majority of the scientific community fail to recognise the potential danger from a change in the chemical envelope of the Earth. They thus fail to consider the possibility of defining a limit to nuclear activity, which can be universally agreed upon and could set the stage for arms control. So long as the nuclear problem is viewed as one of international rivalry there can be little prospect of any control because it will always seem that one nation can gain an advantage over another. Kahn's dictum that a Doomsday Machine can never be used is a reasonable enough basis for arms control. But this depends on our being able to recognise such a machine.

The main motivation for stratospheric research should be the desire to delimit the effects of general contamination and to define the absolute limit of nuclear activity which would constitute 'The Doomsday Machine'.

The fine structure (both spatial and temporal) of the reactive minor constituents deserves careful study. Johnston *et al.*¹ and Goldsmith *et al.*³ present arguments that it is the long term global effects of nuclear tests on the ozone content, and not short term local effects, which

should be considered. This is an oversimplification. The form of polar stratospheric circulation and the characteristics of the auroral belt were abnormal during the winter of these tests. It is always possible that there have been more complex interrelationships between the tests and the photochemical regime than has been supposed.

All the parameters involved must be studied, and all potential, alternative models of stratospheric chemical behaviour must be included in order to avoid a superficial interpretation which fits the selected facts visible from the fraction of the iceberg that can be seen at present.

- ¹ Johnston, H. S., Whitten, G., and Birks, J., *J. geophys. Res.*, **78**, 6107-6135 (1973).
- ² Foley, H. M., and Ruderman, M. A., *J. geophys. Res.*, **78**, 4441-4451 (1973).
- ³ Goldsmith, P., Tuck, A. F., Fott, J. S., Simmons, E. L., and Newson, R. L., *Nature*, **244**, 545-551 (1973).
- ⁴ Berkner, L. V., and Marshall, L. S., *Discuss. Faraday Soc.*, **34**, 122-141 (1964).
- ⁵ Kahn, H., *On Thermonuclear War* (Princeton University Press, Oxford and Princeton, 1960).
- ⁶ Roberts, W. O., and Olson, R. H., *Rev. Geophys. Space Phys.*, **11**, 731 (1973).

Hopping losses in polarisable dielectric media

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The loss in most dielectric materials shows a component that is 'flat' in frequency over several decades and does not depend strongly on temperature. The usual interpretation as the sum of many Debye-like loss peaks is criticised and a new model is proposed in which charge carriers hop between localised sites in a polarisable medium.

THE dielectric loss $\chi''(\omega)$ is defined as the imaginary component of the complex dielectric susceptibility

$$\chi(\omega) = [\epsilon(\omega)/\epsilon_0] - 1 = \chi'(\omega) - i\chi''(\omega) \quad (1)$$

where $\epsilon(\omega)$ is the dielectric permittivity, ϵ_0 is the permittivity of free space and ω is the radian frequency. The corresponding a.c. conductivity is $\sigma'(\omega) = \epsilon_0\omega\chi''(\omega)$ and this may exceed the d.c. conductivity σ_0 by some orders of magnitude. The experimentally observed frequency dependence of dielectric loss in a wide range of materials may be expressed by the empirical relation

$$\chi''(\omega) = \Lambda(T)\omega^{n-1}$$

where $\Lambda(T)$ is weakly dependent on temperature but is not generally characterised by a simple activation energy and the exponent n lies in the range $0 < n < 1$, with typical values between 0.6 and 0.95. This implies that the loss is 'flat' in frequency, often over many decades in the normally accessible range between sub-audio and microwave frequencies¹⁻⁵. In general, there seems to be no correlation between the values of σ_0 and $\sigma'(\omega)$ for various materials.

There are two different approaches to the theoretical interpretation of the flat loss given by equation (2). The first is applicable to materials which are believed to contain molecular dipoles and it involves the assumption of a suitably wide distribution function $g(\tau)$ of dipolar relaxation times τ , covering several powers of ten⁶.

The second approach is that of the electronic hopping school which considers the frequency dependence of the localised charge carriers hopping in a random array of centres^{3-5,7,8}. This 'sequential hopping' model is in many ways equivalent to the former approach, replacing the distribution of dipolar relaxation times with a corresponding distribution of hopping times. But it also provides for the d.c. conductivity in the limit of zero frequency. Given a sufficiently wide distribution of intercentre spacings and of their relative energy differences, it is possible to obtain a frequency dependence of the type given by equation (2) and extending over a reasonably wide range of frequency. Another explanation invoking local atomic movements in disordered solids has been proposed by Pollak and Pike⁹.

Hopping model

I have expressed some doubt about the universal applicability of the sequential hopping model in view of the fact that a very wide range of different materials show a very similar dependence on frequency and the absolute values of the dielectric loss fall in a relatively narrow range². The lack of correlation between σ_0 and $\sigma'(\omega)$ is also unexpected in the hopping model, as is the apparent independence of $\sigma'(\omega)$, but not of σ_0 , on pressure^{10,11}. Furthermore, the same frequency dependence is seen in relatively thick samples in which sequential hopping represents a plausible model and in very thin (1 to 3 nm) samples, in which there is no scope for more than one hop¹².

So I believe that there is sufficient uncertainty about the origin of the flat dielectric loss due to hopping carriers to make it desirable to propose an entirely new physical model in order to evaluate its merits and consequences. I consider that an essential feature of this model must be its general applicability to a wide range of semiconducting and dielectric systems. It is relevant to recall here that it has been suggested that the flat loss in some conventional dielectric materials may also be due to electronic hopping, in view of the known presence in these materials of low mobility carriers^{13,14}. A detailed analysis of the behaviour of dipolar systems will be published elsewhere.

Hopping in a polarisable medium

In the conventional picture one considers hopping as occurring between two localised levels, each of which is uniquely defined in space and in energy (Fig. 1a). This model is applicable to media in which the polarisation responds sufficiently rapidly to the appearance of an electron on any one site so that the transition may be said to occur effectively into the final state. But if the polarisation of the dielectric medium responds relatively slowly in comparison with the time taken by the tunnelling process, as is the case almost by definition in all materials showing a strong dispersion of conductivity with frequency, then the energy level picture must be as shown in Fig. 1b. The 'empty' and the 'occupied' states are separated by a polarisation energy W_p , due to the relaxation of the surrounding lattice and carriers.

In this case, the transition from a localised level i into an equivalent unoccupied level j having the same energies may be considered to occur in three stages (Fig. 1c). Process 1 is the thermal excitation of the carrier into a virtual state corresponding to the unrelaxed energy of an empty state. The following tunnelling transition (2) is not activated and the process is completed by a gradual relaxation, 3, into the ground state of the newly occupied centre.

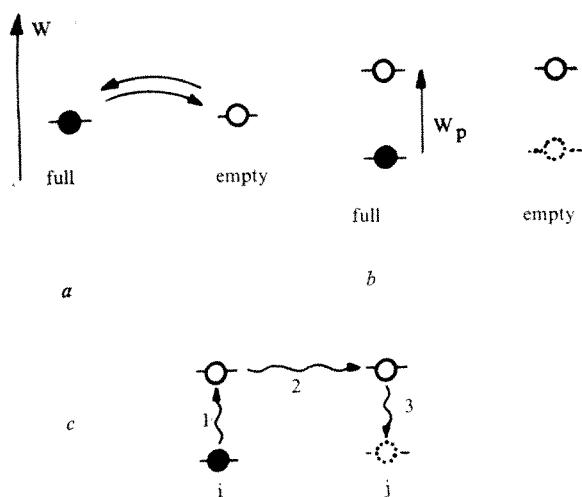


Fig. 1 Localised energy levels in hopping between pairs of centres. *a*, Hopping in a medium in which polarisation relaxes very rapidly—the conventional hopping system; *b*, full and empty centres in a medium in which polarisation relaxes slowly; *c*, three stages of hopping between two centres as in *b*.

In thermal equilibrium detailed balancing determines the transition rates $\gamma_{ij}^0 = \gamma_{ji}^0 = 1/\tau_0$ at which the gain of energy from process 3 exactly balances the energy required by process 1. For this mechanism to be applicable we require that the equilibrium transition time τ_0 should satisfy the condition

$$\tau_0 \gg \tau_p \quad (3)$$

where τ_p is the relaxation time for the polarisation process, since otherwise the carrier would be re-excited before it has had a chance to relax. This condition may be expected to be satisfied at low temperatures, such that $kT < W_p$, and at higher temperatures the system becomes effectively the same as the model of Fig. 1*a* corresponding to a rapidly polarisable medium, since in this case the model has no time to respond to the rapid transition rate and adjusts itself only to the mean occupancy.

The proposed slow polarisation mechanism is not necessarily confined to materials with polar lattices—it may also be expected in otherwise non-polar materials which contain hopping carriers responding to any changes of the spatial distribution of other carriers in their neighbourhood.

I now consider an assembly of N identical pairs of localised sites, each pair containing one electron capable of jumping between the two sites constituting the pair. I shall assume for simplicity that the sites have the same energies—this does not entail any loss of generality of the subsequent argument, while simplifying the discussion. In the absence of external fields the occupancies of the individual sites are equal on average and are $f_0 = 1/2$.

If an external field is applied to the system favouring 'downstream' transitions and discouraging 'upstream' ones, the occupancies of the corresponding centres change by $\pm f'$ resulting in a net polarisation

$$P = ea \sum_s f'_s \quad (4)$$

where a is the distance between sites and the index s denotes summation over pairs of centres.

The perturbation of the occupancies of the system by the applied field must result in a net loss of energy W_p for each transition in excess of the equilibrium rate $1/\tau_0$. We argue that at this excess transition rate the energy derived from the relaxation process 3 in Fig. 1*c* can no longer be recovered and is lost to the phonon system as heat. The rate of energy loss may thus be expressed in the form

$$dW/dt \propto W_p |\gamma_{12} - \gamma_{21}| = W_p |df'/dt| \propto |dP/dt| \quad (5)$$

since the excess occupancies f' are directly related to the excess

transition rates. The absolute value signs imply that the energy loss occurs for either sign of the rate of change of f' . Assuming now an alternating electric field at a frequency ω , $E = E_0 \sin \omega t$, we may define a frequency-dependent complex susceptibility $\chi(\omega) = P/E$, implying a phase lag between P and E due to the presence of loss.

The physical nature of this loss may be envisaged by considering the extra energy required from the alternating field to cause the increased transition rate from the relaxed states on reversal of the sense of polarisation. This energy is derived from the phase lag between P and E .

Frequency dependence of the susceptibility

To obtain the energy loss per cycle, W_c , we must integrate the rate given by equation (5) over the half period during which $dP/dt > 0$. During the other half cycle the loss arises on the opposite sites in the pairs whose occupancy is being increased at the time. The loss per cycle is therefore proportional to the amplitude of the polarisation P , but the frequency ω does not appear in the result of the integration. So

$$\chi'' \propto |\chi(\omega)| = [\chi'^2 + \chi''^2]^{1/2} \quad (6)$$

regardless of the frequency ω . However, this relation can only be satisfied generally if

$$\chi''(\omega) \propto \chi'(\omega) \quad (7)$$

that is, if the real and imaginary components of the complex susceptibility are the same functions of frequency, implying that

$$\chi''(\omega)/\chi'(\omega) = \text{constant with respect to frequency} \quad (8)$$

I note, however, that $\chi'(\omega)$ and $\chi''(\omega)$ must obey the universally applicable Kramers-Kronig relations which in the extended frequency range $(-\infty, \infty)$ may be written in the form of a Hilbert transform

$$\chi'(\omega) = (1/\pi) \int_{-\infty}^{\infty} \frac{\chi''(x) dx}{(x - \omega)} \quad (9)$$

and a similar expression for the real and imaginary parts reversed and with a negative sign in front of the integral. The integral is to be taken here in the sense of the Cauchy principal value.

It is a property of Hilbert transforms that equation (9) and its counterpart can only be satisfied with the condition (7) by the following functions¹⁸:

$$\chi''(\omega) = A \operatorname{sgn} \omega |\omega|^{n-1} \quad (10)$$

and

$$\chi'(\omega) = A \tan(n\pi/2) |\omega|^{n-1} \quad (11)$$

where A is a constant and the exponent of the power law satisfies the condition $0 < n < 1$. This implies that the ratio (8) satisfies the condition:

$$\chi''(\omega)/\chi'(\omega) = \cot(n\pi/2) = \tan[(n-1)\pi/2] \quad (12)$$

so that the ratio of the real and imaginary parts of the susceptibility is directly related to the exponent n .

The upper frequency limit for the applicability of the relations (10) and (11) may be expected to be $1/\tau_0$, the equilibrium transition frequency, since beyond this limit the polarisation would cease to respond to the applied field, just as in the case of the Debye dipolar or hopping processes beyond the reciprocal relaxation time. At the lower end of the frequency spectrum, the ultimate requirement is that the loss must go to zero as ω tends to zero, so that $\chi''(\omega) \propto \omega$ at very low frequencies. In many non-polymeric systems this region is not accessible experimentally because of the onset of d.c. conduction which dominates the dielectric loss. The expected frequency dependence of loss for a single set of hopping pairs is therefore as shown in Fig. 2.

Temperature dependence of loss

Up to this point the analysis was deliberately confined to the discussion of the frequency dependence of dielectric loss, since this could be obtained with the minimum of specific assumptions relating to the relaxation mechanisms. An extension of the

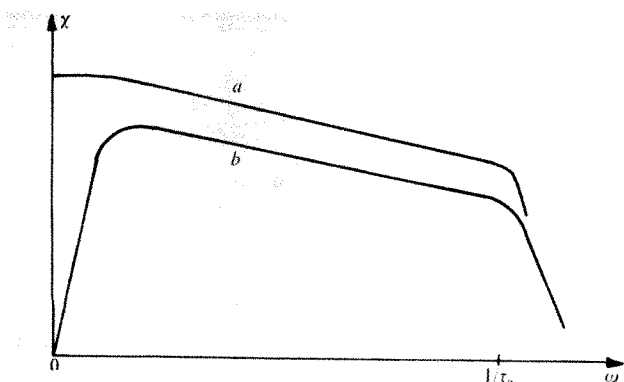


Fig. 2 The frequency dependence of the real and imaginary parts of the complex dielectric susceptibility, $\chi(\omega) = \chi'(\omega) - i\chi''(\omega)$ showing the 'flat' loss accompanied by a similar frequency dependence of χ' and the cutoff at high frequencies above the reciprocal equilibrium transition time, $1/\tau_0$. a, $\chi' \propto \omega^{n-1}$; b, $\chi'' \propto \omega^{n-1}$.

analysis to cover the dependence of loss on temperature would require more detailed assumptions about the nature of the hopping transitions than we are able to make at present.

It may be stated, however, that the temperature would be expected to affect several parameters relevant to the present analysis. The equilibrium transition rate $1/\tau_0$ is certainly thermally activated and the polarisation relaxation time τ_p might be likewise, although the extent of their dependence on temperature may be quite different. Since the relative magnitudes of τ_0 and τ_p determine the applicability of the present analysis, equation (3), as distinct from the conventional Debye-like hopping, some of the contributing mechanisms may be either brought in or eliminated according to the prevailing circumstances.

Distribution of hopping parameters

The idealised model used so far in the present analysis is unrealistic in several important respects. Any real hopping system must contain a distribution of hopping parameters and it may also involve spatial distributions of centres which make possible multiple sequential hopping. In addition, the spatial distribution of the axes of the hopping pairs is normally isotropic so that the effective field is not E but $E \cos \theta$, where θ is the polar angle of the pair with respect to the field E .

The point to be noted in the present analysis is that in the presence of several sets of hopping parameters, or even of a continuous distribution, we are summing flat-loss characteristics instead of summing loss peaks. The final shape of the $\chi''(\omega)$ curve is therefore much less sensitive to the nature of the distribution of hopping parameters than would be a sum of Debye-like loss mechanisms. Of special significance is the point that the range of hopping relaxation times τ_0 does not have to correspond to the experimentally observed range of validity of flat-loss power-law dependence on frequency.

It can easily be envisaged that one of the principal effects of the presence of a distribution of hopping parameters may be an increased temperature dependence, as some of the more strongly activated mechanisms become progressively 'frozen out' with falling temperature.

Discussion

Here I have proposed a hopping model in which the carriers suffer an almost frequency-independent loss of energy arising from a relatively slow relaxation of polarisation in the dielectric matrix in which they hop. This should be contrasted with the frequency-dependent loss normally associated with hopping conduction in nonpolarisable media and also with dipolar

rotations, both of which give rise to characteristic loss peaks as functions of frequency.

One direct consequence of the present model is the fact that both the real and the imaginary parts of the complex dielectric susceptibility $\chi(\omega)$ have the same frequency dependence. The power-law dependence given by equations (10) and (11) then follows as a natural consequence of the Kramers-Kronig relations, leading to a flat frequency response even in the presence of only one single set of hopping parameters. This means that in the present model it is not necessary to invoke any special distribution of hopping or dipolar relaxation times to explain the very widely experimentally observed flat frequency dependence of dielectric loss, as would be the case with the summation of individual Debye-like loss peaks. In particular, it is not necessary to postulate sequential hopping over many sites distributed randomly in space and in energy, as is clearly the case with d.c. conductivity. This makes it much easier to understand the similarity of the experimentally observed behaviour of relatively thick and of very thin samples. It is also clear that the dielectric loss has nothing to do with the d.c. conductivity σ_0 of the material, since they are due to essentially different processes. The lack of dependence of loss on hydrostatic pressure is also understood in terms of the insensitivity of the polarisation energy W_p to pressure, as opposed to the strong dependence of the hopping probabilities themselves, especially the most difficult ones determining the d.c. conductivity.

The present analysis is deliberately confined to a very general model, making the minimum of assumptions about the specific properties of any particular model under consideration. I feel that when we are confronted with the case of a very widely observed behaviour, the first task is to show that this behaviour is consistent with a model postulating only a very generally applicable property—in the present instance the existence of a slow relaxation of polarisation in the dielectric matrix in which charge carriers move by hopping.

A comment is indicated about the relation between the approach proposed in the present paper and the earlier theories of sequential hopping^{3-5,9}. These theories may well be applicable to certain materials which have the required density and distribution of localised levels, for example chalcogenide glasses in which there is also a reasonable correlation between the d.c. and a.c. conductivities. I suggest, however, that the present model offers a wider framework in which it is possible to understand the observed behaviour of many materials to which the other theories could not be applied so easily.

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- ¹ Jonscher, A. K., *Electronic and Structural Properties of Amorphous Semiconductors* (edit. by Le Comber, P. G., and Mort, J.), (Academic Press, 1973).
- ² Jonscher, A. K., *J. Phys. C*, **6**, L235-L239 (1973).
- ³ Pollak, M., and Geballe, T. H., *Phys. Rev.*, **122**, 1742-1753 (1961).
- ⁴ Pollak, M., *Phys. Rev.*, **138**, A1822-1826 (1965).
- ⁵ Scher, H., and Lax, M., *Phys. Rev.*, **B7**, 4491; **B7**, 4502 (1973).
- ⁶ McCrum, N. G., Read, B. E., and Williams, G., *Anelastic and Dielectric Effects in Polymeric Solids* (Wiley, 1967).
- ⁷ Austin, I. G., and Mott, N. F., *Adv. Phys.*, **18**, 41-102 (1969).
- ⁸ Moore, E. J., *J. Phys. C*, **7**, 339-351 (1974).
- ⁹ Pollak, M., and Pike, G. E., *Phys. Rev. Lett.*, **28**, 1449-1451 (1972).
- ¹⁰ Le Sueur, E. J., thesis, Univ. of London (1972).
- ¹¹ Ivkin, E. B., Kolomiets, B. T., Raspopova, E. M., and Tsandin, K. D., *Soviet Phys. Semiconductors*, **5**, 1558-1560 (1972).
- ¹² Jonscher, A. K., *Proc. Fifth Int. Conf. on Amorphous and Liquid Semiconductors* (Garmisch, 1973).
- ¹³ Jonscher, A. K., 1972 *Annual Report on Conference on Electrical Insulation and Dielectric Phenomena*, 418-425 (Nat. Acad. Sci., Washington DC, 1973).
- ¹⁴ Jonscher, A. K., *Electrets, Charge Storage and Transport in Dielectrics*, (edit. by M. M. Perlman) 269-284, (Electrochemical Society Inc., 1973).
- ¹⁵ *Tables of Integral Transforms* (edit. by Erdélyi, A.), II, 249 (McGraw Hill, New York, 1954).

Chemical and biological evolution of a nucleotide-binding protein

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Three-dimensional alignment of the common nucleotide binding structure in dehydrogenases, kinases and flavodoxins permits the recognition of homologous amino acids when sequence comparisons alone would fail. Minimum base changes per codon can then be used to measure evolutionary distances which suggest that this structure was present during precellular evolution.

A COMMON structural domain¹ whose function is to bind nicotinamide adenine dinucleotide (NAD) has been found in lactate dehydrogenase (LDH)^{2,3}, in soluble malate dehydrogenase (sMDH)⁴, in liver alcohol dehydrogenase (LADH)⁵ and in glyceraldehyde-3-phosphate dehydrogenase (GAPDH)⁶. Rao and Rossmann⁷ showed that the same structure was utilised to bind flavin mononucleotide (FMN) in flavodoxin⁸⁻¹⁰. They also showed that this structure consists of two smaller units the function of each being to bind a mononucleotide. Buehner *et al.*⁶ proposed an evolutionary tree which traces the incorporation and evolution of the mononucleotide-binding structure in various dehydrogenases and in flavodoxin. Further data on the three-dimensional structures and amino acid sequences of some dehydrogenases and flavodoxins are now available which permits the evaluation of the proposed evolutionary tree more precisely. We show here that the position and sequence of the nodes in this tree are consistent with the molecular data, and that a rough time scale for these events can be proposed.

Independent support for the evolutionary tree comes from a study of redox potentials and the evolution of biological electron transport^{11,12}, suggesting a common origin for NAD and flavin-binding proteins. Furthermore, structures similar to the mononucleotide or dinucleotide-binding protein fragment have been found in phosphoglycerate kinase¹³ (Bryant *et al.*¹⁴ suggest an alternative solution) and tentatively in adenyl kinase¹⁵. Both these enzymes need to bind AMP, ADP or ATP. Similarly a flavodoxin-like structure has been recognised tentatively in rhodanese¹⁶ whose biological function is not fully established but is known to bind flavin FAD FMN and NAD.

Structural alignments

The nucleotide-binding protein discussed above consists of a parallel sheet formed by three extended polypeptide strands. The first two are connected by an α -helix, and the last two by

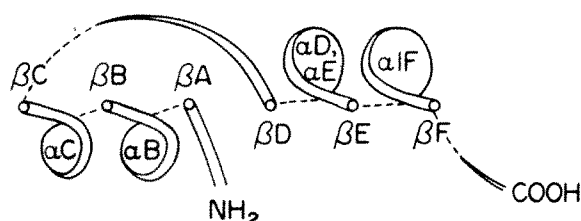


Fig. 1 Diagrammatic representation of a dinucleotide-binding protein. The amino termini of the strands in the β -pleated sheet are closest to the viewer. The dinucleotide binds to the carboxy termini of the strands in the sheet.

either an α -helix or another, less well defined, structure. In dehydrogenases the secondary structural features of the dinucleotide-binding fragment have been termed β A, β B, β C, β D, β E and β F within the β -pleated sheet, while the helices are labelled α B, α C, . . . (Fig. 1). This nomenclature was originally devised for the LDH structure³. Helices α B and α C connect strands β A with β B and β B with β C, respectively. They are on the same side of the sheet. Along the polypeptide chain, the sequence β A, α B, β B, α C, β C forms the first (adenine) nucleotide-binding fragment, while the second (nicotinamide) nucleotide-binding fragment is related by an approximate two-fold axis. The fold of each fragment has the same unique band.

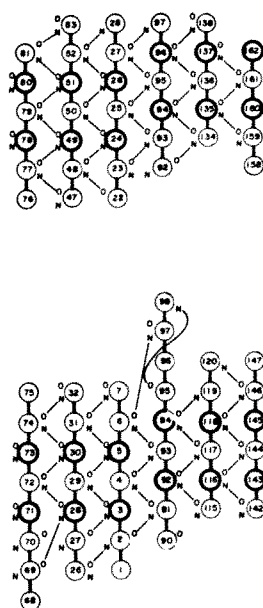


Fig. 2 Hydrogen bonding diagram of β -pleated sheet region in (top) dogfish apo-LDH and (bottom) lobster holo-GAPDH. Amino acids with thick outlines have residues facing into hydrophobic pockets between the β -pleated sheet and α helices. The hydrophobic character of these residues is strongly conserved (Table 1).

There is a greater conservation of the structure of the sheet region than of the helices. For example, in LADH and GAPDH the helix α D is missing, while in LDH and s-MDH it forms part of a flexible loop which undergoes a large conformational change during catalysis. The greater conservation of the parallel pleated sheet region can be seen readily in Fig. 2 where the hydrogen bonding within the sheet has been depicted for dogfish apo-LDH and lobster holo-GAPDH. When these bonds are equivalently aligned, with due regard for the direction of polarity of the peptide chain, then the orientation of the C_{β} atom in each amino acid side chain must also be aligned. Hence superposition of the hydrogen bonding provides a sensitive method of determining homologous amino acids in equivalent β -sheet regions of different nucleotide-binding fragments. The atomic coordinates of the equivalenced C_{α} atoms were taken to set up an approximate rotation matrix to superimpose the two molecules being compared. This was then refined to minimise the sum of the square of the distances between equivalenced atoms⁷. With this alignment other residues, not in the sheet, but nevertheless following the same

Table 1 Nucleotide-binding protein fragment comparison of amino acids

Reference to sequence data	βA	αB	βB	βC	βF
Dogfish LDH 38	2 N K I T V V G 3 B A V 3 G	3 M A D A I S V L M K D L A 4 0 3 1	4 7 D E V A L V D 2 6 3 3	7 6 A K I V S G K 9 1 8 8	8 8 4 D 1 3 4 7 6 E E E
Pig GAPDH 39 Lobster GAPDH 40 Yeast GAPDH 41	1 K V G V D G G 2 S K I G I D G 1 V R V A I D G 9 1 3 2 0 0	3 R L V T R A A A F N S G K V V 2 L L V L R A A A L S C G G 0 R L V M R I A A L S R P B A 5 2 2 0 5 1	6 D I V A I N D D 2 V V A V N D D 2 V V A S B B 2 2 2 2 4 4	9 K K A I T I F F O N E 6 K K I I T V V Y 3 K K I A T T 2 3 6 G A T E C V N P	8 8 4 D 1 3 4 7 6 E E E
Horse LADH 42, 43	3 T C A V F G L G G V G 2 2 4 2 5 5	5 L S V I M G C K A A G - A 2 2 6 6 3 3	7 A R I I G V D 2 2 9 9 8 8	6 G A T E C V N P 2 2 4 4 3 3	8 8 4 D 1 3 4 7 6 E E E
Bovine GluDH 19	5 K T F A V Q G 1 M K I V Y 2 2 0 0	7 H S M R Y L H R F G - A 2 2 3 3 1 1	9 K C V A V G E S 2 2 8 8 4 4	6 G S I 2 2 5 5 4 4	8 8 4 D 1 3 4 7 6 E E E
<i>Clostridium</i> MP 10, 44, 45 Flavodoxin	1 M K I V Y 2 2 0 0	7 E L I A K G I I E S G 2 2 3 3 1 1	8 K D V N T I N 2 2 4 4 3 3	5 S D V N 2 2 4 4 3 3	8 8 4 D 1 3 4 7 6 E E E
Subtilisin 46	2 V I N M S L G G P S G 3 3 1 1	8 S A A L K A A V D K A G 2 2 3 3 1 1	7 V V V A A A 2 2 4 4 3 3	6 G N E G S 2 2 4 4 3 3	8 8 4 D 1 3 4 7 6 E E E
Dogfish LDH 38	8 A G S 9 9 2 2	1 F K F I I P N I V K H S P 2 2 5 5 1 1	1 3 C I L E L H P 3 3 1 1 4 4	1 5 8 H R I I G B G 1 1 4 4 1 1	1 6 4 C 1 4 4 8 8
Pig GAPDH 39 Lobster GAPDH 40 Yeast GAPDH 41	9 A G T A G A V I A I R S 8 7 2 2 6 6	2 M E K A S A H L K K - G G A 1 I E K A S A H L K K - G G A 2 2 7 7 3 3	4 K R V I I S A 4 K R V V I S A 2 2 8 8 7 7	1 K I V S N A S S C 1 M T V V S N A S S C 3 3 1 1 2 2	8 8 4 D 1 3 4 7 6 E E E
Horse LADH 42, 43	0 G G V D F S F E V I G R 4 4 7 7	7 T M V T A L S C C Q - E A Y 6 6 7 7 5 5	8 G V S V I V G 7 7 0 0 8 8	2 R T W K G A I F G 1 1 0 0 8 8	8 8 4 D 1 3 4 7 6 E E E
<i>Clostridium</i> MP 10, 44, 45 Flavodoxin	4 L N E 4 4 7 7	6 H E P F I E E I S T K I S G 7 7 5 5 1 1	8 K K V A L F G 7 7 0 0 8 8	6 G N E G S 2 2 4 4 3 3	8 8 4 D 1 3 4 7 6 E E E

The AMP binding fragment above is aligned below with the NMN binding fragment in flavodoxin. LDH Cys 165 and GAPDH Cys 149 form the essential thiol groups of these two enzymes.

Table 2 Comparison of the NAD-binding domain among various dehydrogenases

		1	2	3	4
Dogfish LDH	1		1.12	1.16	1.13
Lobster GAPDH	2	75		1.13	1.00
Horse LADH	3	74	71		1.13
Bovine GluDH	4				

The top right of the matrix shows the minimum base changes per codon for the alignments in Table 1. The bottom left shows the number of those equivalent amino acids whose C α atoms approach each other to within 3.8 Å.

folding sequence could be equivalenced and incorporated into the refinement procedure.

The amino acid alignments shown in Table 1 are thus based on three-dimensional structure, while the character of the amino acids has been determined chemically. Since Rao and Rossman⁷ have pointed out similarity of structure between a part of subtilisin^{17,18} and a mononucleotide-binding fragment, with the aromatic specificity pocket of subtilisin corresponding to the adenine-binding pocket of the dehydrogenases, this comparison has been included in Table 1. Results for individual comparisons among the dehydrogenases are shown as a matrix in Table 2. The resultant alignment of the bound nucleotides is best seen in a series of stereographic diagrams. The C α

backbone of the NAD-binding fragment of LDH has been used as a standard of comparison. In Fig. 3, LDH is compared with the dehydrogenases GAPDH and LADH, in Fig. 4 comparisons are made with phosphoglycerate kinase¹³ and flavodoxin and Fig. 5 shows a comparison of the AMP-binding fragment with the MNN-binding fragment in LDH.

Sequence homologies with GluDH

The alignment of the glutamate dehydrogenase (GluDH) sequences¹⁹ is not based on structure but on amino acid sequence homologies alone. Different methods of testing sequences for homologies in distantly related proteins have been reviewed by Haber and Koshland²⁰, Barker and Dayhoff²¹ and others. The procedure described by Jukes and Cantor²², which depends on counting minimum base changes per codon, has been used here. By far the best alignment was found in comparing residues 1 to 38 in GAPDH with 245 to 283 in bovine GluDH corresponding to β A, α B and β B (Table 1). This corresponds to the most conserved part of the NAD-binding structure and furthermore the character of important amino acids was maintained.

The glycine in position 28 of LDH is strictly conserved as any larger residue would cause steric hindrance to the binding of the ribose ring. The cause of conservation of the glycine in position 33 of LDH is probably related to its position on helix α B immediately opposite the β -pleated sheet. The conservation of LDH aspartate 53 (changed to glutamate in GluDH) at the end of β B must be related to its function of binding the O2' atom in the adenine ribose³. The alternate hydrophobic residues in the β -pleated sheet is also quite outstanding.

Although GluDH is known to have two independent binding sites for NADH or NADPH²³ the sequence comparison



Fig. 3 Superimposed dinucleotide-binding domains using LDH as a standard. The LDH C α backbone is shown in dark as is its NAD coenzyme. Residue numbers are given for LDH only. Corresponding numbers for the other compounds (shown in open bonds) can be derived from Table 1. Comparisons are with (a) GAPDH and (b) LADH.



Fig. 4 Comparison of (a) phosphoglycerate kinase (PGK) and (b) flavodoxin with LDH. Conventions used are described in Fig. 3. The black ball is the Mg^{2+} site in PGK.

could only identify one truly significant portion of the polypeptide chain which compares with the known NAD binding domains in other dehydrogenases.

The alignment shown in Table 1 for GluDH with GAPDH is different from that given by Smith *et al.*²⁴ or the two comparisons given by Engel²⁵, one of which contains Smith's shorter sequence. The alignment given here shows 1.00 minimum base changes per codon with respect to lobster GAPDH as opposed to 1.48 and 1.53 for Engel's relationships. Furthermore, Engel aligns two GluDH sequences with residues 198–261 of GAPDH. However, this region of GAPDH belongs to the domain which supplies catalytic residues and generates substrate specificity, a domain which might be anticipated to be special to GAPDH. Williams and Wilkins²⁶ point out that Engel's alignments were based on doubtful statistical procedures.

Measuring evolutionary divergence

A relative estimate of the time since the divergence from a common ancestor (or node in an evolutionary tree) can be obtained by comparing amino acids of related proteins. Margoliash and Fitch^{27,28} used the amino acid sequence to show homologous alignments between proteins and then the mean change between the sequence can be measured by various scoring procedures to determine evolutionary distance. In this article (except for the GluDH comparisons) three-dimensional structure has been used to obtain alignments, because of its

greater conservation over amino acid sequence. Every pair of structurally equivalent amino acids can then be examined for their genetic relationship.

It is reasonable to assume that the probability of any one amino acid changing to any other is inversely proportional to the minimum number of base changes. Thus there can be 0, 1, 2 or 3 base changes or on the average 1.5 base changes per codon. If the actual genetic code is taken into account, then the average number of minimum base changes required is 1.57 to change any one amino acid to any other. If, further, the natural frequency of amino acids²⁹ is considered, this number changes to 1.51. Whether or not an observed minimum base change per codon is significantly lower than a random variation will depend on its distance from the assumed random level. When additional independent data (such as three-dimensional structures) are available, comparisons are of greater significance than when only sequence information is at hand. Thus a combination of structure and sequence permits the measurement of more distant evolutionary relationships.

The minimum base change per codon can be taken as a measure of relative evolutionary distance, that is the elapsed time since the occurrence of a common ancestor for divergent events. This measure is unlikely to be exactly linear with time if no correction is made for back mutations. Another cause for possible deviation from linearity is that the rate of accepting point mutations may have been different at different times. In general, however, a uniform acceptance rate is a reasonable assumption for a given protein. For GAPDH the observed minimum base change per codon between the pig and yeast

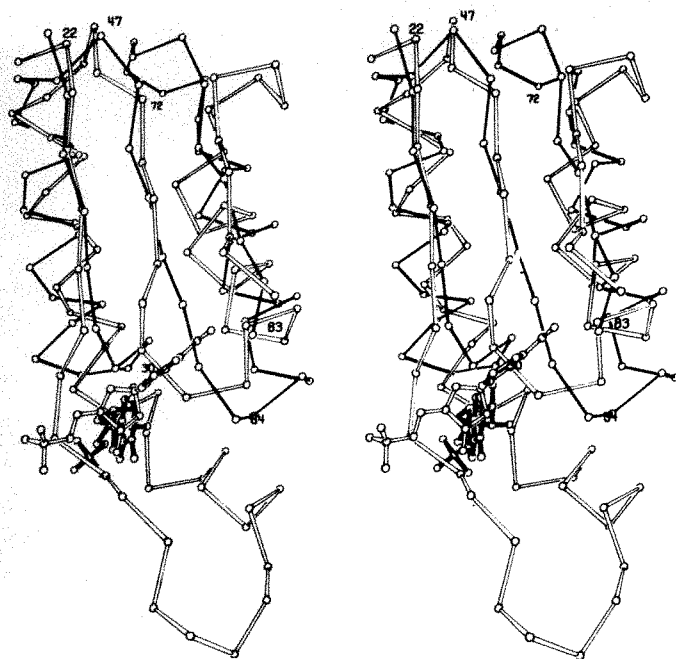


Fig. 5 Superimposed AMP and NMN mononucleotide-binding fragments in LDH. The AMP binding domain is shown with dark bonds and is numbered. The corresponding numbering for the NMN binding fragment can be derived from Table 1.

enzyme in the nucleotide binding protein (residues 1–149) is 0.59, but only 0.35 for residues 150–331. Presumably the differing functions of the two domains within a single polypeptide chain¹ impose different rates of evolution. Thus our estimates of divergence among dehydrogenases have been taken only within the nucleotide-binding domains.

A time scale for evolution

The evolutionary tree suggested by Buehner *et al.*⁶ has been

expanded as shown in Fig. 6. Observed minimum base changes per codon are also related to a probable time at each node. The divergence of the mammalian line from Aves can be placed about 3×10^8 yr ago (node 1, Fig. 6), from Arthropoda about 6×10^8 yr ago (node 2, Fig. 6) and from Fungi about 1.2×10^9 yr ago (node 3, Fig. 6)^{30,31}. The presence of the NAD-binding protein in cytoplasmic and mitochondrial dehydrogenases implies that it originated more than 1.5×10^9 yr ago when mitochondria were possibly incorporated into proto-eukaryotes³². Probably, however, this structure is a good deal older as it would have been required in the earliest prokaryotes for glycolysis around 3.2×10^9 yr ago³³. If it is assumed that simple polypeptides and nucleic acids had gained the ability to perform energy transfer steps and primitive copying processes during precellular evolution³⁴ we might expect to find the mononucleotide-binding protein before 3.2×10^9 yr ago. It is also reasonable to assume that it was formed sometime after the age of the Earth 4.5×10^9 yr ago³⁵.

Figure 6 shows that, the larger the age associated with the node, the greater is the minimum base change per codon consistent with a divergent evolutionary process from a single common ancestor. That alternative nucleotide-binding proteins exist is, however, evident in the structures of RNase³⁶ and staphylococcal nuclease³⁷. Nevertheless, it might be anticipated that the basic structure shown in Fig. 1 will frequently be found where there is requirement (especially an old and basic requirement) for binding nucleotides. Examples might be amino acid tRNA synthetases, ribosomal proteins and virus coat proteins. The recognition of this structure by sequence homology or from X-ray structure determinations may also give guidance as to function where none is definitely known.

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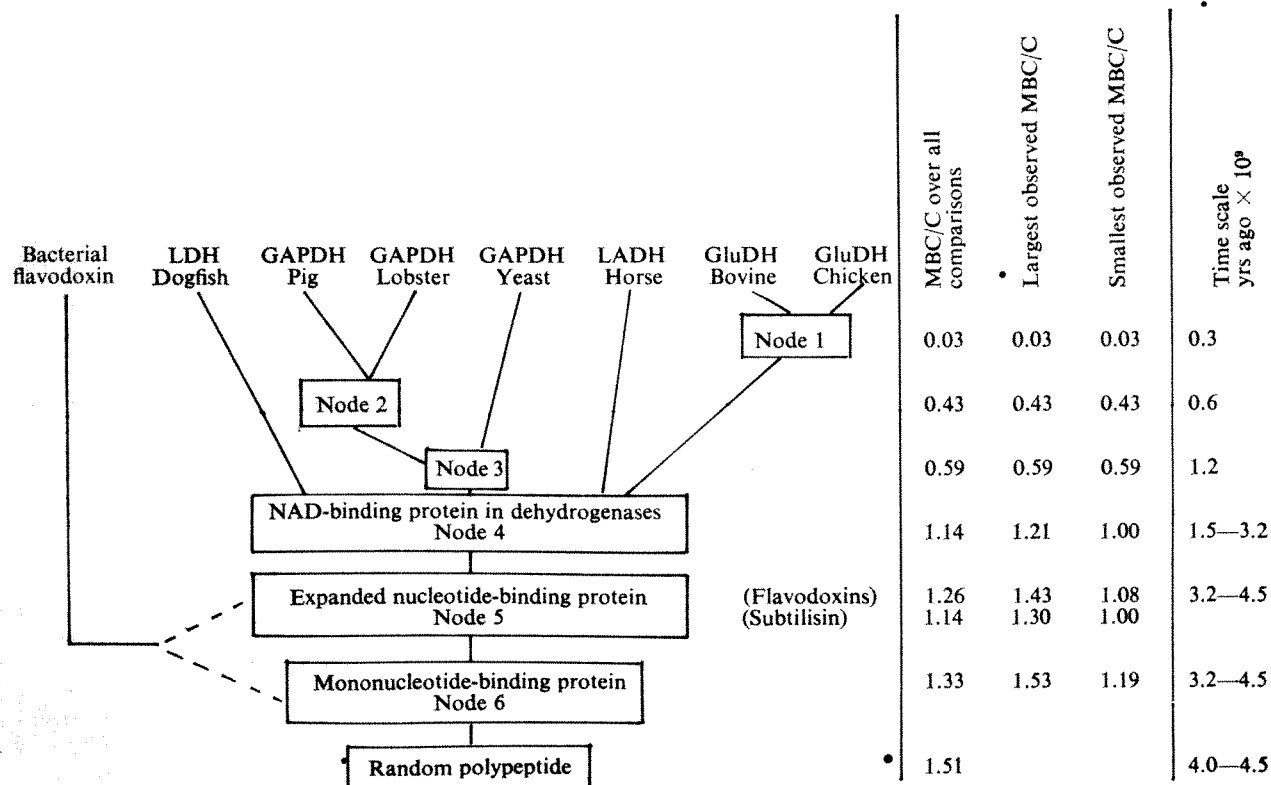


Fig. 6 Evolutionary tree consistent with observed minimum base changes per codon (MBC/C) and possible time scale derived primarily from fossil data.

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- ¹ Rossmann, M. G., and Liljas, A., *J. molec. Biol.* (in the press).
- ² Adams, M. J., Ford, G. C., Koekoek, R., Lentz, jun., P. J., McPherson, jun., A., Rossmann, M. G., Smiley, I. E., Schevitz, R. W., and Wonacott, A. J., *Nature*, **227**, 1098 (1970).
- ³ Rossmann, M. G., Adams, M. J., Buehner, M., Ford, G. C., Hackert, M. L., Lentz, jun., P. J., McPherson, jun., A., Schevitz, R. W., and Smiley, I. E., *Cold Spring Harbour Symp. quant. Biol.*, **36**, 179 (1971).
- ⁴ Hill, E., Tsernoglou, D., Webb, L., and Banaszak, L. J., *J. molec. Biol.*, **72**, 577 (1972).
- ⁵ Brändén, C. I., Eklund, H., Nordström, B., Boiwe, T., Söderlund, G., Zeppezauer, E., Ohlsson, I., and Åkeson, Å., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2439 (1973).
- ⁶ Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossmann, M. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3052 (1973).
- ⁷ Rao, S. T., and Rossmann, M. G., *J. molec. Biol.*, **76**, 241 (1973).
- ⁸ Watenpaugh, K. D., Sieker, L. C., Jensen, L. H., LeGall, T., and Dubourdieu, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3185 (1972).
- ⁹ Andersen, R. D., Apgar, P. A., Burnett, R. M., Darling, G. D., LeQuesne, M. E., Mayhew, S. G., and Ludwig, M. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3189 (1972).
- ¹⁰ Burnett, R. M., Darling, G. D., Kendall, D. S., LeQuesne, M. E., Mayhew, S. G., Smith, W. W., and Ludwig, M. L., *J. biol. Chem.* (in the press).
- ¹¹ Baltscheffsky, H., *Fourth Int. Conf. on the Origin of Life*, Barcelona, 1973 (in the press).
- ¹² Baltscheffsky, H., in *Dynamics of energy transducing membranes* (edit. by Ernster, L., Estabrook, R. W., and Slater, E. C.) (Elsevier, Amsterdam, in the press).
- ¹³ Blake, C. C. F., and Evans, P. R., *J. molec. Biol.* (in the press).
- ¹⁴ Bryant, T. N., Watson, H. C., and Wendell, P. L., *Nature*, **247**, 17 (1974).
- ¹⁵ Schulz, G. E., and Schirmer, R. H., *Nature* (in the press).
- ¹⁶ Smit, J. D. G., thesis, Univ. Groningen (1973).
- ¹⁷ Wright, C. S., Alden, R. A., and Kraut, J., *Nature*, **221**, 235 (1969).
- ¹⁸ Drenth, J., Hol, W. G. J., Jansonius, J. N., and Koekoek, R., *Cold Spring Harbour Symp. quant. Biol.*, **36**, 107 (1971).
- ¹⁹ Moon, K., Piszkiwicz, D., and Smith, E. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1380 (1972).
- ²⁰ Haber, J. E., Koshland, jun., D. E., *J. molec. Biol.*, **50**, 617 (1970).
- ²¹ Barker, W. C., and Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **5**, 101 (1972).
- ²² Jukes, T. H., and Cantor, C. R., in *Mammalian protein metabolism* (edit. by Munro, H. M.), **22** (Academic Press, New York, 1969).
- ²³ Krause, J., Böhner, M., and Sund, H., *Eur. J. Biochem.*, **41**, 593 (1974).
- ²⁴ Smith, E. L., Landon, M., Piszkiwicz, D., Brattin, W. J., Langley, T. J., and Melamed, M. D., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 724 (1970).
- ²⁵ Engel, P. C., *Nature*, **241**, 118 (1973).
- ²⁶ Williams, J., and Wilkins, A. G., *Nature*, **247**, 556 (1974).
- ²⁷ Margoliash, E., and Smith, E. L., in *Evolving genes and proteins* (edit. by Bryson, V., and Vogel, H. J.), **221** (Academic Press, New York, 1965).
- ²⁸ Margoliash, E., and Fitch, W. M., *Science*, **155**, 279 (1967).
- ²⁹ Dayhoff, M. O., Eck, R. V., and Park, C. M., *Atlas of Protein Sequence and Structure*, **5**, 89 (1972).
- ³⁰ Dodson, E. O., in *Evolution: Process and product* (Reinhold, New York, 1960).
- ³¹ Salthe, S. N., in *Evolutionary biology* (Holt, Rinehart and Winston, New York, 1972).
- ³² deDuke, C., *Science*, **182**, 85 (1973).
- ³³ Barghorn, E. J., *Scientific American*, **224**, 30 (1971).
- ³⁴ Carter, C., Kraut, J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 283 (1974).
- ³⁵ York, D., and Farguhar, R. M., in *The Earth's age and geochronology* (Pergamon, Oxford, 1972).
- ³⁶ Richards, F. M., Wyckoff, H. W., Carlson, W. D., Allewell, N. M., Lee, B., and Mitsui, Y., *Cold Spring Harbour Symp. quant. Biol.*, **36**, 35 (1971).
- ³⁷ Cotton, F. A., Bier, C. J., Day, V. W., Hazen, jun., E. E., and Larsen, S., *Cold Spring Harbour Symp. quant. Biol.*, **36**, 243 (1971).
- ³⁸ Taylor, S. S., Oxley, S. S., Allison, W. S., and Kaplan, N. O., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1970 (1973).
- ³⁹ Harris, J. I., and Perham, R. N., *Nature*, **219**, 1025 (1968).
- ⁴⁰ Davidson, B. E., Sajó, M., Noller, H. G., and Harris, J. I., *Nature*, **216**, 1181 (1967).
- ⁴¹ Jones, G. M. T., and Harris, J. I., *FEBS Lett.*, **22**, 185 (1972).
- ⁴² Jörnvall, H., *Eur. J. Biochem.*, **16**, 25 (1970).
- ⁴³ Jörnvall, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2295 (1973).
- ⁴⁴ Tanaka, M., Hanice, M., and Yasunabin, K. T., *Biochem. biophys. Res. Commun.*, **44**, 886 (1971).
- ⁴⁵ Fox, J. L., Smith, S. S., and Brown, J. R., *Z. Naturforschung*, **27b**, 1096 (1972).
- ⁴⁶ Alaitan, S. A., DeLange, R. J., and Smith, E. L., *J. biol. Chem.*, **243**, 5296 (1968).

Three abundance classes in HeLa cell messenger RNA

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Approximately 35,000 different poly(A)-containing RNA sequences are present in HeLa cell cytoplasm. The sequences are grouped in three distinct abundance classes.

THE amount of DNA per haploid genome in the higher eukaryotes is known to be very large, and this has long posed the question of how much of the DNA encodes mRNA. Up to the present time few serious attempts have been made to answer this question. One problem has been the difficulty experienced in attaining sufficiently high concentrations of RNA to drive hybridisation reactions to completion. The discovery of poly(A) stretches in mRNA¹⁻³ has partially removed this difficulty, since poly(A)-containing RNA can now be separated from rRNA^{2,4,5} and much higher concentrations of mRNA can be attained. A second difficulty has been the existence of repetitive sequences in the DNA⁶: the transcript of one repetitive sequence can hybridise with related sequences,

producing falsely high results. This problem was solved by Hahn and Laird⁷ and others⁸⁻¹¹ using a technique devised by Kohne¹². Labelled nonrepetitive DNA sequences are isolated using hydroxyapatite, and annealed with an excess of unlabelled RNA. The DNA-RNA hybrids are then recovered on hydroxyapatite. In this way it was shown that about 10% of nonrepetitive mouse DNA was represented in transcripts found in total brain RNA^{7,9,10}. Similarly 0.9% of nonrepetitive *Xenopus* DNA is represented by transcripts in the mature oocyte⁸.

An even more serious problem has been that whatever answer was obtained, it had to be regarded as a minimum. No matter how much RNA was added, or for how long the samples were annealed, the possibility always remained that classes of RNA were present at a lower concentration than the least concentrated sequences observed to react. Such RNA sequences would fail to react significantly with the complementary DNA sequences, and would go undetected. This problem can now be solved in the following way. It is now possible to synthesise a complementary DNA

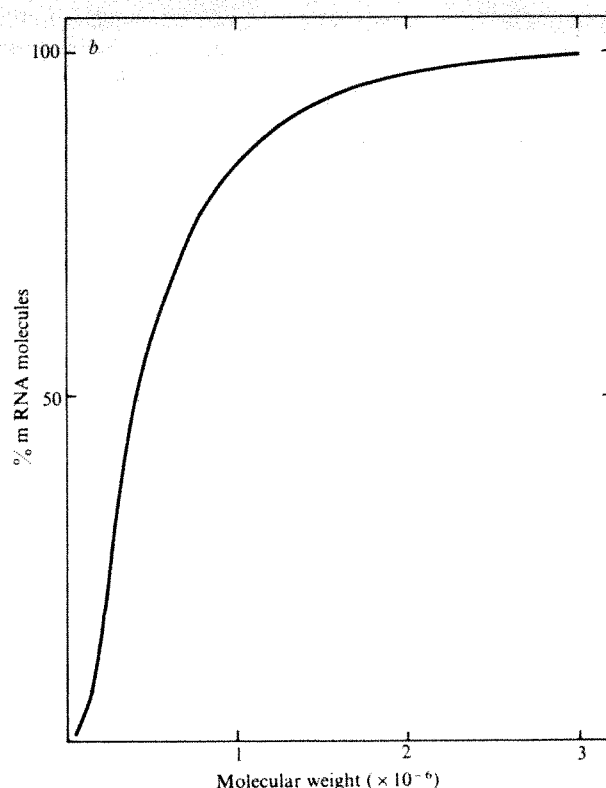
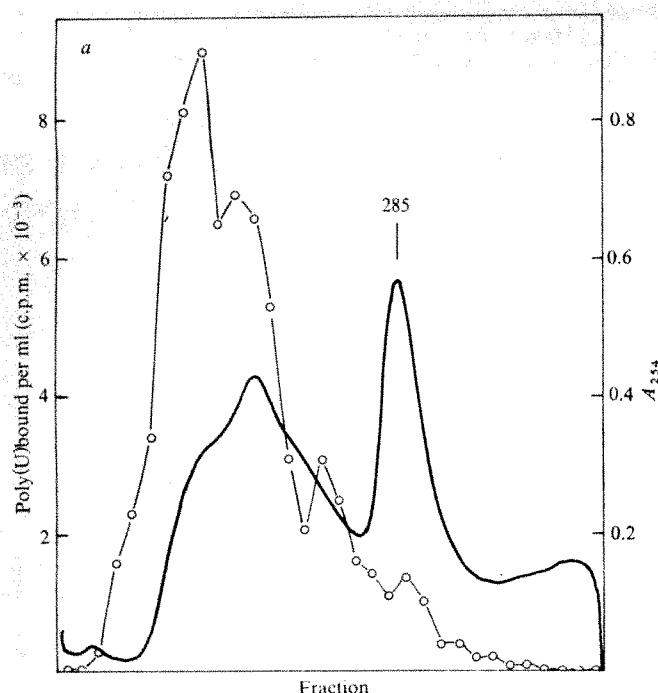


Fig. 1 Molecular weight distribution of HeLa cell mRNA. *a*, Sucrose gradient of enriched mRNA fraction showing A_{254} and results of annealing a portion of each fraction with poly(U)²¹(o). Sedimentation is from left to right. *b*, Cumulative plot of number of mRNA molecules against molecular weight (calculated according to Spirin²³ and Gierer²⁴). Two gradients like that shown in panel *a* were averaged. Approximately 10^9 HeLa cells were washed and lysed with RSB containing 1% NP-40. Nuclei were separated by centrifugation and the outer membrane fraction was removed²⁵ and added to the cytoplasmic fraction. Cytoplasmic RNA was extracted by a procedure²⁶ modified after Parish and Kirby²⁷ and passed repeatedly over oligo(dT)-cellulose⁴ until free of material which reacted with poly(U)²¹. The poly(A)-containing RNA was eluted⁴ and again treated in the same way. The final eluate from oligo(dT)-cellulose was centrifuged for 16 h at 25,000 r.p.m. and 25°C on a 15–30% sucrose gradient in NTE (0.1 M NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA) containing 0.5% SLS (Spinco No. 27 rotor). The mRNA preparations used in this report were prepared by precipitating fractions between 5 and 35S with ethanol, and passing the precipitate, dissolved in 0.3 M NaCl–10 mM Na acetate, pH 5, over a column of Sephadex-SP50 overlying Chelex-100, developed with the same buffer.

transcript (cDNA) on a template of poly(A)-containing mRNA^{13–15}. By annealing this with an excess of the RNA template, we can determine the R_{ot} (product of initial RNA concentration and annealing time) at which the cDNA transcript hybridises completely. The same R_{ot} will be sufficient to obtain hybridisation of all the DNA complementary to mRNA when an excess of unlabelled mRNA is annealed with labelled single-copy DNA. Thus, in principle, the answer obtained should be a definitive one.

The availability of cDNA offers a second important advantage in that the kinetics of hybridisation between cDNA and an excess of unlabelled mRNA are dependent on the sequence complexity of the mRNA^{16,17}. By comparison with a suitable kinetic standard it should be possible to estimate the complexity of the mRNA in favourable cases. When this is so, we have available two independent estimates of mRNA sequence complexity. If they agree, confidence in the estimates will be greatly strengthened.

Preparation of HeLa cell mRNA

The mRNA was prepared by extracting the total cytoplasm of cells lysed with 1% NP-40. Thus, both free and membrane-bound polyribosomes contributed their mRNA populations, together with any mRNA present in the

postribosomal fraction. Poly(A)-containing RNA was prepared using oligo(dT)-cellulose⁴. The evidence at present available^{18,19} indicates that most mRNA contains poly(A). The great advantage of using this method is that it provides a defined, reproducible fraction of the total cellular RNA which is predominantly mRNA. Nevertheless, such preparations contain some 18S and 28S rRNA (Fig. 1). To accommodate this problem, we titrated each mRNA preparation with radioactive poly(U)^{20,21}. Two preparations of HeLa cell mRNA (containing rRNA) bound 8.4% and 8.6% by weight of poly(U). If we take the number-average molecular weight of the mRNA to be 640,000 (2,000 nucleotides) and the average length of the poly(A) to be 150 nucleotides²² the amount of poly(U) bound should be 7.5% by weight (the ribonuclease-resistant complex formed between poly(A) and poly(U) is a 1:1 complex²¹). In practice, we assumed a 10% by weight binding in calculating the mRNA concentration. Minor adjustments are made in the subsequent calculations.

The number-average molecular weight of the mRNA was estimated by annealing sucrose gradient fractions with radioactive poly(U) (Fig. 1). Assuming that poly(A) size is the same irrespective of the size of the mRNA, the number-average molecular weight is readily calculated to be close to 640,000.

Molecular hybridisation

Total HeLa cell mRNA proved to be as efficient as haemoglobin mRNA as a template for cDNA synthesis by avian myeloblastosis virus reverse transcriptase. The reaction was completely dependent upon priming by (pT)₁₀ suggesting that synthesis starts at the 3' ends of the mRNA molecules within the poly(A) sequence^{13–15}.

In vast DNA excess hybridisation experiments, labelled HeLa cell mRNA shows a prominent repetitive component (A. Spradling, S. Penman, S. Campo and J. O. Bishop, manuscript in preparation). To estimate the proportion of

repetitive sequences in the cDNA, it was annealed with an excess of unlabelled HeLa cell DNA. Highly labelled non-repetitive HeLa cell DNA was also annealed with unlabelled DNA to provide a comparison (Fig. 2). The non-repetitive cellular DNA behaved as though completely unique, with a $C_{0t\frac{1}{2}}$ of close to 10^3 mol s l^{-1} . The cDNA showed evidence of a 10% repetitive component. The $C_{0t\frac{1}{2}}$ of the second, major cDNA transition is also close to 10^3 mol s l^{-1} .

The hybridisation of the cDNA with an excess of mRNA (template) is shown in Figs 3 and 4. Figure 3 shows a linear-log plot of the data. Three transitions are seen, with midpoints ($R_0t\frac{1}{2}$, mol s l^{-1}), of 0.05, 0.9 and 45. That these are real is shown more clearly in Fig. 4. The linear-log plot tends to obscure the inflections between transitions which are more easily seen in straightforward linear plots. Three of these are needed to cover adequately the range of Fig. 3. Figure 4a shows the first inflection clearly, Fig. 4b shows both, and Fig. 4c shows the second clearly. Note the differences in the scale of the three abscissae of Fig. 4.

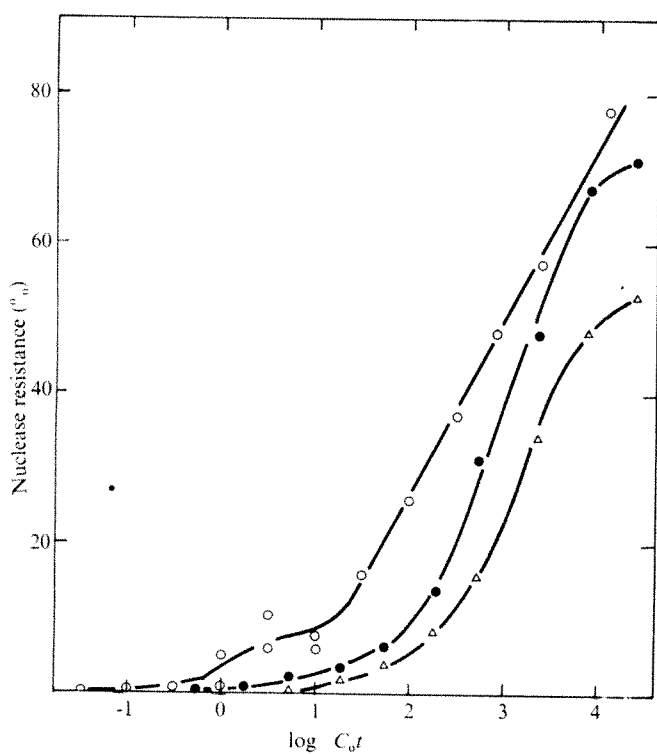


Fig. 2 Renaturation experiments with an excess of unlabelled HeLa cell DNA. The labelled species were (●) nonrepetitive HeLa cell DNA; (○) cDNA prepared against total cytoplasmic poly(A)-containing RNA; and (△) the fraction of nonrepetitive HeLa cell DNA which hybridises with mRNA at a R_0t of 350. cDNA was synthesised using as template mRNA prepared as described in Fig. 1 (ref. 28, except that the reverse transcriptase was purified through CM-Sephadex²⁹ and the substrate was ³H-dCTP (13.3 Ci mmol⁻¹)). HeLa cell DNA was purified as described³⁰, sheared at 50,000 p.s.i. in a Sorvall French Press at 10°–15° C in 0.3 M NaCl–10 mM Na acetate, pH 5, and passed over a Sephadex-SP-50-Chelex-100 column developed with the same buffer. DNA prepared in this way is 300–400 nucleotides long. Labelled DNA (2.4×10^5 cpm μg^{-1}) was prepared in the same way from cells labelled with ³H-thymidine. Nonrepetitive DNA was purified by annealing to a C_{0t} of 10 mol s l^{-1} and isolating the fraction eluting from hydroxyapatite between 0.12 M and 0.4 M PB (equimolar Na-phosphate buffer) at 65° C. DNA renaturation was carried out as described³¹, but using 0.24 M PEB (equimolar Na-phosphate buffer containing 1 mM EDTA) at 70° C. Samples were diluted to a final DNA concentration of 250–300 μg ml⁻¹ and divided into two aliquots, one of which was treated at 50° C for 40 min with nuclease S₁ (ref. 32) in a solution with the final composition: 48 mM PEB, 0.2 mM EDTA, 20 mM NaCl, 30 mM Na acetate, pH 4.5, 0.6 mM ZnSO₄, 0.052 N acetic acid.

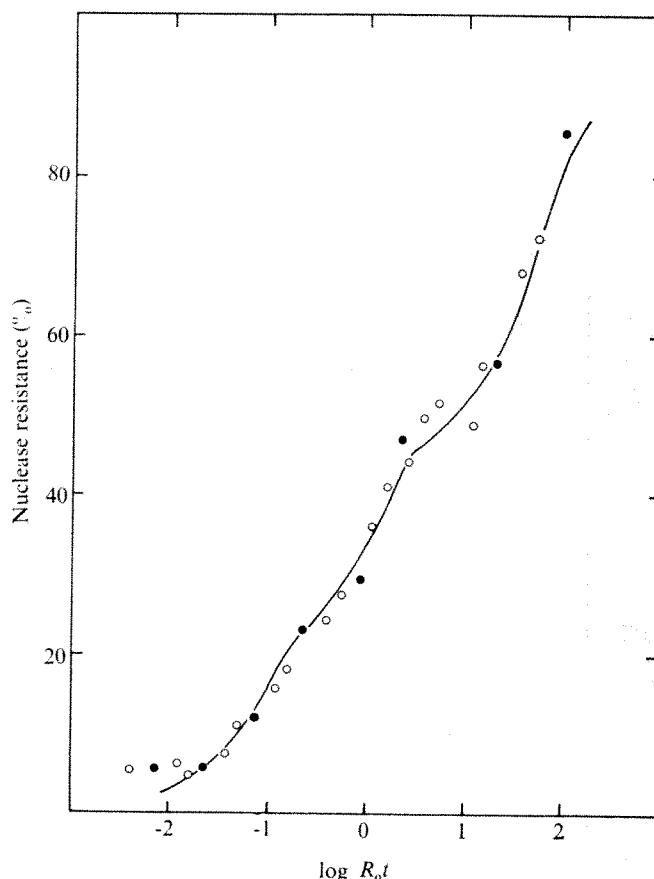


Fig. 3 Hybridisation between cDNA and its mRNA template. Three experiments are shown, two (○) performed with mRNA from cells in spinner culture, one (●) with mRNA from a monolayer culture grown in burlers. In all, five reaction mixtures were used, containing 38, 43 and 295 μg ml⁻¹ (○) and 20 and 210 μg ml⁻¹ (●) of mRNA, assuming a 10% poly-(A) content. Samples were annealed at 70° C in 0.24M PEB and challenged with nuclease S₁ in the presence of 300 μg ml⁻¹ of denatured duck DNA (Fig. 2). The continuous line was drawn using the relationship

$$(d/D_0) = 1 - \sum P_n / e^{k_n t} P_n R_0$$

where D_0 and d represent respectively the initial amount of DNA and the amount duplexed at time t (s) and R_0 the amount of mRNA present (all in mol l^{-1}). The subscript n denotes the transition (1st, 2nd or 3rd). In each transition, P denotes the proportion of the cDNA represented, and k the rate constant of DNA–RNA hybridisation (1 mol⁻¹ s⁻¹), given by $k = 0.69/R_0t_{1/2}$ (ref. 33). The values taken for the first, second and third transitions were: P , 0.22, 0.28, 0.50; k , 62.7, 2.74, 0.307.

The interpretation of the hybridisation data depends on the availability of a suitable standard. This is provided by the work of N. D. Hastie, M. G. Ferace, K. B. Freeman and J. O. Bishop (unpublished). The $R_0t\frac{1}{2}$ of the transition observed when an excess of rabbit haemoglobin α -chain mRNA is annealed with homologous cDNA is 3×10^{-4} mol s l^{-1} . When total ($\alpha + \beta$) haemoglobin mRNA is annealed with homologous cDNA, the $R_0t\frac{1}{2}$ is 6×10^{-4} mol s l^{-1} . Since the molecular weight of haemoglobin mRNA is about 200,000 (refs 34, 35) we take 9×10^{-4} mol s l^{-1} to be the $R_0t\frac{1}{2}$ of an RNA with a molecular weight of 6×10^5 .

The data which can be extracted from Fig. 3 are shown in Table 1. The proportion of the cDNA which reacts in each of the three transitions is shown in the first column, and the observed $R_0t\frac{1}{2}$ of each in the second. The line shown in Fig. 3 was drawn by summing three ideal first order reactions calculated according to these characteristics. The good fit of the line to the data indicates that it is reasonable to interpret each of the transitions as an

Table 1 Numerical analysis of the data shown in Figs. 3 and 4

Transition	<i>P</i>	$R_{ot\frac{1}{2}}$ (observed)	$R_{ot\frac{1}{2}}$ (if pure)	No. of 640,000 dalton sequences		Molecules of each sequence per cell
				If 90% saturation	If 100% saturation	
1	0.22	0.05	0.015	17	15	8,000
2	0.28	0.90	0.335	370	330	440
3	0.50	45	29.9	33,000	36,250	8

For the definition of *P* see Fig. 3.

ideal pseudo-first-order reaction. (The reaction is pseudo-first- rather than second-order because the RNA is in great excess over the cDNA). If the RNA responsible for any one transition were present on its own, thus contributing all of the RNA used in calculating R_{ot} , the $R_{ot\frac{1}{2}}$ of its transition would be lower. The values of $R_{ot\frac{1}{2}}$ have also been corrected for the 7.5% poly(A) content of mRNA (Table 1, column 3). The numbers of sequences of average molecular weight 640,000 required to generate the three transitions are obtained by dividing the corrected $R_{ot\frac{1}{2}}$ values by 9×10^{-4} (Table 1, column 4). Interpreting Table 1, we would say that HeLa cell poly(A)-containing cytoplasmic RNA contains three distinct fractions: 22% of the RNA comprises only 17 different sequences, 28% comprises 370, and 50% comprises 33,000 different sequences, a total of close to 35,000 sequences. Some qualification is necessary because of the small amount of repetitive sequence found in the cDNA

(Fig. 2) which may contribute disproportionately to the first or second of the transitions seen in Fig. 3. This could affect the absolute value in one case by a factor of 1/3 at the very most.

These numbers were derived by assuming that no more than 90% of the cDNA can form hybrids, as they are measured here. This is true for cDNA prepared against haemoglobin mRNA, where it can be convincingly demonstrated. If the theoretical upper limit were 100%, the numbers would not change greatly (Table 1, column 5). Of course, in the case of any of the transitions, the number-average molecular weight of the RNA molecules need not be exactly 640,000. If so, the calculations would require some adjustment.

The data can also be used to calculate the numbers of copies per cell for RNA sequences of each class. By analogy with complex DNA renaturation patterns⁶ we see that these depend on the observed $R_{ot\frac{1}{2}}$ but not upon either RNA molecular weight or the percentage of the total reaction which each transition occupies. Taking 10^6 HeLa cells to contain 12.5 μ g of RNA of which 5% is poly(A)-containing mRNA, the number of copies per cell equals 400 (observed $R_{ot\frac{1}{2}}$). Thus (Table 1, column 6) there are on average 8,000 RNA copies per cell in the highest frequency class, and about 9 copies per cell in the lowest.

A total of 35,000 RNA sequences of average molecular weight 640,000 represents a total sequence complexity of 2.2×10^{10} daltons. Approximately 20% of the HeLa cell mRNA (prepared in the same way) is repetitive (A. Spradling, S. Penman, M. S. Campo and J. O. Bishop, manuscript in preparation). Accordingly, the overall complexity of the non-repetitive sequences in the mRNA is about 1.8×10^{10} daltons. The analytical complexity of mammalian DNA is 1.8×10^{12} daltons, and of this about 70% or 1.3×10^{13} daltons is non-repetitive. Thus, the cDNA hybridisation kinetic data predict that 1.4% of 'single-copy' HeLa cell DNA is complementary to our mRNA preparation.

Sheared, purified single-copy DNA, highly labelled with ³H-thymidine, was annealed with enriched mRNA and the double-stranded fraction, containing both DNA-RNA hybrid and DNA duplex, was isolated using hydroxyapatite (HAP). This fraction was dialysed against a low-salt buffer, digested exhaustively with ribonuclease and again fractionated on HAP. This moves the DNA which was hybridised with RNA into the single-strand fraction, while leaving duplexed DNA in the double-strand fraction¹². The results of two such experiments are shown in Table 2. The average of these is 1.05%, in good agreement with prediction. In one experiment the single-copy DNA complementary to mRNA was recovered from the second HAP column and annealed with an excess of unlabelled HeLa cell DNA. Although it failed to renature completely, presumably due to degradation during the very extensive handling it experienced, this fraction clearly did not contain repetitive DNA sequences (Fig. 2).

Number of mRNA species in HeLa cells

One source of uncertainty which remains is the possibility that different mRNA species are not equally transcribed by reverse transcriptase. This is not, however, so serious

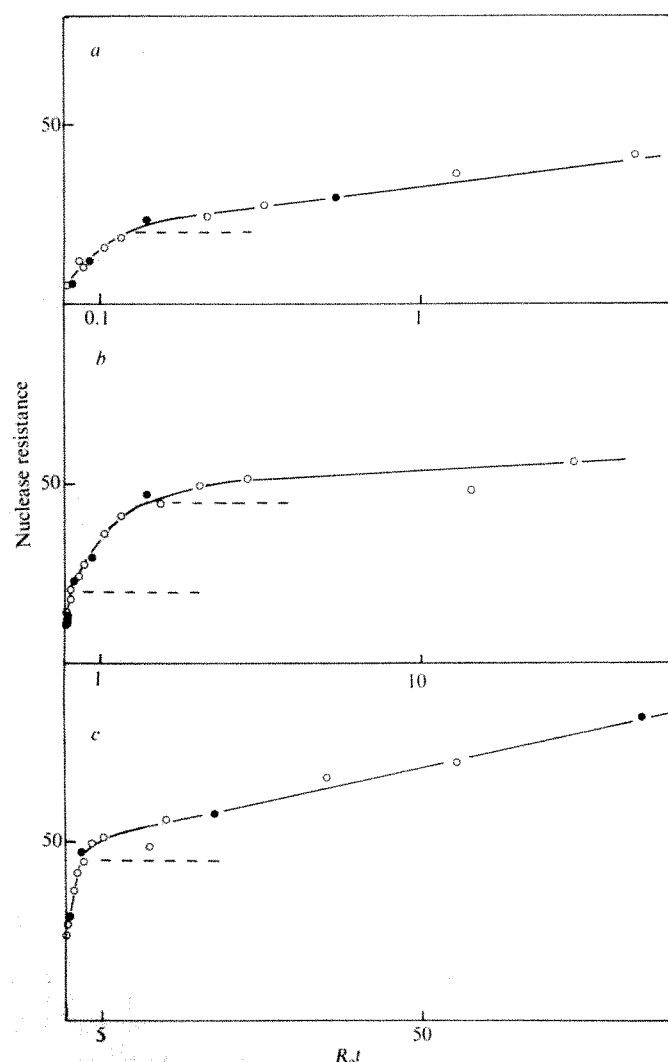


Fig. 4 Linear plots of hybridisation between cDNA and its mRNA template. The data are the same as for the linear-log plot shown in Fig. 3. Dotted lines indicate inflections between transitions.

as might be thought at first sight. Provided that the three frequency classes were transcribed with similar efficiency, even though that efficiency were low, the $R_{ot}^{1/2}$ values would be approximately correct, since they depend on the overall mRNA concentration. If an entire frequency class of mRNA molecules were not transcribed, and if this were the lowest frequency class (in terms of mRNA molecules per cell) the cDNA data would be misleading. This possibility is, however, extremely unlikely. A lower frequency class of RNA molecules which formed a significant proportion of the total would necessarily be complementary to a large part of the single-copy DNA. The good agreement between the kinetic data and the DNA saturation experiments virtually excludes the possibility that such a fraction exists.

Recently, Grady and Campbell³⁷ found that 8.5% of mouse single-copy DNA would form duplexes with total RNA from a mouse cell line, while 15% formed duplexes with RNA from the same line transformed with polyoma. Using a double-reciprocal method to extrapolate the reactions to completion, they estimated that the respective RNA preparations were complementary to about 20% and 30% of the single-copy DNA. If most of the complexity of total cellular RNA is due to HnRNA, the difference between their values and ours is compatible with current ideas on the processing of HnRNA³⁸.

We cannot be sure that all of the poly(A)-containing sequences we have studied are in fact mRNA sequences, although the literature on the subject points strongly in this direction. Assuming that they are, the number 35,000 is much greater than the number of 'genes' estimated by genetic means to be present in the genome of *Drosophila* (for example refs 39–41) and on the same order as the number of protein-encoding sequences estimated by Ohta and Kimura⁴² to be present in the human genome. As far as the human situation is concerned, we may consider three alternative possibilities. (1) HeLa cells may be completely or almost completely derepressed, expressing most of the potential of the human genome. (2) The total potential of the genome may be much greater than suspected, and HeLa cells express only a minor part of it. (3) Human cells have a large number of common functions, and differences between them are determined by relatively few mRNA species: HeLa cells therefore contain the common sequences as a large proportion of their total mRNA population. The answer to these questions will no doubt come from further work of a similar nature with HnRNA and mRNA from cell lines and from tissues.

The different frequency classes of mRNA offer interesting grounds for speculation. In this laboratory, M. S. Campo has obtained evidence which strongly suggests that a small proportion of rat myoblast mRNA contains a high proportion of repetitive sequences. Some of the mRNA

in the higher abundance (molecules per cell) classes may therefore be transcribed from repetitive genes, comparable to histone genes^{43,44}. In HeLa cells, two mRNA populations with different half lives have been described^{45,46}. The more abundant classes of mRNA discovered here may belong to the longer-lived class of mRNA. Lastly, as to function, there are obvious candidates in the protein structure of the cell for more abundant mRNA species, such as actin, tubulin and fibrin, which are present in large amounts in exponentially growing cells, and therefore are very probably synthesised from relatively abundant species of mRNA.

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By applying the same techniques to a *Drosophila melanogaster* cell line (Schneider line 3) we find that it contains a much smaller number of cytoplasmic poly(A)-containing RNA sequences, of the order of 4,000 (M. Izquierdo and J. O. Bishop, unpublished experiments).

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- ¹ Darnell, J. E., Wall, R., and Tushinski, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1321–1325 (1971).
- ² Lee, S. Y., Mendecki, J., and Brawerman, G., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1331–1335 (1971).
- ³ Edmonds, M., Vaughan, M. H., and Nakazato, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1336–1340 (1971).
- ⁴ Aviv, H., and Leder, P., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1408–1412 (1972).
- ⁵ Kates, J., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 743–752 (1970).
- ⁶ Britten, R. J., and Kohne, D. E., *Science*, **161**, 529–540 (1968).
- ⁷ Hahn, W. E., and Laird, C. D., *Science*, **173**, 158–161 (1971).
- ⁸ Davidson, E. H., and Hough, B. R., *J. molec. Biol.*, **56**, 491–506 (1971).
- ⁹ Brown, I. R., and Church, R. B., *Biochem. biophys. Res. Commun.*, **42**, 850–856 (1971).
- ¹⁰ Grouse, L., Chilton, M. D., and McCarthy, B. J., *Biochemistry*, **11**, 798–805 (1972).
- ¹¹ Gelderman, A. H., Rake, A. V., and Britten, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 172–176 (1971).
- ¹² Kohne, D. E., *Biophys. J.*, **8**, 1104–1121 (1968).
- ¹³ Ross, J., Aviv, H., Scolnick, E., and Leder, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 264–268 (1972).
- ¹⁴ Verma, I. M., Temple, G. F., Fan, H., and Baltimore, D., *Nature new Biol.*, **235**, 163–167 (1972).
- ¹⁵ Kacian, D. L., Spiegelman, S., Bank, A., Terada, M., Metafora, S., Dow, L., and Marks, P. A., *Nature new Biol.*, **235**, 167–169 (1972).
- ¹⁶ Laird, C. D., and McCarthy, B. J., *Genetics*, **60**, 303–312 (1968).
- ¹⁷ Bishop, J. O., *Biochem. J.*, **113**, 805–811 (1969).
- ¹⁸ Greenberg, J. R., and Perry, R. P., *J. molec. Biol.*, **72**, 91–98 (1972).
- ¹⁹ Adesnik, M., Salditt, M., Thomas, W., and Darnell, J. E., *J. molec. Biol.*, **71**, 21–30 (1972).
- ²⁰ Gillespie, D., Marshall, S., and Gallo, R. P., *Nature new Biol.*, **236**, 227–231 (1972).
- ²¹ Bishop, J. O., Rosbash, M., and Evans, D., *J. molec. Biol.*, **85**, 75–86 (1974).
- ²² Sheiness, D., and Darnell, J. E., *Nature new Biol.*, **241**, 265–268 (1973).
- ²³ Gierer, A., *Z. Naturforsch.*, **13**, 788–793 (1958).
- ²⁴ Spirin, A., *Prog. Nucl. Acid Res.*, **1**, 301–332 (1963).
- ²⁵ Penman, S., *J. molec. Biol.*, **17**, 117–128 (1966).
- ²⁶ Spradling, A., Penman, S., Campo, M. S., and Bishop, J. O., *Cell* (in the press).
- ²⁷ Parish, J. H., and Kirby, K. S., *Biochim. biophys. Acta*, **129**, 554–562 (1966).
- ²⁸ Bishop, J. O., and Rosbash, M., *Nature new Biol.*, **241**, 204–207 (1973).
- ²⁹ Kacian, D. L., Watson, K. F., Burny, A., and Spiegelman, S., *Biochim. biophys. Acta*, **246**, 365–373 (1971).
- ³⁰ Bishop, J. O., and Pemberton, R., in *Biochemistry of Cell Differentiation* (edit. by Monroy, A., and Tsanev, R.), 23–39 (Academic Press, London, 1972).
- ³¹ Bishop, J. O., *Biochem. J.*, **126**, 171–185 (1972).
- ³² Sutton, W. D., *Biochim. biophys. Acta*, **240**, 522–531 (1971).
- ³³ Bishop, J. O., *Acta endocr., suppl.*, **161**, 247–276 (1972).

Table 2 Complementarity between nonrepetitive HeLa cell DNA and enriched mRNA

Exp.	R_{ot} (mRNA)	C_{ot} (DNA)	Percentage of total DNA recovered	
			Duplex fraction (First fractionation)	Single-strand fraction (Second fractionation)
1	335	8	4.5	1.2
2	360	8	5.2	0.9

Nonrepetitive HeLa cell DNA was prepared by annealing highly labelled (150,000 c.p.m. μg^{-1}) sheared DNA to a C_{ot} of 10 mol s^{-1} and fractionating by means of hydroxyapatite (HAP)³⁶. Aliquots were annealed with mRNA at 70° C in 0.24 M PEB, diluted to 0.02 M PB and adsorbed to HAP. The duplex fraction was eluted between 0.12 and 0.4 M PB. This was dialysed exhaustively against 10 mM tris, pH 7.5, and then digested for 2 h at 37° C with 20 $\mu\text{g ml}^{-1}$ of RNase. The sample was then adjusted to 0.02 M PB and the fractionation was repeated. The single-stranded fraction was eluted between 0.02 and 0.12 M PB.

- ³⁴ Gaskill, P., and Kabat, D., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 72-75 (1971).
³⁵ Gould, H. J., and Hamlyn, P. H., *FEBS Lett.*, **30**, 301-304 (1973).
³⁶ Miyazawa, Y., and Thomas, C. A., *J. molec. Biol.*, **11**, 223-237 (1965).
³⁷ Grady, L. J., and Campbell, W. P., *Nature new Biol.*, **243**, 195-198 (1973).
³⁸ Scherrer, K., and Marcaud, L., *Bull. Soc. chim. Biol.*, **47**, 1697-1713 (1965).
³⁹ Demerec, M., *Cold Spring Harb. Symp. quant. Biol.*, **2**, 110-115 (1934).

- ⁴⁰ Herskowitz, I. H., *Am. Nat.*, **84**, 255-260 (1950).
⁴¹ Judd, B. H., Shen, M. W., and Kaufman, T. C., *Genetics*, **71**, 139-156 (1972).
⁴² Ohta, T., and Kimura, M., *Nature*, **233**, 118-119 (1971).
⁴³ Weinberg, E. S., Birnstiel, M. L., Purdom, I. R., and Williamson, R., *Nature*, **240**, 225-228 (1972).
⁴⁴ McCarthy, B. J., and Farquhar, M. N., *Brookhaven Symp. Biol.*, **23**, 1-17 (1972).
⁴⁵ Singer, R. H., and Penman, S., *J. molec. Biol.*, **78**, 321-334 (1973).
⁴⁶ Greenberg, J., *Nature*, **240**, 102-104 (1972).

LETTERS TO NATURE

PHYSICAL SCIENCES

Ultraviolet spectra of Capella

IN December 1973, during observations from the space observatory Orion 2 on Soyuz 13, three spectrograms of Capella (α Aur, GO III, $m_v=0.2$) were obtained in the wavelength region 2,000-3,000 Å. The spectrograms were made by a wide-angle meniscus telescope of the Cassesgrain system, with an aperture diameter of 240 mm, an equivalent focal length of 1,000 mm, and a 4-grade quartz prism objective. The dispersion of the spectrograph was 170, 280 and 550 Å mm⁻¹, at wavelengths of 2,000, 2,500 and 3,000 Å, respectively.

Spectrograms of the Capella were obtained from exposure times of 15 s (film F-19), 1.5 min (F-20), and 18 min (F-21) (Fig. 1). Even the spectrogram with an

exposure time of 15 s at wavelengths of 2,700-5,000 Å was overexposed. Increasing the exposure time resulted in an extension of the photographic background on the film at the expense of faint stars. This accounts for a regular diminution of the mean photometric amplitude between the background of the film and the darkness, passing from the F-19 record over to the F-21 record.

At spectral resolutions of 8, 14 and 28 Å at wavelengths of 2,000, 2,500, and 3,000 Å, respectively, the Orion 2 spectrograms were originally to be used only for a study of the continuous spectra of stars. Often the quality of the spectrograms, however, proved so satisfactory that stronger

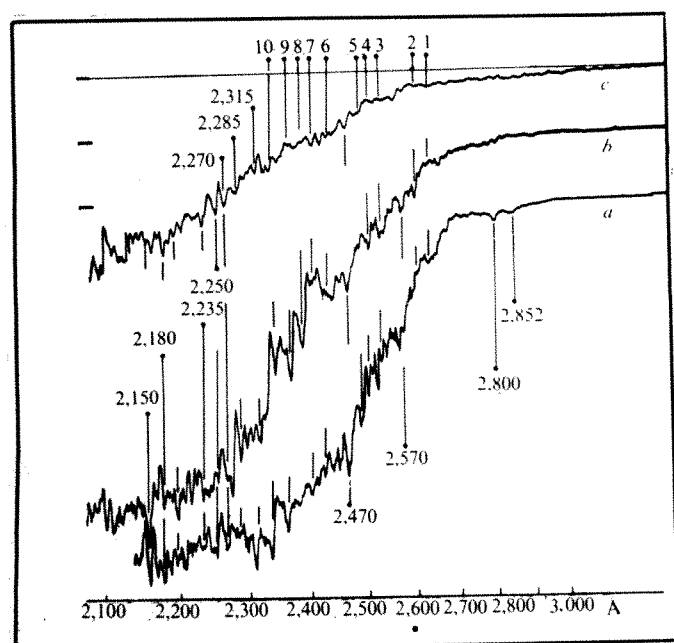


Fig. 1 Microphotometric records of the three ultraviolet spectrograms: a, 15s (F-19 film); b, 1.5 min (F-20 film); c, 18 min (F-21 film). All wavelengths identified in Table 1. 1-10 as in Table 2.

Table 1 Ultraviolet absorption lines in the spectra of Capella

Observed length (Å)	Proposed identification		
2,852	2,851.6 MgI	2,852.2 MgI	2,851.8 FeI
2,800	2,795.5 MgII	2,802.7 MgII	
2,570	2,570.8 FeII	2,571.0 TiII	
2,470	2,470.7 FeII		
2,365	2,364.8 FeI	MoII	NiII
2,315	2,316.0 NiII	2,316.0 TiII	2,315.6 MoII
2,285	2,286.2 CoII	2,284.1 FeII	2,284.8 CoII
2,270	2,270.2 NiI		
2,250	2,251.2 SnI	FeII	TiII
2,235	2,236.3 CuI	MoII	FeII
2,180	2,180.5 NiII		
2,150	2,148.7 SnI	2,152.2 SnII	FeII

absorption lines or bands could be distinguished. This was the case with the spectrograms of Capella.

The continuous spectrum of Capella extends to about 2,100 Å (Fig. 1). In the interval 2,850-2,150 Å, nearly 10 absorption lines (bands) can be distinguished with certainty (Fig. 1, and Table 1). Strictly speaking, each of these 'lines' is a band resulting from the blending of contiguous absorption lines. There is some doubt about the reality of line 2,150 Å, although it occurs with enough certainty, simultaneously on F-19 and F-20 film.

Moreover, about 10 absorption lines can be marked out less confidently (Fig. 1 and Table 2). In fact, however, the true number of measurable absorption lines exceeds those indicated in both Tables, especially in the region shorter

Table 2 Ultraviolet lines in the spectra of Capella

No. of line in Fig. 1	Observed length (Å)	Proposed identification		
1	2,640	TiII	FeII	
2	2,610	FeII	NiII	
3	2,540	FeI		
4	2,510	FeI	NiI	
5	2,490	FeI		
6	2,440	FeI	MgII	TiII
7	2,405	NiII	MoII	FeII
8	2,385	FeII	MoII	
9	2,365	MoII		CuII
10	2,345	FeII	NiII	

than 2,300 Å. In some cases even the weak line is distinctly visible in all three spectrograms (although using F-21 film) at wavelengths shorter than 2,250 Å, the short-wave tail is veiled by diffuse light and by the field from Capella itself.

The final location of such lines, however, must await a more thorough examination.

At the wavelengths under consideration (2,800–2,150 Å) the absorption lines in the spectrum of Capella pertain largely to the neutral metals and their ions, which have ionisation potentials of less than 8 eV. These are: FeII, NiII, CuII, MgII, TiII, SnII, SiII, MoII, MnII, and so on.

The 'elevation' in the continuous spectrum at 2,350–2,430 Å, is clearly seen on F-20 film, less clearly seen on F-21 film and only partly seen on F-19 film (Fig. 1). It is unlikely that the elevation results from a blending of the emission lines; rather, it simply forms part of the continuous spectrum. This must lead to the conclusion that there is a large zone of depression on both sides of the elevation, extending to nearly 2,700 Å from the long-wave side, and to 2,100 Å from the shortwave side. Future observations will show whether this conclusion is correct.

A more detailed analysis of the spectrograms will be discussed elsewhere.

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Faraday rotation studies in Africa during the solar eclipse of June 30, 1973

Four stations were set up in southern and eastern Africa (Table 1) to observe variations of total electron content during the eclipse of the Sun on June 30, 1973. Each station was equipped with a conventional Faraday rotation recording system consisting of a receiver and a mechanically rotating aerial. We recorded the amplitude fading of the 137 MHz signals from the geostationary satellite, Intelsat IIF3, located at approximately 14°W. The four stations were operating for a total period of 10 d, including June 30, the day of the eclipse.

Chimonas and Hines¹ first suggested that internal atmospheric gravity waves should be generated as a result of the Moon's shadow travelling with supersonic speed during an eclipse of the Sun. In the case of the solar eclipse of March 7, 1970 they suggested that the magnitude of the pressure perturbation would be as much as 10% at an

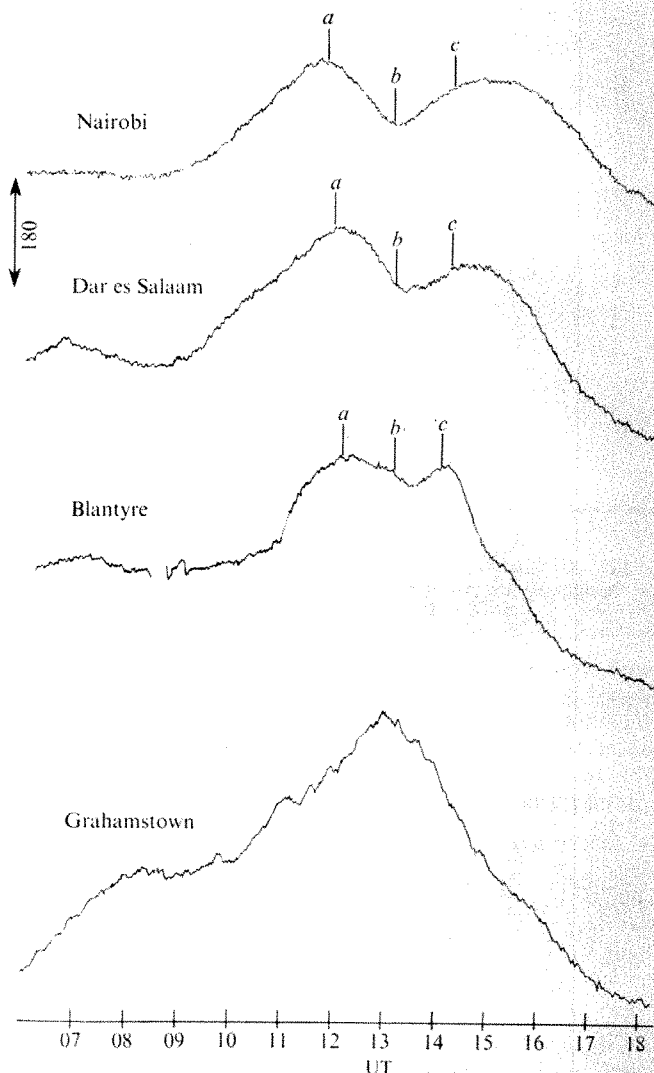


Fig. 1 The variation of the Faraday rotation angle of the 137 MHz transmission received from Intelsat IIF3 at four stations in eastern and southern Africa during the eclipse of June 30, 1973. *a*, Start of the eclipse; *b*, maximum phase of the eclipse; *c*, end of the eclipse.

altitude of 200 km. Davis and da Rosa² observed fluctuations of 1.5% in total electron content on signals from two geostationary satellites, which they tentatively associated with the eclipse. Other workers^{3,4} also observed ionospheric disturbances at times approximately appropriate for eclipse generation. Frost and Clark⁵ conclude that there is insufficient evidence to associate the observations positively with the eclipse. Beer and May⁶ computed the expected paths of bow waves generated by the solar eclipse of June 30, 1973 and concluded that the waves would come to a focus in southern Africa. It had been estimated⁷ that in the region associated with this focusing, the bow wave amplitude should be at least 10 times greater than in California in 1970.

During the 1970 eclipse the directions of the bow waves,

Table 1 Stations established in Africa during the 1973 eclipse

	Station coordinates		Coordinates of the ionospheric point	
	Latitude	Longitude	Latitude	Longitude
Nairobi	1.3° S	36.8° E	1.2° S	32.3° E
Dar es Salaam	6.8° S	39.2° E	6.3° S	34.2° E
Blantyre	15.8° S	35.0° E	14.7° S	30.4° E
Grahamstown	33.3° S	26.7° E	22.2° S	30.8° E

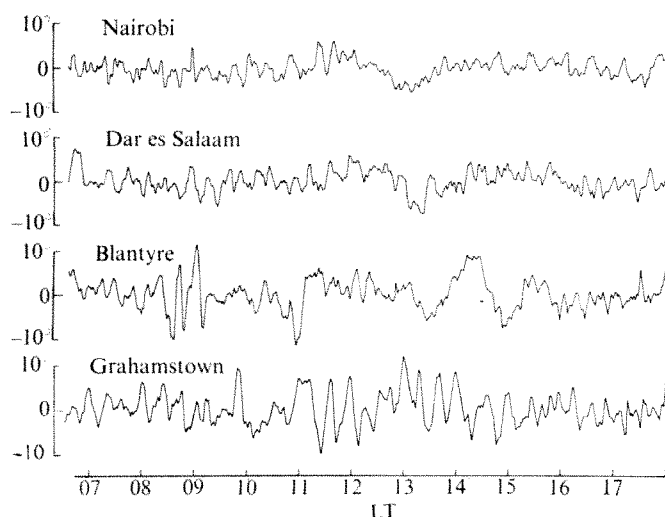


Fig. 2 The raw data after filtering to remove long period changes in the total electron content. The filtered data are shown for a band pass of 5–45 min.

with respect to the local magnetic fields and the radio signal paths, were not so favourable for the observation of travelling ionospheric disturbances induced by the eclipse as in 1973. The greater ionospheric coupling in 1973, and the long duration of the eclipse, encouraged expectations of perturbations of the total electron content of at least 15%.

The positions of the ionospheric points have been calculated for an ionospheric height of 350 km. The Faraday rotation records were read at intervals of 1 min. Totality in East Africa occurred at about 1300 UT, and the estimated time of arrival of the bow wave^{5,6} near Grahamstown was 1500–1700 UT. The expected perturbation of some 15% in the total electron content at Grahamstown could easily have been recorded. The observations, however, show no fluctuations identifiable with the eclipse at any of the four stations (Fig. 1). These results agree with the observations of Schödel *et al.*⁷

The raw data have been filtered to reduce noise and to remove the long period changes (mainly diurnal) in the total electron content (Fig. 2). The results show that travelling ionospheric disturbances with amplitudes of 3% were frequent at Grahamstown; there was, however, no way of associating perturbations on the records with the eclipse at any of the four stations. We conclude that if there were any travelling ionospheric disturbances, induced by the eclipse, they produced variations of less than 1% in the total electron content. Thus, it seems that the disturbances were very much smaller than had been anticipated.

The failure to observe eclipse induced fluctuations of the total electron content under such apparently favourable conditions must raise doubts about the validity of the theories which predict them, and about the interpretation of previous observations. In this connection, we draw attention to the suggestion of Beer^{6,8} concerning the production of travelling ionospheric disturbances by the terminator travelling at supersonic speed. Titheridge⁹ rarely observed travelling ionospheric disturbances associated with sunrise and sunset.

The times of maximum eclipse and of the beginning and end of the eclipse have been computed by the Nautical Almanac Office for a height of 350 km at the ionospheric points corresponding to the stations at Nairobi, Dar es Salaam and Blantyre. The time delay between the local maximum of the eclipse and the subsequent minimum in the electron content was about 5 min at Nairobi—much shorter than expected. At Dar es Salaam and Blantyre it was around 20 min, which agrees with earlier observations

elsewhere. Klobuchar *et al.*¹⁰ also observed a delay of only 5 min between the minimum in the electron content and the local time of maximum solar observation at Akjoujt (20.1°N, 14.0°W) during the 1973 eclipse. Nairobi and Akjoujt are situated at about 10° on either side of the geomeric dip equator in Africa.

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¹ Chimonas, G., and Hines, C. O., *J. geophys. Res.*, **75**, 875 (1970).

² Davis, M. J., and da Rosa, A. V., *Nature*, **226**, 1123 (1970).

³ Lerfeld, G. M., Jurgens, R. B., Vesecby, J. F., and Washburn, T. W., *J. atmos. terr. Phys.*, **34**, 727 (1972).

⁴ Sears, R. D., *J. atmos. terr. Phys.*, **34**, 733 (1972).

⁵ Frost, A. D., and Clark, R. R., *J. geophys. Res.*, **78**, 3995 (1973).

⁶ Beer, T., and May, A. N., *Nature*, **240**, 30 (1972).

⁷ Schödel, J. P., Klostermeyer, J., and Röttger, J., *Nature*, **245**, 87 (1973).

⁸ Beer, T., *Nature*, **242**, 34 (1973).

⁹ Titheridge, T. E., *J. geophys. Res.*, **73**, 243 (1968).

¹⁰ Klobuchar, J. A., Taves, E. H., and Menzel, D. H., *Joint Satellite Studies Group Report No. 5* (Lannion), 89 (1974).

Liquid immiscibility between silicate and carbonate melts in naturally occurring ijolite magma

PORTIONS of the fluids and melts responsible for the crystallisation of rocks and minerals may be trapped and preserved as small inclusions within a crystal, providing much information about the physical and chemical conditions prevailing during crystallisation^{1,2}.

Studies of inclusions in apatites from some East African carbonatites and ijolites³ reveal that carbonate-rich and silicate-rich melts can coexist as immiscible fractions in naturally occurring ijolite magmas. The apatites come from ijolite pegmatites collected from two localities within the Usaki complex of West Kenya⁴. The distinction between primary inclusions (those formed during the actual growth of the crystal) and secondary inclusions (those formed after growth has terminated) was immediately apparent since the primary inclusions characteristically occur as long (up to 150 μm) tubular cavities parallel with the *c* axis of the apatite crystal. Only rarely were small, curved planes of minute (<10 μm) secondary inclusions observed.

Four different primary inclusion types were recognised in the apatites from ijolite pegmatite (U366);

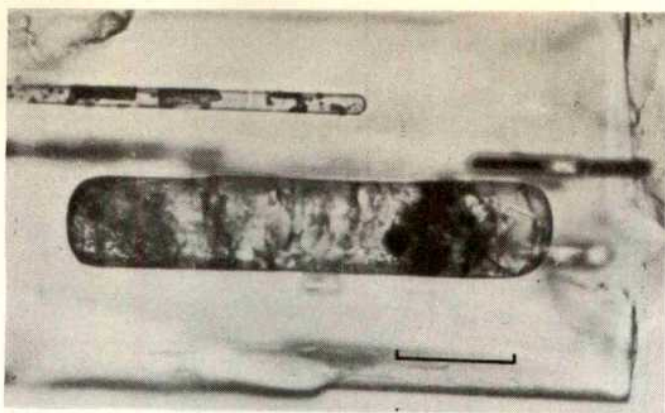


Fig. 1 Large, tubular, multisolid, carbonate-rich inclusion (type A) in apatite from ijolite U366. Most of the colourless, crystalline material within the inclusion exhibit high order interference colours. On the right of the inclusion, several small, black, magnetic, crystalline phases are apparent. Bar = 20 μm .

- (1) Silicate melt inclusions consisting of minute, crystalline specks in a glassy matrix ($n \sim 1.55$) but no vapour bubble.
- (2) Carbonate-rich melt inclusions which consist of at least 60% anisotropic, colourless, crystalline material (Fig. 1) and usually a small amount of aqueous fluid and vapour. This colourless material reacted instantly, dissolving with rapid effervescence, when the inclusions were opened in dilute, acidified (HCl) glycerol on a microscope crushing stage of the type described by Roedder⁵. Most of this material, however, remained insoluble in pure deionised water. This behaviour in acid media and the high order interference colours of these crystals indicate that they are carbonate-bearing minerals. Small crystals of a black, opaque, magnetic ore mineral are also commonly present.
- (3) Nahcolite-bearing inclusions which represent a trapped aqueous fluid phase. These inclusions contain variable amounts of Nahcolite (NaHCO_3 , ref. 6) aqueous fluid, and a CO_2 -rich vapour bubble.
- (4) Gaseous inclusions representative of a vapour phase. These consist almost entirely of highly compressed (sometimes liquefied) carbon dioxide.

In many instances, all four inclusion types occur side by side in the same apatite crystal. Since they are primary³, they represent low temperature equivalents of the trapped portions of phases present in the ijolite magma during the crystallisation of the apatites.

The apatites from ijolite pegmatite (U1256) also contain

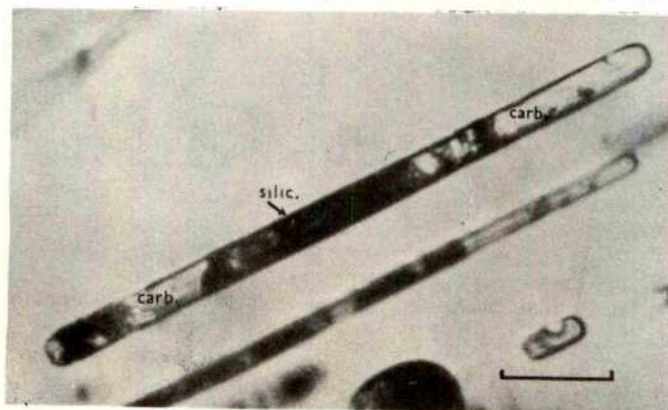


Fig. 2 Complex carbonate-rich/silicate melt inclusion in apatite from ijolite U1256. This inclusion shows a dark, central patch of brown, green and black crystalline phases; predominantly silicate minerals (silic.). The colourless solids are principally carbonate-bearing phases (carb.). Bar = 20 μm .

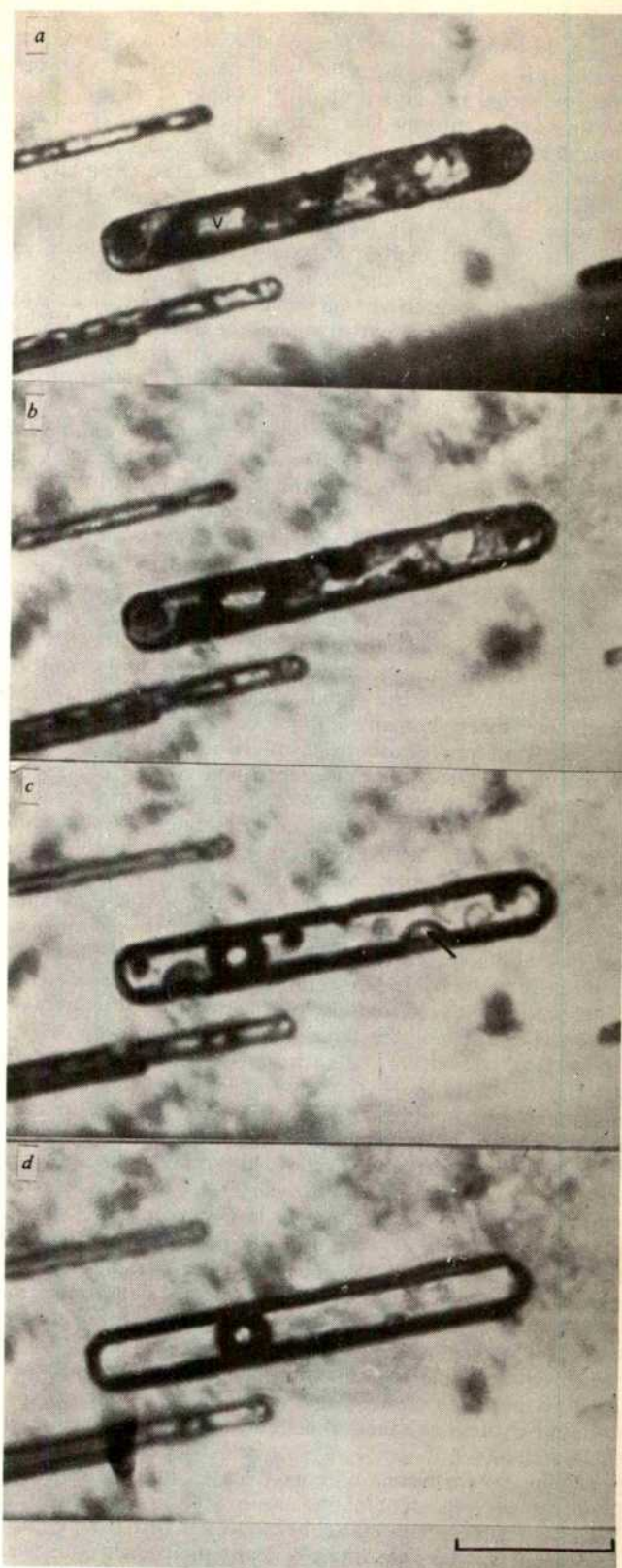


Fig. 3 The behaviour of the contents of a complex, multi-solid carbonate-rich/silicate melt inclusions on heating (type B from U1256). Bar = 20 μm . a, At 23° C, the inclusion as seen at room temperature. Note the presence of a large vapour bubble (V); b, At 565° C, melting of the carbonate phase begins; c, At 890° C, all the carbonates have melted to a colourless liquid. Small globules of a clear green liquid (one of which is arrowed) have also formed from the dark silicate minerals. Thus, two immiscible liquids are present in the inclusion at this temperature; d, At 960° C, complete homogenisation of the carbonate-rich melt and silicate melt has taken place. The vapour bubble, although somewhat smaller in volume, still remains.

silicate inclusions (glassy matrix, $n \sim 1.56$) and carbonate-rich inclusions, but very few of the aqueous, nahcolite-bearing or gaseous inclusion-types. A further type has, however, been recognised in this sample (Fig. 2). These inclusions are intermediate between carbonate melt inclusions and silicate melt inclusions, and consist of various proportions of both silicate and carbonate-rich material. Whereas the individual silicate inclusions and carbonate inclusions represent the separate trapped portions of two distinctly different melts, the complex silico-carbonate inclusions result from the simultaneous entrapping of a portion of carbonate-rich melt and silicate-rich melt. This demonstrates that these two melts coexisted in the ijolite magma as separate phases at the time the apatites crystallised. Heating studies were made on the inclusions using a Leitz 1350 microscope heating stage. The details will be published elsewhere but it is interesting to note the behaviour of the complex silicate/carbonate-rich melt inclusions as they are heated and cooled. Two immiscible liquids were observed at elevated temperatures (Fig. 3c).

When simple carbonate-rich melt inclusions (here called type A) from both samples were heated (U366 and U1256), their crystalline, carbonate-bearing mineral phases began to melt at about 500° C and were almost entirely molten at temperatures between 640° and 750° C. At these temperatures, only a small amount of solid material, including the small, black, opaque, magnetic, crystalline speck, remained together with a small vapour bubble. These phases usually dissolved in the melt at higher temperatures. On cooling, the inclusion contents recrystallised completely and even rapid cooling failed to produce a glass. When the complex silico-carbonate-rich inclusions from U1256 were heated (Fig. 3), however, the colourless, carbonate-rich fraction melted at temperatures between 575° and 640° C but the silicate fraction, seen as small optically isotropic and anisotropic, green, brown and black patches within the inclusion, did not dissolve completely in the resulting melt. Instead, they melted independently to form pale green coloured globules at temperatures between 820° and 900° C. Where these globules occupied up to 20% of the total volume of the inclusions (type B), they gradually dissolved in the carbonate-rich melt at temperatures ranging from about 950° C up to 1,100° C. But in those instances where the globules occupied a volume greater than about 20% (type C), they still remained, coexisting with the carbonate-rich melt, even at 1,100° C (the maximum temperature attainable on the apparatus).

When inclusions of type B were cooled from the temperature at which the two melts had become miscible, the molten contents separated into two distinct immiscible liquid phases; green silicate globules and a carbonate-rich melt (Fig. 4). Continued cooling caused a small portion of the silicate globules to crystallise but most quenched to a pale green-coloured, isotropic glass. The colourless, carbonate-rich melt crystallised at temperatures between about 575° and 500° C.

Cooling of inclusions of type C gave similar results and some of the silicate globules were seen to coalesce into larger globules which subsequently quenched to a glass.

Heating studies on simple silicate melt inclusions were generally unsuccessful owing to leakage. When two small inclusions were heated to 950° C, then cooled, their contents were, however, seen under oil immersion to have quenched to the same pale green-coloured, isotropic glass as the green globules in the complex inclusions.

The exact composition of the two immiscible melts is unknown, but one is certainly a silicate-rich melt and the other carbonate-rich. Other phases such as CO₂ vapour and an aqueous, saline, carbonate-bearing fluid (seen as nahcolite-bearing aqueous inclusions) coexisted with these two melts in the ijolite magma.

Several authors⁷⁻⁹ have suggested that immiscibility may

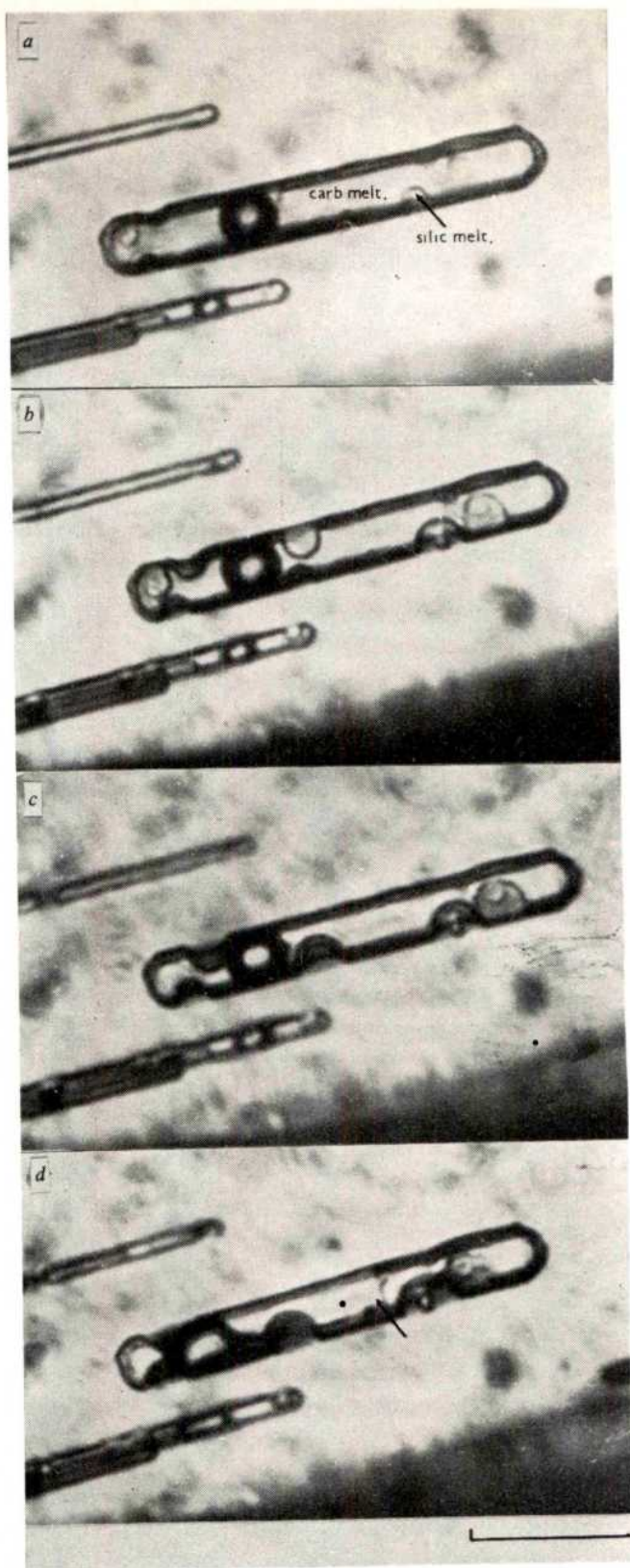


Fig. 4 The behaviour of the contents of the inclusion shown in Fig. 3d, as it is cooled from 960° C. Bar = 20 μ m. a, At 950° C, initial separation (unmixing) of the homogeneous melt takes place. Silicate melt globules (one of which is arrowed) form, leaving a predominantly carbonate-rich melt; b, At 910° C, the silicate globules grow in size and unmixing of the two melts is almost complete; c, At 805° C, some of the silicate globules have coalesced, others begin to crystallise; d, At 575° C, the onset of crystallisation of the carbonate-rich melt (arrowed). The silicate globules have already crystallised or quenched to a glass.

be an important process in the derivation of carbonatitic fluids from parent carbonated silicate magmas. Experimental studies by Koster van Groos and Wyllie¹⁰⁻¹² on selected joins in the system $\text{CaO}-\text{Na}_2\text{O}-\text{Al}_2\text{O}_3-\text{SiO}_2-\text{CO}_2-\text{H}_2\text{O}$ at higher temperatures have confirmed the existence of immiscibility between carbonate-rich and silicate-rich melts in soda-rich synthetic systems, though it does not appear in more lime-rich systems¹³. Since the inclusions studied here represent trapped portions of a natural magmatic system, it is evident that immiscibility between carbonate-rich melts or fluids, and silicate melts can take place in natural ijolitic magmas. These data may be interpreted to show two different possible relationships between carbonatitic and ijolitic melts. First, a parental ijolite magma can, by immiscibility differentiation, produce carbonatite and silicate (?nepheline-syenite) partner magmas; or second, a hyperalkaline silicate parent magma can produce immiscible carbonatite and ijolite partner magmas. The former provides the simplest solution but we believe the field evidence indicates the latter.

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¹ Roedder, E., *U.S.G.S. prof. Pap.*, 440-JJ (1972).

² Roedder, E., in *Geochemistry of Hydrothermal Ore Deposits* (edit. by Barnes, H. L.), 515 (Holt, Rinehart, New York, 1967).

³ Rankin, A. H., *thesis*, Univ. Leicester (1973).

⁴ King, B. C., Le Bas, M. J., and Sutherland, D. S., *J. geol. Soc. Lond.*, **128**, 173 (1972).

⁵ Roedder, E., *Schweizer. miner. petrogr. Mitt.*, **50**, 41 (1970).

⁶ Rankin, A. H., and Le Bas, M. J., *Miner. Mag.* (in the press).

⁷ von Eckermann, H., in *Ultramafic and related rocks* (edit. by Wyllie, P. J.), 302 (Wiley, New York, 1967).

⁸ Ferguson, J., and Currie, K. L., *J. Petrology*, **12**, 561 (1971).

⁹ Barth, T. F. W., and Ramberg, I. B., in *Carbonatites* (edit. by Tuttle, O. F., and Gittins, J.), 254 (Interscience, New York, 1966).

¹⁰ Koster van Groos, A. F., and Wyllie, P. J., *Am. J. Sci.*, **264**, 234 (1966).

¹¹ Koster van Groos, A. F., and Wyllie, P. J., *Am. J. Sci.*, **266**, 432 (1968).

¹² Koster van Groos, A. F., and Wyllie, P. J., *Am. J. Sci.*, **273**, 465 (1973).

¹³ Watkinson, D. H., and Wyllie, P. J., *J. Petrology*, **12**, 357 (1971).

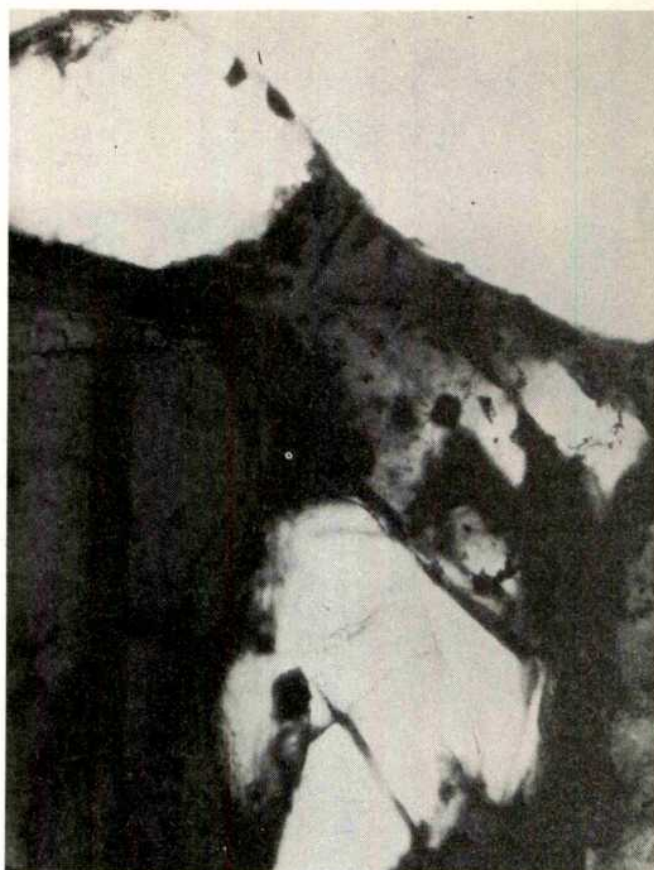


Fig. 1 Transmitted light photograph ($\times 96$) of glass-kaersutite contacts from nodule 1718, Volcan de Tenequia. Kaersutite showing rounded margins is on the left, clinopyroxene is on the right. The glass contains a few microlites and minor olivine. The white areas are gas vesicles.

Nodules containing kaersutite, from the Volcan de Tenequia eruption of 1971, were collected on the southern tip of La Palma, Canary Islands. The wehrlite nodules are seldom greater than 10 cm in the largest dimension, are generally ovoid in shape, and consist of olivine (Fo_{80}), clinopyroxene, opaques and kaersutite. The most notable feature of the nodules is the presence of glass in amounts of 1–5% in several of the nodules. The glass is invariably associated with kaersutite and is highly vesiculated. Petrographic examinations show that the glass is not contamination from associated lavas. The glass-kaersutite relationships are shown in Figs 1 and 2. Melting of kaersutite is indicated clearly by both the rounded edges of the amphibole (Fig. 1) and the cusped shape of kaersutite margins in contact with the glass (Fig. 2). Occasionally, glass is present along olivine-kaersutite margins. Clinopyroxene shows no evidence of involvement in the melting event. Olivine (Fo_{80}) and very small amounts of clinopyroxene have crystallised from the melt, and are clearly distinguishable from the cumulate phases, both in composition and appearance (Fig. 2). The melting seems to be congruent.

Compositions of kaersutite and glass were determined by microprobe analysis of two separate nodules (Table 1). Compared with kaersutite, the glass is enriched in alkalis and alumina, is slightly enriched in silica, and is strongly depleted in magnesia. Iron, titanium, and calcium values are comparable. The compositional differences can only be attributed to the crystallisation of olivine and pyroxene from the melt.

We propose that the melting relationships shown here represent a model for the production of alkali basaltic magma through the melting of kaersutitic amphibole at depth. An important factor in this proposal is the striking similarity in compositions between kaersutites and alkali basalts. This is particularly true when comparing kaersutites to ankaramitic

Kaersutite is a possible source of alkali olivine basalts

STUDIES into the origin of basaltic magmas have led to the belief that the upper mantle contains a hydrous phase, either phlogopite or amphibole, as a source of water and potassium¹⁻³. Here we present data concerning the role of kaersutite, a possible upper mantle phase, in the genesis of alkali basalts. Specifically, we have observed the melting of kaersutite within ultramafic nodules from La Palma. Both the kaersutite and the glass produced through melting show strong compositional similarities to members of the alkali olivine basalt series. The observed relationships strongly indicate that alkali basalts may be derived from a melting event in which kaersutite is the principal participant.

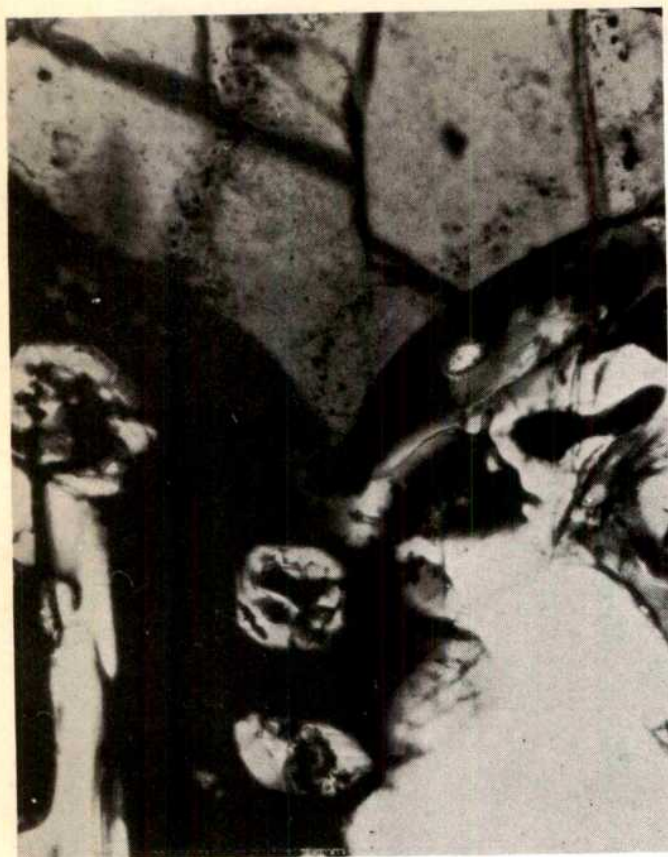


Fig. 2 Transmitted light photograph ($\times 96$) of kaersutite (top), with a cusped shaped margin in contact with glass. A large olivine crystal with a glass inclusion at its centre is at the left. The small equant grains are clinopyroxene. The white area (lower right) is a gas vesicle.

rocks (Table 1) which may represent a parental magma for the alkali basalt series. Ankaramites are characterised by a relatively high K_2O content of $<1-2\%$ with a SiO_2 content of $41-44\%$. Kaersutites, usually noted for a high TiO_2 content, typically have K_2O abundances of $1-2\%$, a value substantially higher than in other aluminous amphiboles.

The observed differences in alumina, magnesia and alkali contents between kaersutite and the associated glass, lie within the variation of rock chemistry shown in the alkali basalt series. Differentiation trends are marked by a rapid decrease in MgO ,

Table 1 Chemical compositions of kaersutite and glass

	*1	2	3	4	5	6	7	8
SiO_2	42.2	44.4	41.4	43.3	43.26	43.2	42.53	43.30
TiO_2	5.25	3.95	3.85	4.14	3.71	3.4	4.78	4.02
Al_2O_3	13.4	15.6	10.3	16.6	13.68	9.69	16.61	16.95
Fe_2O_3	—	—	—	—	3.92	3.66	3.80	3.62
FeO	10.0 ^a	10.8	10.6	10.25	9.39	8.97	7.84	7.70
MnO	0.12	0.18	0.17	0.09	0.22	0.16	0.13	0.06
MgO	11.4	4.05	14.6	4.53	9.22	12.64	5.56	5.72
CaO	12.2	9.61	11.4	10.8	10.28	12.10	10.03	10.49
Na_2O	3.07	4.74	2.93	5.42	3.60	1.59	3.85	4.74
K_2O	1.23	2.77	1.12	2.08	1.47	1.18	1.96	2.34
H_2O	—	—	—	—	—	1.79	1.27	—
Totals	98.87	96.10	96.37	97.21	98.75	99.72 ^b	99.71 ^c	98.94

*1, Kaersutite from xenolith in ash, Volcan de Tenequia, La Palma (No. 1718); 2, glass from xenolith No. 1718; 3, kaersutite, cumulate phase, xenolith from Volcan de Tenequia, La Palma (No. 1719); 4, glass from xenolith (No. 1719); 5, ankaramite, La Palma¹⁰; 6, ankaramite, Vallee de Paparoo, Tahiti-nui¹¹; 7, basanite, Vaitepiha River, Taurapu, Tahiti¹²; 8, basanite, Las Manchas, La Palma¹²; a, total Fe determined as FeO ; b, analysis total includes $0.67 H_2O$ — and $0.61 P_2O_5$; c, analysis total includes $0.45 H_2O$ — and $0.90 P_2O_5$.

with increases in Al_2O_3 and total alkalis at essentially constant levels of SiO_2 , TiO_2 , FeO and CaO at least to the basanite stage. The glass compositions closely approximate those of basanites (Table 1). As the glass composition is apparently controlled by the crystallisation of olivine, basalt compositions between ankaramites and basanites could easily be produced by fractional crystallisation of this mineral.

There is no evidence that the observed glass-kaersutite relationship actually represents melting of a hydrous phase at depth. Glass has been observed previously in ultramafic inclusions, and has been attributed to incipient fusion of the xenolith immediately before eruption^{4,5}. Similarly, the composition of the cumulate olivine does not indicate a mantle origin. Although the observed melting may, however, have occurred at shallow depths, it is reasonably certain that, compared with likely mantle assemblages, kaersutite would remain the mineral with the lowest melting temperature at depth (ref. 2).

The origin of kaersutites in ultramafic nodules has been generally attributed to the crystallisation of this mineral from alkali basalt magmas under hydrous conditions. It is entirely possible, however, that kaersutite is formed as a product of tholeiitic magmatism as well. Helz⁶ has shown that at $1,000^\circ C$ and $5 K_b PH_2O$, amphibole crystallising from a tholeiitic liquid contains 4.27% of TiO_2 and is comparable in composition to most reported kaersutites. Thus, a widespread kaersutite occurrence may result from the crystallisation and accumulation of the amphibole under hydrous conditions at midoceanic ridges or any site of tholeiitic activity. Gravitational settling of kaersutite may provide a zone enriched in amphibole which could then act as a reservoir for alkali olivine basalt production. At the ridge, melting of some of the stored amphibole, at temperatures in excess of those required for the production of tholeiitic liquids, would account for late-stage alkaline activity. If accumulation has resulted from crystallisation at midoceanic ridges, the lithosphere thus produced would contain at its base a potential source for alkali olivine magma over a large geographic area. Passage of the plate over a 'hot spot' could account for oceanic volcanism at sites far removed from ridges.

Previous arguments for the involvement of amphibole in the generation of basaltic magmas have been based on compositional similarities to basalts⁷⁻⁹, the requirement for a source of potassium¹, titanium⁸, and water, and experimental evidence from whole-rock melting experiments in the presence of vapour^{2,3}. The data presented here strongly suggest that kaersutite is entirely suitable as the principal source of alkali olivine basalts.

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- Oxburgh, E. R., *Geol. Mag.*, **101**, 1 (1964).
- Lambert, I. B., and Wyllie, P. J., *Nature*, **219**, 1240 (1968).
- Green, D. H., *Trans. Leicester lit. phil. Soc.*, **64**, 1 (1970).
- White, R. W., *Contr. Miner. Petrol.*, **12**, 45 (1966).
- Vilminot, J. C., *Bull. Soc. fr. Minér. Cristallogr.*, **87**, 143 (1964).
- Helz, R. T., *J. Petrology*, **14**, 249 (1973).
- Varne, R., *J. Petrology*, **9**, 169 (1968).
- Varne, R., *Contr. Miner. Petrol.*, **27**, 45 (1970).
- Mason, B., *Mineralog. Mag.*, **36**, 997 (1968).
- Chaigneau, M., and Fuster, J.-M., *C. r. hebdom. Séanc. Acad. Sci., Paris*, **274**, 2948 (1972).
- McBirney, A. R., and Aoki, K., *Mem. geol. Soc. Am.*, **116**, 523 (1968).
- Órtiz, D. J. R., *Boln. Inst. geol. min. Esp.*, **63**, 3 (1951).

Elastic energy and plate tectonics

A GLOBAL, quadrupolar mechanical analysis of the energy stored in the Earth, based on the concept of a 'reference elastic distortion'^{1,2}, has shown that quadrupolar continental drift, and in particular the westerly polar motion could be understood as resulting from a yielding process which operates as a consequence of the elastic energy in the Earth.

Reduction of elastic energy is, however, not the only driving force available for continental drift; thermal stresses and thermal convection certainly play an important role³. Nevertheless, it is instructive to study the extent to which the elastic energy reduction principle alone can explain the finer details of continental drift. We here give a preliminary report of the calculations which have been made; a more extended account will be published elsewhere.

We have utilised various spherical harmonic analyses of the terrestrial topography⁴⁻⁶ and have taken density fluctuations into account by various degrees of quenching or total vanishing of g fluctuations. The gross features of our results were influenced very little by these effective isostatic compensations. To make things as simple as possible, we have also ignored compressibility and any radial variation of the density ρ or rigidity μ ; these, too, do not seem to alter appreciably our qualitative results, which show a significant correlation between regions of high elastic energy density and regions known to be seismically active. The predicted surface displacements are also in good agreement with accepted directions of continental drift.

The equations for self-gravitating elastic bodies⁷, have a local elastic energy density (EED) term

$$\varepsilon(\mathbf{r}) = \mu u_{ik}^2(\mathbf{r}) \quad (1)$$

which can be replaced by

$$\varepsilon(\mathbf{r}) = \mu [u_{ik}(\mathbf{r}) - u^{(0)}_{ik}(\mathbf{r})]^2 \quad (2)$$

$$u_n(\mathbf{r}) = (R/2g) \{ [(n+2)/(n-1) - ((n+3)/n) (r/R)^2] \times \nabla \Phi_n^{\text{eff}} + (2r/R^2) \Phi_n^{\text{eff}} \} \quad (3)$$

$$k_n = \{ 1 + (\mu \rho g R) / (2n^2 + 4n + 3/n) \}^{-1} \quad (4)$$

$$\Phi_n^{\text{eff}} = k_n \Phi_n + (1 - k_n) \Phi_n^{(0)} \quad (5)$$

where u_{ik} is the strain tensor, ($= \partial_k u_i + \partial_i u_k$); $u^{(0)}_{ik}$ is the 'reference' strain tensor which, for simplicity, we assume to be

derivable from a reference displacement $\mathbf{u}^{(0)}$ by $u^{(0)}_{ik} = \partial_k u_i^{(0)} + \partial_i u_k^{(0)}$. Again for simplicity, we assume that $\mathbf{u}^{(0)}$ represents the effect of an effective 'reference potential' $\Phi^{(0)}$ on the fluid body. That is, equation (2) is the elastic energy density for a self-gravitating body which froze when an external potential $\Phi^{(0)}(\mathbf{r})$ was acting on it, and is now distorted differently as a result of the replacement of $\Phi^{(0)}$ by the actual current potential Φ . Expanding all quantities in spherical harmonics, and denoting by subscript n any quantity belonging to the harmonic manifold of order n , the modified equations can be solved to obtain

$$u_n(\mathbf{r}) = R/2g \{ (n+2/n-1 - n+3/n (r/R)^2) \nabla \Phi_n^{\text{eff}} + 2r/R^2 \Phi_n^{\text{eff}} \} \quad (3)$$

where $n \geq 2$. The $n=0,1$ terms only determine the volume and centre of the initial sphere.

Where R and g are the radius and free fall surface acceleration, k_n is related to the n th order Love number⁸

$$k_n = [1 + (\mu \rho g R) (2n^2 + 4n + 3/n)]^{-1} \quad (4)$$

and

$$\Phi_n^{\text{eff}} = k_n \Phi_n + (1 - k_n) \Phi_n^{(0)} \quad (5)$$

For $\Phi_n^{(0)} = 0$, that is, for departures from spherical shape, equation (5) gives the well known results, whereas with $\Phi_n^{(0)}$ present, the body behaves as if fluid is under a potential which is a weighted average (with weight $k_n < 1$) of the reference and actual potentials.

For actual computation, it is easier to expand, in the usual way,

$$\Phi_n(\mathbf{r}) = gR(r/R)^n \sum_m \varepsilon_n^m y_n^m(\theta, \phi) \quad (6)$$

$$\Phi_n^{\text{eff}}(\mathbf{r}) = gR(r/R)^n \sum_m \eta_n^m y_n^m(\theta, \phi) \quad (7)$$

$$\Phi_n^{(0)}(\mathbf{r}) = gR(r/R)^n \sum_m \varepsilon_n^m(0) y_n^m(\theta, \phi) \quad (8)$$

and the surface radial displacement, which is the terrestrial topography, can be expanded to give

$$u_r(R, \theta, \phi) = R \sum_{n,m} u_n^m y_n^m(\theta, \phi) \quad (9)$$

Equation (5) is then transformed to give

$$\eta_n^m = k_n \varepsilon_n^m + (1 - k_n) \varepsilon_n^m(0) \quad (10)$$

and from equation (3):

$$\eta_n^m = - [2(n-1)/2n+1] u_n^m \quad (11)$$

Given η_n^m from the topography analyses and knowing ε_n^m from the actual external potentials, both $\varepsilon_n^m(0)$ and, therefore, the displacement $\mathbf{u} - \mathbf{u}(0)$, which causes the strain which determines the elastic energy density, can be calculated. Note that $\mathbf{v}_n = \mathbf{u}_n - \mathbf{u}_n(0)$ is, again, the displacement of an equivalent fluid body, acted upon by a potential $k_n(\Phi_n - \Phi_n^{(0)})$.

The variables controlling the irreversible release of elastogravitational energy are the $\varepsilon_n^m(0)$ values. In general, the total elastogravitational energy can be written, for $\varepsilon_n^m = 0$, as a sum of squares of the $\varepsilon_n^m(0)$; thus the various processes will act in such a way as to decrease the latter on the average. (Of course, over short time scales some of these could grow at the expense of others, as long as the total energy is decreasing.) The energy reduction process manifests itself in real body and surface motion, as shown by equation (9)–(11). Such a motion, which we shall tentatively consider as a form of continental drift, will depend strongly on the exact way in which the various $\varepsilon_n^m(0)$ values change. In order to try to understand this, we have assumed that the important physical processes are local, so that:

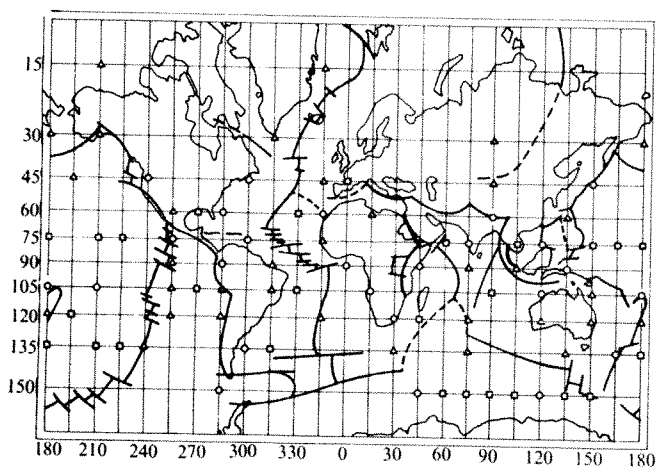


Fig. 1 The movement of the Earth's crust. The arrows represent du_r/dt as calculated from equation (13) in the special case that $\beta = 0$. A solid arrow indicates that du_r/dt is negative, corresponding to crustal sinking; a dotted arrow indicates that du_r/dt is positive, corresponding to crustal rising. Background map taken from Stacey, F. D., *Physics of the Earth*, (Wiley, New York, 1969.)

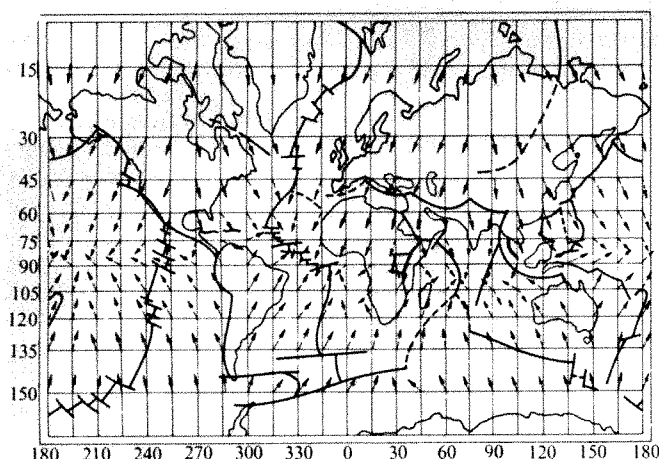


Fig. 2 A plot of EED maxima as calculated with the spherical harmonics coefficients of the Earth's topography in the presence of the Earth's rotational potential⁶. The figure is drawn for a spherical shell at 0.95 Earth radius. Triangles, latitudinal maxima (along a given latitude); boxes, longitudinal maxima (along a given longitude); circles, maxima in both directions. The background heavy lines represent known regions of seismic activity. Background map taken from Stacey, F. D., *Physics of the Earth* (Wiley, New York, 1969.)

$$d/dr[u_n(r) - u_n^{(0)}(r)] = -\alpha' [u_n(r) - u_n^{(0)}(r)] \exp[\beta \varepsilon(r)]$$

with suitable values of α' and β . This expression is equivalent to (with $\Phi_n = 0$):

$$d/dr u_n(r) = -\alpha u_n(r) \exp[\beta \varepsilon(r)] \quad (13)$$

The actual process of strain release described is considerably more complicated.)

We can apply our formulation to a simple model study—the behaviour of a self-gravitating sphere deformed by

$$\begin{aligned} u_r &= 0 & \text{for } \theta \geq \theta_0 \\ u_r &= -aR(1 - \cos(\theta - \theta_0)) & \text{for } \theta < \theta_0, \end{aligned} \quad (14)$$

where θ is the spherical latitude. This is a 'one-ocean' model of the Earth; θ_0 is the half angular width of the ocean; R the terrestrial radius; and a is a parameter specifying the depth of the ocean, chosen so that the centre of gravity of the deformed body is not too different from the undeformed body.

For this model, we have chosen θ_0 to be 60° , and examined both the EED distribution pattern and the displacement from the reference sphere (a sphere having the same mass and the same centre of gravity as that under consideration). We find that:

(1) the elastic energy is a maximum (of height 10 in arbitrary units) at the centre of the ocean, and falls smoothly to a minimum of 0.65 at 46° , rising again to a maximum of 3.2 at 64° , just off the coast; it falls smoothly again to a low of 0.06 at 118° and finally rises to a maximum of 1.16 at 180° .

(2) the radial component of the displacement vector from the reference sphere $u_r = \sum_{n \geq 2} u_{nr}$ is negative from 0° to 48° ,

positive from 50° to 112° , then negative again from 114° to 180° .

(3) the θ component of the displacement vector from the reference sphere, $u_\theta = \sum_{n \geq 2} u_{n\theta}$, is zero at both poles,

positive from 0 to 78° and negative from 80° to 180° .

We therefore infer that:

(1) it is reasonable to associate regions of maximal elastic energy density with seismically active regions. If our model ocean is regarded as a first approximation for the Pacific Ocean, then we predict seismically active regions in the centre of the ocean, (the Hawaiian Islands), the coasts, (Japan, South-east Asian coasts, the Indonesian islands, and the American Pacific coast) and opposite the ocean centre (the Middle East). These regions are, indeed, seismically active.

(2) Fault lines and plate boundaries can be induced inside the continental land mass as a result of the strain created by the ocean. If we assume, that $\tilde{u} \propto \Delta u$ on the basis of equation (13), we would expect fault lines at 50° and 114° , and a plate boundary at 80° . Moreover, the ocean tends to close in order to reduce the overall strain energy.

A second model study was devoted to the investigation of possible interference effects between two oceans, ocean A and ocean P, which are both described by expressions of the form of equation (14). Their maximum depths were chosen to have the ratio 4:7. Ocean A has a half-angular width of 25° and P has a half-width of 75° . The angle between the centres of A and P was chosen as 120° . We found that maxima in EED still exist on the coast and centre of P, and on the far side of the coast of A away from P. There is no EED maximum diametrically opposite to the centre of P because it is too close to the far side of the coast of A. This model shows that interference is quite important. Of course, this model is too rough to match the actual description of the Atlantic and Pacific Oceans.

In the first spherical harmonic analysis of the Earth's lithosphere and hydrosphere⁴, and to a certain extent, in subsequent analysis^{5,6}, the Mid-Atlantic Ridge was not prominently reproduced. As a result, we expect that our calculation of EED and displacements in the Atlantic region is less meaningful than the corresponding calculation for the Pacific Ocean. Using the various sets of spherical harmonic coefficients of the Earth's topography, we have calculated the EED distribution in various spherical shells in the Earth at distances of 1.0, 0.975, 0.95, 0.925, 0.9, 0.85, 0.8, 0.7, 0.6, 0.5, and 0.4 Earth radii for regions of 15° by 15° . We have carried out calculations both with and without the Earth's rotational potential. In each case, we have scanned for latitudinal maxima of the EED (along a given latitude) and longitudinal maxima of the EED (along a given longitude) for each spherical shell. We have associated regions of maximal EED with seismically active regions. Also, at a given angular coordinate, we searched for maxima in the function $r^2 \varepsilon(r)$ along the radius. We have associated such maxima with the 'focal points' of seismic regions. We have also calculated the displacement vectors

$$\mathbf{u} = \sum_{n \geq 2} \mathbf{u}_n \text{ and } \mathbf{u} - \mathbf{u}^{(0)} = \sum_{n \geq 2} (\mathbf{u}_n - \mathbf{u}_n^{(0)})$$

According to the simple model expressed by equation (13), the Earth's crust, at a point r , will move with a velocity proportional to the magnitude of \mathbf{u} , but oppositely directed to it. Figure 1 shows the plot of du/dr , calculated with the Prey coefficients⁴ with rotation included but without ocean waters as an external load and without isostasy, in the special case that β is 0. It clearly illustrates that oceans tend to close and that mountains tend to disperse.

Space does not permit presentation of all our EED results, which include calculations in each shell for each case considered. All shells in the region between 0.8 to 1.0 Earth radii have an EED pattern fairly close to the experimentally known distribution of seismically active regions, but not quite the same. In most cases, the shells at 0.95 and 0.925 Earth radii give best fits to observed seismic regions. Furthermore, inclusion of the Earth's rotational potential leads to closer agreement with observation. Figure 2 shows the EED maxima distribution in the 0.95 Earth radius shell, calculated with the most recent data⁶ in the presence of the rotational potential.

If only the latitudinal maxima are considered, there seems to be a better fit to the observed seismically active region. A way of understanding this is to suggest that the EED maxima, both longitudinal and latitudinal, are there. Latitudinal maxima are, however, sensitive to Coriolis forces, and so seismic activities may be more associated with latitudinal maxima than longitudinal maxima.

Along a seismic plate boundary, points on the same plate have EED maxima in the same spherical shell or in adjacent shells. In general, EED maxima for points on coastal plates lie closer to the Earth's surface than those on mid-oceanic plates.

A deformed body possesses elastic energy which is gradually released in irreversible processes. It may be possible to understand general features of seismic activity and continental drift as global phenomena related to irreversible strain releases. Although we have not reproduced EED maxima along the Mid-Atlantic Ridge, a ridge does tend to form in the middle of the Atlantic Ocean (Fig. 1). Our EED maxima distribution, in and around the Atlantic Ocean, could well correspond to a situation before the Mid-Atlantic Ridge was formed.

Refinements in the spherical harmonic analysis of the Earth's topography, and inclusion of the effects of isostasy and local variations in μ and g are desirable. Coupling to thermal convection is probably important, as thermal energy seems to be sufficient to move the continental plates³. Core-mantle coupling which would include the effects of the existence of the liquid core may also play an important role. The relaxation process described by equation (13) is obviously too simple and needs to be improved; this will require a better understanding of the solid-state physics of the Earth's crust. We hope to return to these and related questions elsewhere.

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- ¹ Pines, D., and Shaham, J., *Phys. Earth planet. Interiors*, **6**, 103 (1972).
- ² Pines, D., and Shaham, J., *Nature phys. Sci.*, **243**, 122 (1973).
- ³ Runcorn, S. K., *Continental drift*, 145-194 (Academic Press, New York, 1962).
- ⁴ Prey, A., *Abhandlungen der Koniglicher Gesellschaft der Wissenschaften zu Göttingen, Mathematisch-Physikalische Klasse, neue Folge* **11**, 1 (1922).
- ⁵ Lederstege, K., *Handbuch der Vermessungskunde*, Band V (J. B. Metzlersche Verlagsbuchhandlung und Carl Ernst Poeschel Verlag GmbH in Stuttgart), 848-856 (1956/1969).
- ⁶ Balmirio, G., Lambeck, K., and Kaula, W. M., *J. geophys. Res.*, **78**, 478 (1973).
- ⁷ Love, A. E. H., *A treatise on the mathematical theory of elasticity*, fourth ed. (Dover Publications, New York, 1944).
- ⁸ Munk, W. H., and MacDonald, G. J. F., *The Rotation of the Earth*, 15-37 (Cambridge University Press, Cambridge, 1960).

Remote sensing and lake eutrophication

AN infrared photograph of part of Clear Lake, California (Fig. 1) shows beautiful, complex patterns of blue-green algal blooms which were not observed by conventional limnological techniques. Repeated observations of patterns such as these can be used to chart the surface movement of these buoyant algae and can also be used to help control algal scums in eutrophic lakes.

A considerable amount of microstructure is visible in the photograph, including plume and wave-like concentration. We use the term 'microstructure' because the distances between what are obviously very different concentrations of phytoplankton are only of the order of a few metres, which are small distances relative to the area of the lake (17,000 ha). Although sampling teams on the lake could see some evidence of surface algal concentrations, they did not observe the microstructure at all despite the



Fig. 1 Infrared aerial photograph of surface algal concentrations in the Upper Arm of Clear Lake, California, taken on May 11, 1973 with an International Imaging Systems Mark I multispectral camera with four 100 mm F. L. lenses and Kodak Type 2424 film. The image is 5 km across. a, Wake of boat. Images were simultaneously recorded in red (590-690 nm), green (470-590 nm) and blue (400-470 nm) light. The infrared light used to record this image (730-950 nm) should not be confused with emitted thermal radiation (5,000-14,000 nm). Solar elevation above the horizon was 35°. On the water, dip samples from the first 10 cm of the water column were measured for turbidity and analysed spectrophotometrically for soluble phycocyanin-c and particulate chlorophyll *a* after methanol extraction³. Secchi disc depths and field notes were recorded at each station. One of us (A.J.H.) devised an aerial photographic technique for surface blooms, which is more available to the average limnologist. A conventional hand-held SLR camera with a through-the-lens light meter, 35-mm lens, standard colour film and a rotatable polarising filter gave a fair impression of the bloom microstructure from a small highwing airplane at an altitude of 2,000 m. Polarised light brought out algal surface patterns but in less detail than the multispectral pictures.

fact that the boat tracks are visible in Fig. 1. Measurements from the boat showed that the lake is virtually free of suspended sediment and that it supports a near monoculture of *Aphanizomenon flos-aquae*¹. Lake surface turbidity, including algae, was only 3 Jackson Turbidity Units. Concentrations of chlorophyll *a* were measured as 14 and 255 $\mu\text{g l}^{-1}$ in two of the photographed areas and we are therefore sure that the pattern resulted from a single species of blue-green algae. Complex patterns such as these cannot be determined using conventional techniques because major changes in algal distribution can occur in a few minutes and the details are invisible from a moving boat. Sky reflections, internal reflections of upwelling light and the disturbance from the boat itself all add to the problem.

Buoyant, gas-vacuolate blue-green algal blooms are a considerable nuisance in Clear Lake. For example, when the Upper Arm of Clear Lake was photographed (Fig. 1), the surface patterns were very different from those in the Oaks Arm. Algae formed dense streaks several metres wide and 10 cm deep and had chlorophyll *a* concentrations of approximately $10^3 \mu\text{g l}^{-1}$ relative to adjacent values of approximately $10^2 \mu\text{g l}^{-1}$. Physical concentrations such as these, if prolonged, would be catastrophic for both algae, which would die, and humans, who would suffer from the smell. Blue phycocyanin-c, a paint-like pigment virtually unique to blue-green algae, is lost from their cells upon death. Even by 09.00 LT the streaks contained up to 500 times more free phycocyanin-c than was observed in nearby areas, indicating considerable decay of the surface phytoplankton. In spring *Aphanizomenon* can fix atmospheric

nitrogen gas into cellular material and is responsible for up to 50% of the lake's annual intake of nitrogen¹⁻⁴ and therefore, by definition, for much of its eutrophic state. Observations of the destruction of *Aphanizomenon* are important in the control of the extreme eutrophic state of the lake.

Although we believe that most of the observed patterns resulted from *Aphanizomenon* (we also observed a few which resulted from suspended sediment), spectral signatures of the algal patterns varied. On May 11, 1973 algal concentrations were bright in infrared light (Fig. 1); a photograph of the same area in red light provided a similar, but less contrasting, response and photographs in blue and green light showed no patterns. A different spectral signature occurred on June 21, 1973: algal concentrations which were still bright in infrared light, appeared as similar, though darker patterns in blue, green and red light. The spectral signature observed on May 11, however, only occurred in limited areas. Importantly, lysed (dead) algal streaks were bright in all spectral regions. Temporal and spatial signature variations such as these result from any combination of several factors: algal distribution and concentration in the water column⁵, gas vacuolation in the algae⁶, or background turbidities or anomalous concentration effects⁷.

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- ¹ Horne, A. J., and Goldman, C. R., *Limnol. Oceanogr.*, **17**, 678-692 (1972).
- ² Goldman, C. R., and Wetzel, R., *Ecology*, **44**, 283-294 (1963).
- ³ Horne, A. J., Dillard, J. E., Fujita, D. K., and Goldman, C. R., *Limnol. Oceanogr.*, **17**, 693-703 (1972).
- ⁴ Horne, A. J., Javornicky, P., and Goldman, C. R., *Limnol. Oceanogr.*, **16**, 684-689 (1971).
- ⁵ Horne, A. J., *Third Annual Report, Clear Lake Algal Research Unit* (Clear Lake Algal Research Unit, Lakeport, California, 1973).
- ⁶ Walsby, A. E., *Bact. Rev.*, **36**, 1-32 (1972).
- ⁷ Duntley, S. Q., in *NASA, Manned Spacecraft Center, Fourth Annual Earth Resources Program Review*, NASA-TM-X-68563 102 (1)-102(25) (1972).
- ⁸ Talling, J. F., and Driver, D., in *Primary productivity measurement, marine and freshwater* (edit. by Doty, M. S.), USAEC TID-7633, 142-146, Office of Technical Services, Washington, (1961).

X-ray photoemission spectroscopy

We wish to report the first X-ray photoemission spectroscopy (XPS) experiments performed at Stanford Synchrotron Radiation Project, using synchrotron radiation from the Stanford Positron Electron Accelerator Ring (SPEAR) facility. The photoemission technique has been used extensively in the study of the electron properties of materials^{1,2}.

The main emphasis was on the study of the feasibility of using the favourable properties of synchrotron radiation to achieve high resolution XPS. A resolution of 0.6-1.0 eV (or 0.9-1.5 eV) is achieved in commercially available XPS machines with (or without) monochromatised radiation. We have obtained a total experimental width of 0.42 eV, implying a resolution of about 0.25 eV. Studies in the X-ray region have been limited to the few photon energies determined by characteristic X-ray emission lines (mainly Al $K\alpha_{1,2}$ and Mg $K\alpha_{1,2}$ radiation at 1486.7 eV and 1253.6 eV, respectively, the intensities of which are high enough and the characteristic linewidths of which are small enough to be useful as a monoenergetic X-ray source³. Synchrotron radiation⁴ provides a continuous wavelength spectrum, which for the SPEAR storage ring with a critical wavelength⁴ of $\lambda_c = 4.6 \text{ \AA}$ (at a beam energy of 2.5 GeV) extends down to a fraction of an angstrom ($\approx 1/10 \times \lambda_c$) with considerable intensity. The advantages of using synchrotron radiation for XPS experiments have been reviewed⁵, and parameters of the SPEAR storage ring have been summarised.

In a photoemission experiment the sample is irradiated with monochromatic radiation and a kinetic energy analysis is performed on the photoemitted electrons. Our experimental apparatus, consisting of a beam extractor, a crystal monochromator and an electron spectrometer (Fig. 1). The beam extractor is a 10 m long ultrahigh vacuum pipe ($\Phi = 8$ inches) which is used to channel the synchrotron light from the storage ring out to the experimental area. The beam extractor contains, among other things, instrumentation for collimating the synchrotron beam in both the horizontal and vertical planes. The X rays are allowed to pass two Be foil filters to reduce long wavelength radiation, and through a Be window (250 μm thick), which provides a vacuum tight connection between the ring (10^{-9} torr) and the monochromator (atmospheric He). The crystal monochromator uses two flat Si (220) crystals in the parallel mode and a Si (440) channel-cut crystal in the anti-parallel mode. A third crystal is optional and was not used for these experiments. The first crystal deflects the X-ray beam downwards in the vertical plane and the beam emerges from the second crystal horizontally about 20 cm below the centre-line of the original beam. The monochromator is positioned inside the radiation shielding of the storage ring and must be remotely controlled. The monochromatic X-ray beam (8,000 eV) passes through a hole in the radiation shielding and into the electron spectrometer through a thin (50 μm) Be window, about 15 m from the source. The electron spectrometer consists of an ultra high vacuum (UHV) chamber (base pressure 4×10^{-11} torr) housing an energy analyser, a sample flange, and accessories for the preparation of UHV samples. The energy analyser is a double-pass cylindrical mirror analyser with a channeltron as the electron detector. Standard techniques for fast pulse counting were used for the data acquisition.

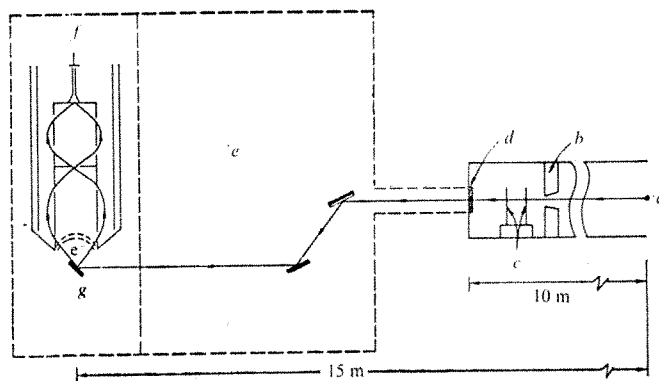


Fig. 1 Schematic figure of the experimental apparatus. *a*, origin of synchrotron radiation; *b*, water cooled collimators; *c*, absorbing Be foils (65 μm); *d*, Be window (250 μm); *e*, crystal monochromator (silicon crystals deflect the beam); *f*, energy analyser; *g*, sample.

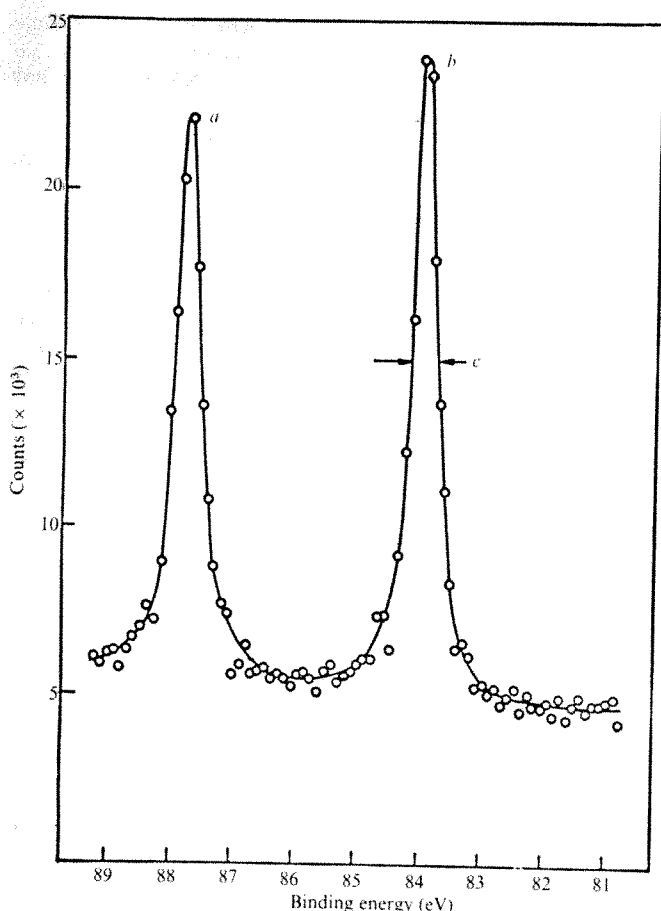


Fig. 2 Electron distribution curve for the 4f doublet of gold. The Fermi level is chosen as the reference level for the binding energy. a, $4f_{5/2}$ peak; b, $4f_{7/2}$ peak; c, 0.42 eV (FWHM).

The performance of our XPS machine was tested by measurements of photoemitted electrons from inner core levels in the noble metals Cu, Ag and Au. The measurements were taken on thin films evaporated *in situ* in the UHV chamber. The observed distribution curve for the well known and extensively examined 4f doublet in Au is shown in Fig. 2. The total accumulated number of counts is plotted against the binding energy of the electrons, using the Fermi energy as the reference level. The counting rate is normalised to an *e*-beam current of 20 mA in SPEAR. During these measurements the ring was run at a beam voltage of 2.5 GeV and an *e*-beam current of 20–40 mA (the beam current typically decays from 30 to 20 mA in 3–4 h, after which the beam is dumped and the ring refilled). The horizontal scale has been calibrated so as to give the binding energy for the $4f_{7/2}$ level, which was reported by Ley *et al.*⁷. The most remarkable thing about the spectrum (Fig. 2) is the extremely narrow width of the lines, demonstrating the high resolution capability of the XPS machine. A linewidth of 0.42 eV at full width at half maximum (FWHM) is obtained from Fig. 2, which is about a factor of two better than results from X-ray tube sources without a monochromatised Al $K\alpha_{1,2}$ source⁸. The splitting between the two peaks, and their relative heights (Fig. 2a, b), are in excellent agreement with earlier results³. Signal-background and signal-noise ratios are expected to be improved after a minor modification of the experimental set up. Three factors contribute to the observed linewidth of 0.42 eV at FWHM: the inherent width of the 4f photoline; the resolution of the energy analyser; and the resolution of the crystal monochromator (including the effects of slits and collimators). The energy resolution of the doublepass cylindrical mirror analyser is well known, and is 0.6% of the pass energy for the electrons. We chose a pass energy of 20 V, thus, the contribution to the resolution from the energy analyser is 0.12 eV. It is more

difficult to give an exact figure for the contribution from the crystal monochromator, because of the complexity of the slits and collimators, and because of the difficulty of estimating the source size. A consideration of all of the geometrical factors, however, suggests that it should lie somewhere between 0.25 eV and 0.35 eV. With the arbitrary assumption of Gaussian line shapes the inherent linewidth would then be 0.2–0.3 eV. It can be assumed, however, that the line shapes are neither pure Gaussian nor Lorentzian. All that we can therefore conclude at present is that the intrinsic linewidth is certainly less than 0.3 eV but probably 0.10–0.15 eV. We are not aware of any theoretical estimate of the different physical processes (for example, radiative and non-radiative Auger transitions) contributing to the linewidth. Published calculations of Auger transition rates do not extend out to the 4f shell⁹.

The calculated photon flux after the monochromator, using the SPEAR parameters already given, is 10^9 – 10^{10} photons $s^{-1} cm^{-2}$ which is consistent with our flux measurement using an ionisation chamber. The useful flux on the sample is determined by the effective aperture of the energy analyser. Finally, we should comment on the counting rate which under the present running conditions of the storage ring and the electron spectrometer is 25 counts s^{-1} on the 4f peaks (Fig. 2). Obviously, this counting rate excludes the possibility of conveniently studying valence bands and core lines with considerably weaker intensity than the Au 4f levels. The intensity of 8,000 eV X rays will, however, increase about 50 times, after the upgrading of the SPEAR storage ring (planned for summer 1974), and thus the required data acquisition time will be considerably shortened.

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¹ Spicer, W. E., in *Optical Properties of Solids* (edit. by Abeles, F.), 755–858 (Elsevier, Amsterdam, 1972).

² *Electron Spectroscopy* (edit. by Shirley, D. A.), (Elsevier, Amsterdam, 1972).

³ Siegbahn, K., *et al.*, *Nova Acta R. Soc. Scient. upsal.* **IV**, **20** (1967).

⁴ Godwin, R. P., *Springer Tracts in Modern Physics*, **51**, 1 (1969).

⁵ Codling, K., *Rep. Prog. Phys.*, **36**, 541 (1973).

⁶ Winick, H., *Bull. Am. phys. Soc.*, **18**, 1600 (1973).

⁷ Kowalczyk, S. P., McFeely, F. R., Ley, L., Pollak, R. A., and Shirley, D. A., *Phys. Rev.*, **B 7**, 5228 (1973).

⁸ Siegbahn, K., *Electron Spectroscopy* (edit. by Shirley, D. A.), (Elsevier, Amsterdam, 1972).

⁹ Bambynek, W., *et al.*, *Rev. mod. Phys.*, **44**, 716 (1973).

Electrically driven instability in elastic liquids

We report here a striking phenomenon which we have observed in some elastic liquids. The phenomenon was discovered during the cleaning out of a glass vessel which contained a newly prepared solution of a polyisobutene of high molecular mass in a polyisobutene with a low molecular mass. The liquid was rather viscous, and so a good proportion of it remained on the walls of the

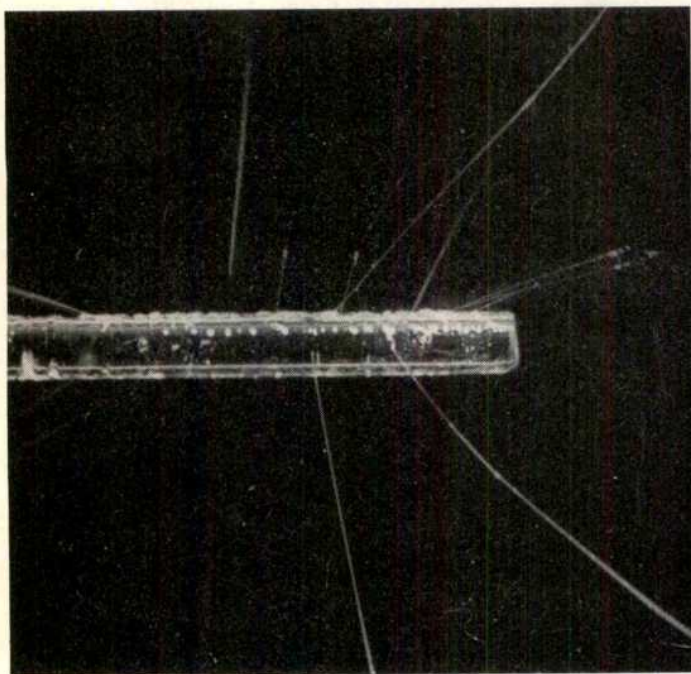


Fig. 1 The formation of fine jets of liquid after a polyisobutene solution has been torn away from a glass surface. The glass rod in the photograph has a diameter of 6 mm.

container. We decided to recover this by utilising the elastic properties of the solution and 'winding' it onto a glass rod. Using this method the liquid can be torn completely free from the walls of the container. Having thus removed some of the liquid from the container we found that the liquid on the rod spontaneously developed a number of fine streamers (Fig. 1). These emanated from irregularities on the surface of the liquid and their tips moved away from the rod at speeds of several cm s^{-1} .

Using an electroscope we established that the process of tearing the liquid from the walls of the container resulted in a separation of electrical charge. We also noticed that the streamers were readily attracted to nearby objects.

It has been known for many years¹ that the horizontal free surface of a liquid in a vertical electric field can be unstable, resulting in the formation of vertical jets of liquid if the field intensity is sufficiently large. We believe that the present phenomenon is an example of this instability. As the winding-on procedure produces irregularities in the free surface, the charge that is generated in the process of tearing the liquid from the container will not be uniformly distributed over the surface, thus giving rise to local field intensities near these irregularities much larger than the average. Presumably these 'local' fields can exceed the critical field intensity required for the described instability to occur, and a jet (or streamer) of liquid forms in exactly the same way as that described by Taylor¹. We have found that these streamers grow to great lengths (to many thousands of diameters) without breaking and can be so fine as to be quite difficult to see with the naked eye. This remarkable cohesion is most probably aided by the very good spinnability of many elastic liquids², which allows them to be drawn into extremely fine threads without breaking. (Taylor found in his experiments¹ that, if he used a conducting liquid, a steady uniform jet of liquid was formed when the field was sufficiently strong. If, however, the liquid was a poor conductor, such as transformer oil, the jet did not properly establish itself and took the form of a series of droplets. We repeated Taylor's experiment using the polyisobutene solution, which has an extremely low conductivity, and found that the instability resulted

in the formation of a steady uniform jet. We attribute this to the good spinnability of the non-Newtonian polyisobutene solution, as opposed to the Newtonian behaviour of transformer oil.)

We tried to reconstruct our observation by spreading a small quantity of the liquid (taken from the stock solution) on a glass plate and then 'tearing' the liquid from the plate by winding it on to a rod. The phenomenon was not observed immediately, but after the liquid had been left on the plate for a day in the open air, we found that the phenomenon could once more be produced easily. Further investigation indicated that the composition of the plate (whether glass, perspex, or a metal) did not affect the experiment noticeably, but that it would not work when the rod was a conductor.

At this stage we decided that the charge separation might occur in a similar manner to that in which charge arises when petrochemical mixtures flow through pipelines. In such cases it has been found that the formation of the charge depends on the presence of trace polar impurities³. In order to test this idea we placed some fresh liquid on a glass plate, put the plate in a desiccator for a week, and tried to observe the phenomenon. No streamers were found but after the liquid on the plate had been left exposed to the air for a few hours we were again able to produce the instability. It seems, therefore, that in our experiments a small amount of water from the air was necessary for the charge generation.

We believe that the extremely low electrical conductivity of polyisobutene solutions is an important feature of the experiment, as it is essential that the field is maintained while the streamers are forming, and this requires that the charge should not leak away too quickly. The phenomenon has also been observed with a solution of polystyrene in di-phthalate.

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¹ Taylor, G. I., *Proc. R. Soc. A*, **313**, 453 (1969).

² Lodge, A. S., *Elastic Liquids*, 229 (Academic Press, London, 1964).

³ Lyle, A. R., and Strawson, H., *Phys. Bull.*, **23**, 453 (1972).

Bordoni peak formation as a result of a martensitic transformation

THE indium-thallium alloys in the composition range 16–31 atom% Tl undergo a martensitic phase transformation from the higher temperature f.c.c. form to the lower temperature f.c.t. modification^{1,2}—a transformation which is well suited to ultrasonic studies. Here we report ultrasonic attenuation measurements, which have revealed the development of an internal friction peak which results from the passage of 27 atom% Tl alloy single crystals through the phase transition (which occurs at 127 ± 2 K for this composition³). The general behaviour of this peak strongly suggests a relaxation type of effect similar to that first observed by Bordoni in copper⁴. Such peaks are either absent or negligibly small in well annealed metals; but they are produced by plastic deformation and are considered to arise from thermally activated processes involving dislocations⁵. We can therefore expect that any dislocations which are formed as a result of the martensitic transition would give rise to such a peak, and that it could be detected

ultrasonically, provided that the investigated ranges of ultrasound frequency and temperature are appropriate to the activation energy involved.

Measurements were made on [110] oriented, single crystal samples of the alloy, of composition 27 atom% Tl, cut from a boule of about 4 cm³ which had grown in a horizontal zone furnace. Ultrasonic pulse-echo measurements were made at 14 MHz and 42 MHz with longitudinal waves and [100] polarised shear waves, respectively (Fig. 1). A large absorption peak occurs at the transition temperature T_c (refs 3 and 6). The peak temperature is independent of frequency, although it does differ somewhat between warming and cooling, as would be expected for this transition. The additional feature of the attenuation behaviour (shown in more detail in Fig. 2) is the peak which occurs at temperatures higher than T_c and which moved to higher temperatures when the driving frequency was raised. During each experiment the sample was taken through a cycle of cooling from room temperature to below T_c (thus producing a banded twin f.c.t. structure) and then warming back to 300 K to reverse the process and to re-establish the single crystal form; the temperature change was continuous, except at the end points. For the first few (≈ 5) cycles following the original spark cutting and planing of the crystal, the additional attenuation peak could be seen only during the warming part of the cycle and was not seen during cooling (Fig. 1). After a further few cycles, however, the peak became established during both warming and cooling (Fig. 1). Subsequent measurements over a long period of time showed that the peak height decreased gradually, until after about eight months it had vanished almost completely.

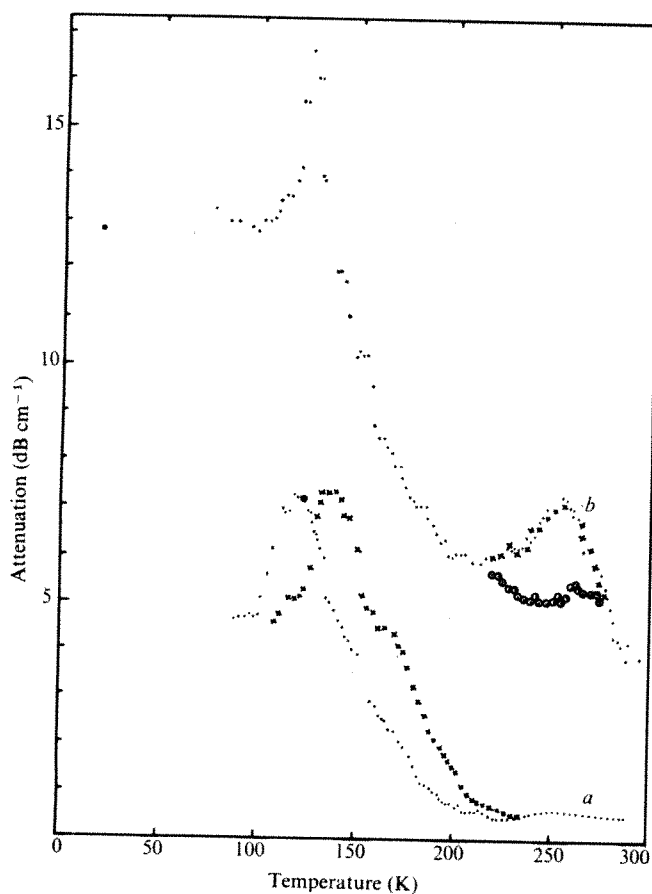


Fig. 1 The temperature dependence of the attenuation of ultrasonic waves propagated along the [110] direction in an In-27 atom% Tl alloy. *a*, 14 MHz curves (longitudinal waves); *b*, 42 MHz curves (shear waves, polarised [001]); ●, cooling; ×, warming; ○, initial cooling. The peaks at 127 K (on cooling) result from the phase transition, and the Bordoni peak appears at about 250 K.

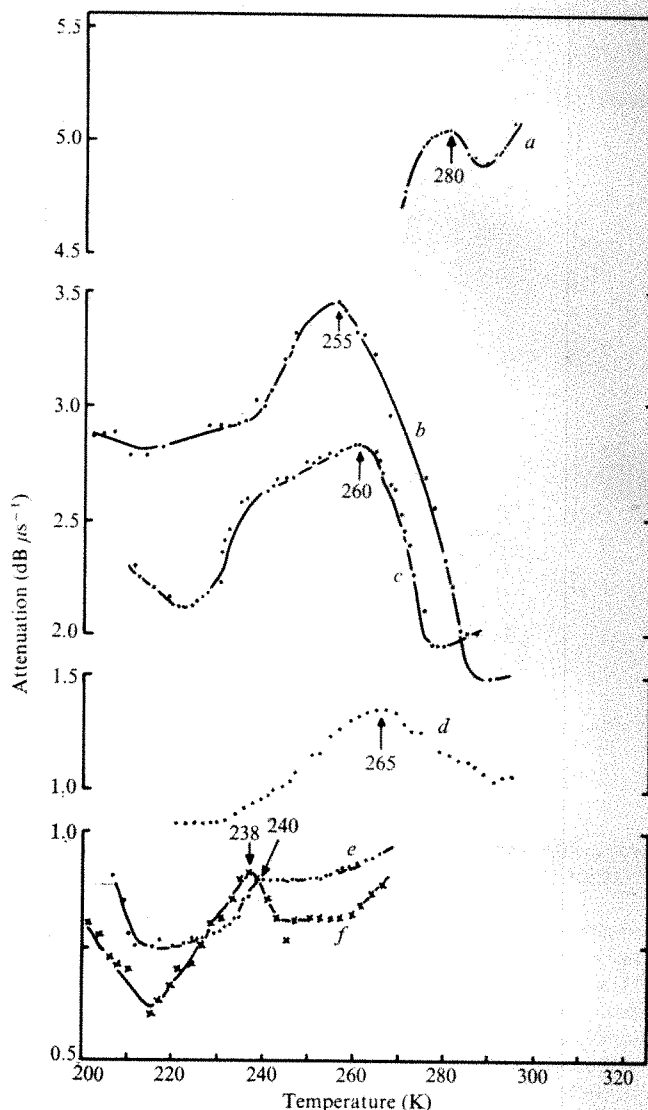


Fig. 2 Collected data of the Bordoni peaks found for ultrasonic waves propagated in the [110] direction in In-27 atom% Tl crystals. *a*, 70 MHz curve, shear wave (sample A); *b*, 42 MHz curve, shear wave (sample A); *c*, 42 MHz curve, longitudinal wave (sample B); *d*, 42 MHz curve, longitudinal wave (sample A); *e*, 14 MHz curve, longitudinal wave (sample B); *f*, 14 MHz curve, shear wave (sample A). These data were obtained during the period when the peaks could be seen on both warming and cooling, and before their amplitude had become too small to measure.

In general, the observed effects are characteristic of Bordoni peaks in f.c.c. metals. For example, Bordoni⁴ first found that the internal friction peak in copper was reduced by about 30% after annealing at 150° C for 10 h. Room temperature annealing of the Bordoni peak, of the kind observed here, is well known in aluminium^{7,8}. In indium-thallium alloys the peak temperature does not depend appreciably upon the number of passes through the transition. This corresponds with the usual finding: that the Bordoni peak position is not very sensitive to prestrain, annealing or impurities. The results in Fig. 2 can be used to test a relaxation process hypothesis. An activation energy, E , is defined, and the peak temperature T_m , and driving frequency, ν , can be related by an Arrhenius type of expression

$$\nu = \nu_0 \exp(-E/kT_m) \quad (1)$$

in which ν_0 is an attempt frequency. A straight line on an Arrhenius plot (Fig. 3) gives a reasonable fit to the present results. Several determinations were made of T_m at each frequency with different ultrasound wave polarisations, on two different [110] samples cut from the same boule, and the

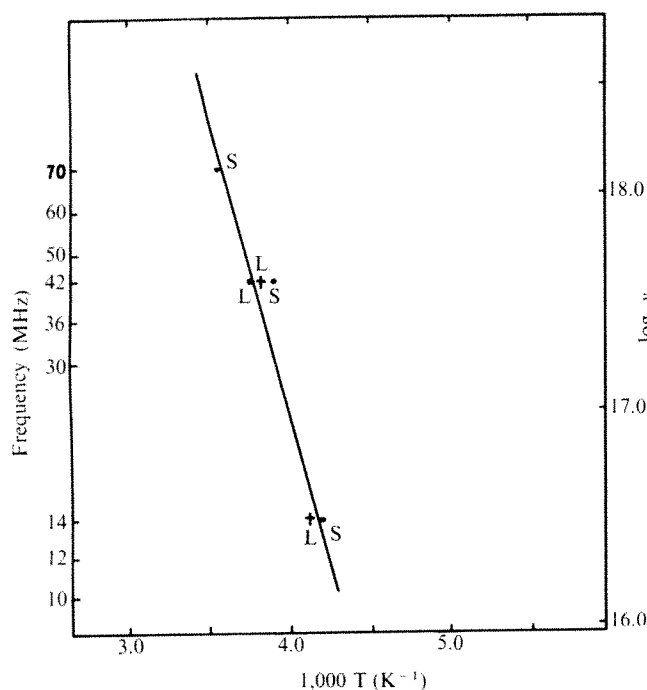


Fig. 3 Arrhenius plot to determine the activation energy and attempt frequency corresponding to the Bordoni peak, induced by passing through the transition. ●, Sample A; +, sample B; L, longitudinal waves; S, shear waves.

variations were found to be small. The activation energy, E , was found to be 0.24 ± 0.05 eV and the attempt frequency, ν_0 , $1.5 \pm 0.4 \times 10^{13}$ Hz. Applied to Bordoni peaks in f.c.c. metals in general this procedure gives activation energies of the order of 0.1 eV: for copper, E is 0.1–0.15 eV (with $\nu_0 = 10^{10}$ – 10^{13} Hz); for aluminium, E is 0.25 eV ($\nu_0 = 10^{14}$ Hz) (ref. 5). The peak parameters obtained for indium-thallium alloy studied here, fall into the same range. It is interesting that there is no significant difference, within the limits of experimental error, between the activation energies obtained for the longitudinal and the transverse modes. A difference can occur, however, when the resolved stress component differs on the slip plane corresponding to the polarisations of the longitudinal and transverse ultrasonic waves⁹.

The general features of the peak in the ultrasound attenuation in the alloy are consistent with those of Bordoni peaks. In other materials such peaks have been shown previously to result from dislocation motion, and to occur at high dislocation densities. The peaks in the 27atom% Ti alloy were formed after passage of the crystals through the martensitic transition, and back. If a dislocation damping mechanism is assumed, then not only is the frequency dependence of the peak temperature accounted for, but so is the observation that the peak appears initially only on warming. The strains involved during the transformation must produce a large number of dislocations which then interact with the ultrasound waves as the sample is warmed up, and anneal out when it is held at room temperature for several hours (300 K is approximately 0.7 of the melting temperature). Therefore, subsequent cooling does not result in the reappearance of the peak. But if after several cycles, the dislocations become pinned sufficiently for short period annealing to become ineffective in causing their removal, then the peak will appear in both the cooling and the warming parts of the cycle, as is observed. The attenuation peak results provide experimental evidence of the production of dislocations during the martensitic transformation.

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- ¹ Guttman, L., *Trans. Am. Inst. Min. Engrs.*, **188**, 1472 (1950).
- ² Bowles, J. S., Barrett, C. S., and Guttman, L., *Trans. Am. Inst. Min. Engrs.*, **188**, 1478 (1950).
- ³ Gunton, D. J., and Saunders, G. A., *Solid State Commun.*, **14**, 865 (1974).
- ⁴ Bordoni, P. G., *J. acoust. Soc. Am.*, **26**, 495 (1954).
- ⁵ Niblett, D. H., *Physical Acoustics, Vol. IIIA*, (edit. by Mason, W. P.), 77 (Academic Press, London, 1966).
- ⁶ Pace, N. G., and Saunders, G. A., *Proc. R. Soc.*, **A326**, 521 (1972).
- ⁷ Birnbaum, H. K., and Levy, M., *Acta metall.*, **4**, 84 (1956).
- ⁸ Baxter, W. J., and Wilks, J., *Acta metall.*, **11**, 979 (1963).
- ⁹ Saunders, G. A., and Seddon, T., *Physics Chem. Solids*, **31**, 2495 (1970).

Determination of the gas constant by an acoustical method

A NEW value for the gas constant, R_0 , has been obtained from measurements of the velocity of sound, c , in argon at the temperature of the triple point of water. Velocity measurements were made using a low frequency variable-path acoustic interferometer, operating at a frequency of 5.6 kHz (ref. 1). Ninety eight independent measurements of sound velocity were made over a range of pressures from 0.3–2.0 atm. A quadratic least squares fit was made of c^2 against pressure, and the resulting acoustic isotherm was extrapolated to zero pressure to yield a value for c_0^2 , the velocity in the limit of low pressures. The gas constant was then obtained from the relationship

$$c_0^2 = \gamma R_0 T / M$$

where $\gamma = 5/3$, $T = 273.16$ K, $M = 39.9478$ g mol⁻¹ and c_0^2 was the experimentally determined value of $94,768.9 \pm 2.1$ m² s⁻². The uncertainty quoted for c_0^2 is the standard error on the intercept of the acoustic isotherm.

The value for the gas constant thus obtained is

$$R_0 = 8,315.59 \pm 0.18 \text{ J K}^{-1} \text{ kmol}^{-1}$$

The uncertainty quoted here is the standard error in the result, which stems from the standard error in c_0^2 arising from random errors in the measurements of sound velocity. It is equivalent to a standard error of 22 parts per million (p.p.m.) in R_0 .

The largest systematic uncertainty is that arising from the uncertainty in the isotopic composition of the argon used in the measurements, and is thought not to exceed 7 p.p.m. Errors in temperature measurement, acoustic frequency and the measurement of displacement in the interferometer are considered negligible, and errors resulting from chemical impurities in the argon are not thought to lead to a systematic uncertainty greater than 3 p.p.m. The major uncertainty in the result is thus that arising from random errors in the measurement of velocity.

Full details of the experimental method and results will be published.

This new value for R_0 is greater by $1.18 \text{ J K}^{-1} \text{ kmol}^{-1}$ (142 p.p.m.) than that based upon measurements of the density of oxygen and quoted as $8,314.41 \pm 0.26 \text{ J K}^{-1} \text{ kmol}^{-1}$ (ref. 2).

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- ¹ Quinn, T. J. A., *Atomic masses and fundamental constants 4: proceedings of the international conference, National Physical Laboratory 1971* (edit. by Sanders, J. H., and Wapstra, A. H.), 529–33 (Plenum Press, London, 1972).
- ² Cohen, E. R., and Taylor, B. N., *Journal of Physical and Chemical Reference Data*, **2**, 566 (1973).

Solution evaporation method for solid state ESCA studies

ALTHOUGH considerable effort in electron spectroscopy for chemical analysis (ESCA) has been channelled into the development of narrow line sources¹⁻⁴, there has been little attention to the problem of increasing ESCA resolution by decreasing the large linewidth contribution from involatile non-conducting solid samples. For such samples, most workers have spread a thin layer of solid on to sticky tape^{5,6}, or have used a metal mesh as host matrix⁷⁻⁹. Al has also been suggested as a sample backing by P. E. Larsen. These procedures generally provide widths approximately 1 eV broader than those from the gas phase¹, thin condensed phase¹⁰, or from conductor¹ spectra. The procedures normally involve the use of a few mg of compound, even though ESCA should be sensitive to $\sim 10^{-8}$ g of a material^{2,11}.

We describe here a simple technique for evaporating very dilute solutions of compounds on to acid-etched Al plates which, first, usually appreciably decreases the linewidths of non-conducting samples; second, enables good spectra to be obtained using μg amounts of compound; and third, minimises charging effects and calibration problems.

All spectra were recorded using an ESCA-36 McPherson Instrument and Mg K α radiation. To avoid decomposition of samples, low X-ray power (15 mA \times 8 kV) was used for as short a period as possible. Spectra were calibrated using the Au evaporation technique^{12,13}, and an argon ion gun (10 keV argon ions) was used to etch a Sn metal foil *in situ* in order to obtain a spectrum of pure Sn metal. Besides the energy levels of direct interest in this study

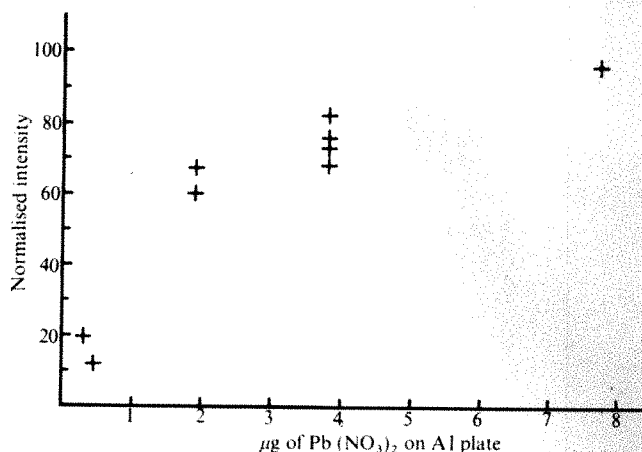


Fig. 2 Normalised intensity of the Pb 4f_{7/2} line against amount of Pb(NO₃)₂ (μg) on the Al plate.

(Pb 4f, and Sn 3d), spectra of energy levels of other elements (such as C 1s, N 1s and O 1s) in the compounds were always recorded.

Alcan AA 3003 aluminium plates (2 cm \times 1 cm \times 0.06 cm) were lightly etched for 2-3 min in 3 M HCl until they were readily wettable with H₂O. Purer Al (Fischer 99.9% purity) was also tried with similar results. More extensive etching or roughening with fine emery paper invariably leads to the poorer results. Pt foil further reduced charging effects but did not wet as well. The etched Al plates were washed with H₂O and acetone and dried in an oven at 70° C.

Generally, solutions of between 10^{-2} M and 10^{-4} M of a given compound were prepared and 3-20 μl pipetted on to the Al plate. Volatile solvents such as benzene readily spread over the plate. Because of the high surface tension of H₂O, the end of the micropipette was used to spread aqueous solutions over the plate; if the plate was sufficiently etched, the water would wet the entire plate. The solvent was then allowed to evaporate in air.

The use of such small amounts of samples is an aid to minimising vacuum problems encountered with slightly volatile or unstable compounds.

An adequate spectrum of 0.4 μg of Pb(NO₃)₂ on a 2 cm² plate can be obtained in 75 min, using low X-ray power, and the linewidths (1.60 eV) are narrower than those (1.70 eV) for the 6 mg of Pb(NO₃)₂ solid (Fig. 1c). It is interesting to compare our results with those obtained by Hercules *et al.*¹¹ using complexing surface groups. We obtained the spectrum on 0.4 μg of compound, whereas they used 2,400 scans (for an unspecified length of time) to obtain a spectrum on ~ 2 μg of Pb.

Considering the area of the Al plates (2.0 cm²), assuming that the area taken up on the surface by a Pb(NO₃)₂ molecule is ≈ 20 Å² and that there is an even distribution of Pb(NO₃)₂ molecules on the surface, 1 μg of Pb(NO₃)₂ corresponds to 1.8 monolayers. Thus, the spectrum in Fig. 1c corresponds to that for about one monolayer of Pb(NO₃)₂. This suggests that considerably smaller amounts of Pb(NO₃)₂ could be detected at higher X-ray powers and longer scan times.

Furthermore, the reproducibility of the results (Fig. 2) is surprisingly good considering the potential difficulties in obtaining an even distribution of molecules on a surface. It is also interesting that the intensity begins to level off after a few μg of compound, although we have found that the normalised intensity continues to increase slowly with increasing amounts of Pb(NO₃)₂ on the plate. The reproducibility, combined with the narrower linewidths and the levelling off of the intensities, strongly indicates a reasonably even distribution of molecules on the surface.

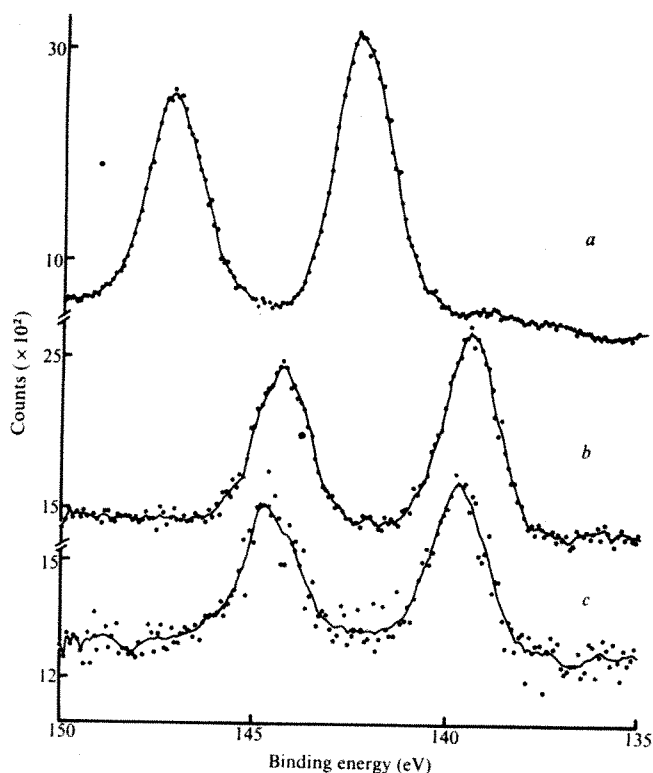


Fig. 1 Pb 4f spectra of Pb(NO₃)₂ taken using a Mg anode and an X-ray power of 120 W. The binding energies are uncorrected for charging effects. See Table 1 for corrected values. The lines drawn through the spectra are seven point smooths. a, 6.1 mg of solid Pb(NO₃)₂ spread evenly onto an acid etched Al plate; time of accumulation = 15 min. b, 3.8 μg of Pb(NO₃)₂ from 10 μl of a 1.6×10^{-3} M Pb(NO₃)₂ solution in H₂O; time of accumulation = 75 min. c, 0.4 μg of Pb(NO₃)₂ from 10 μl of a 1.16×10^{-4} M Pb(NO₃)₂ solution in H₂O; time of accumulation = 75 min.

Table 1 Pb 4f binding energies

No. of spectra	Amount of Pb (NO ₃) ₂ on plate (μg)	Pb 4f uncorrected (± 0.1 eV)	Pb 4f uncorrected with Au (± 0.1 eV)	Au 4f _{7/2} (± 0.1 eV)	Pb 4f corrected (± 0.2 eV)*
2	1.9	144.3	144.3	84.6	143.7
		139.5	139.3		138.7
4	3.8	144.5	144.5	84.8	143.7
		139.6	139.6		138.8
1	7.6	144.4	not obtained		
		139.6			
2	76	144.5	144.1	84.3	143.7
		139.6	139.2		138.8
3	6,100 solid none	147.2	146.8	87.0	143.8
		142.4	141.9		138.9
				84.2	

* Au 4f_{7/2} = 84.0 eV.

Figure 2 indicates that with further improvement of the technique, there could be two very important uses both in ESCA and Auger spectroscopy: calibration curves for quantitative analysis, and escape depth studies.

The results (Fig. 1 and Table 1) also indicate that charging effects are minimised in the solution evaporated samples, relative to the solid spectrum, although the corrected binding energies are the same within the error for all samples. Pb(NO₃)₂ from solution evaporation is still about 0.5 eV compared to 139.6 eV for a solution evaporated sample, whereas the corrected Pb 4f_{7/2} peak is at 138.9 eV and 138.8 eV, respectively. The Au calibrations show that the Pb (NO₃)₂ from solution evaporation is still about 0.5 eV charged relative to Au on the Al plate. Thus, the Au 4f_{7/2} line (84.0 eV for Au metal) is at 84.2 eV on the etched Al plate, but is at 84.6 ± 0.2 on the Al plates with evaporated Pb(NO₃)₂. Au linewidths of 1.40 ± 0.05 eV were obtained for all Au evaporations.

It is apparent that the solution evaporation technique is ideally suited to internal calibration as used in other forms of spectroscopy, and in ESCA spectra of volatile solids in which a compound such as perfluorocyclohexane

(C₆F₁₂) is cocondensed with the compound of interest¹⁴.

For many molecular Sn(IV) compounds, the solution evaporation method leads to significant decreases in linewidths relative to spectra taken in the conventional manner, especially when large charging shifts are present. The minimum Sn 3d linewidths for a conductor (Sn metal) obtainable on our equipment are 1.05 ± 0.05 eV (Fig. 3a) whereas that for a molecular non-conductor (Ph₄Sn) is 1.20 ± 0.05 eV. In contrast, typical 3d linewidths on Ph₄Sn solid are 1.50 ± 0.05 eV. Sn 3d linewidths for a number of other Sn compounds are 1.30 ± 0.05 eV, but others such as Me₂Sn (acac)₂ (acac = anion of acetylacetone) give markedly larger linewidths of 1.65 ± 0.05 eV. Solid samples have larger linewidths probably because of differential charging⁹. For some molecular Sn compounds, narrow linewidths can also be obtained by using very thin solids spread on to the etched Al plates.

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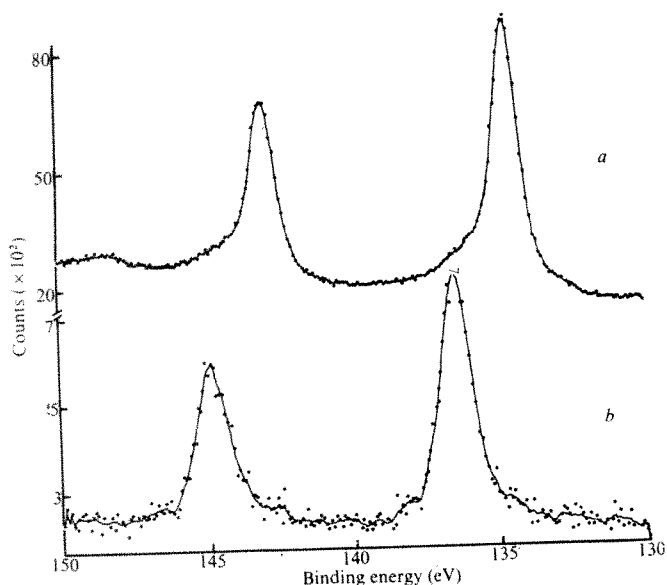


Fig. 3 Sn 3d spectra taken at an X-ray power of 120 W. The lines are seven point smooths. a, Sn metal, produced by an 8 min argon ion etch of Sn foil. The small shoulders on the left hand sides of the peaks are because of residual tin oxides. Time of accumulation = 3.7 min. b, 12 μg of (C₆H₅)₄Sn from 3 μl of 9.3 × 10⁻³M benzene solution of (C₆H₅)₄Sn. Time of accumulation = 15 min.

- Gelius, U., Basiliere, E., Svensson, S., Bergmark, T., and Siegbahn, K., *J. Elect. Spectroscopy*, **2**, 405 (1973).
- Siegbahn, K., et al., *ESCA Applied to Free Molecules* (North Holland Publishing, Amsterdam, 1969).
- Siegbahn, K., et al., *ESCA Atomic, Molecular and Solid State Structure Studied by Means of Electron Spectroscopy* (Almqvist and Wiksells, Uppsala, 1967).
- Electron Spectroscopy*, edit. by Shirley, D. A. (North Holland Publishing, Amsterdam, 1972).
- Tolman, C. A., Riggs, W. M., Linn, W. J., King, C. M., and Wendt, R. C., *Inorg. Chem.*, **12**, 2770 (1973).
- Briggs, D., Clark, D. T., Keable, H. R., and Kilner, M., *J. chem. Soc., Dalton Trans.*, 2143 (1973).
- Betteridge, D., Carver, J. C., and Hercules, D. M., *J. Elect. Spectroscopy*, **2**, 327 (1973).
- Citrin, P. H., *J. Am. chem. Soc.*, **95**, 6472 (1973).
- Dickinson, T., Povey, A. F., and Sherwood, P. M. A., *J. Elect. Spectroscopy*, **2**, 441 (1973).
- Clark, D. T., *Chem. Commun.*, 230 (1971).
- Hercules, D. M., Cox, L. E., Onisick, S., Nichols, G. D., and Carver, J. C., *Analyt. Chem.*, **45**, 1973 (1973).
- Hnatowich, D. J., Hudis, J., Perlman, M. L., and Ragaini, R. C., *J. appl. Phys.*, **42**, 4883 (1971).
- Thomas, J. M., Evans, E. L., Barber, M., and Swift, P., *Trans. Faraday Soc.*, **67**, 1875 (1971).
- Conner, J. A., Hall, M. B., Hillier, I. H., Meredith, W. N. E., Barber, M., and Herd, Q., *J. chem. Soc., Faraday II*, 1677 (1973).

BIOLOGICAL SCIENCES

Actinomycin D and RNA transport

ACTINOMYCIN D, a potent inhibitor of RNA chain propagation of DNA-directed RNA transcription¹⁻³, has been widely used for studies of turnover and transport of RNA in a variety of cell systems, to try to demonstrate that nuclear RNA labelled before the addition of actinomycin D is transported to the cytoplasm. It has been found that most labelled RNA remains in the nucleus⁴⁻⁸. It has been shown that the decrease in radioactivity of the heterogeneous nuclear RNA in HeLa cells is not accompanied by transfer of appreciable material to the cytoplasm⁹. Results of such experiments have even indicated that no transfer of rapidly labelled RNA from nucleus to cytoplasm occurs⁵. Recent studies on the effect of actinomycin D have raised doubts as to the validity of conclusions based on experiments including it. A prolonged half-life of mRNA due to actinomycin D has been observed in liver cells¹⁰, and the decay time of mRNA in HeLa cells has been found to be considerably shortened by treatment with this antibiotic¹¹. A non-physiological breakdown of rapidly labelled RNA by actinomycin D in primary chick fibroblasts has also been reported¹².

In studies of RNA transfer from nucleus to cytoplasm by chase experiments with actinomycin D, the length of the labelling period before inhibition is very important. Actinomycin D, being a template inhibitor, binds to DNA, and thereby efficiently blocks elongation of nascent RNA molecules. The use of too short pulse labelling before administration of the drug may imply that, at the onset of inhibition, most of the radioactivity is confined to nascent RNA chains that cannot be released from the chromosomal sites during the ensuing chase with actinomycin D. Here I describe experiments supporting this idea. Transport of labelled chromosomal high molecular weight RNA to the cytoplasm after actinomycin D chase can be demonstrated if the period of prelabelling is sufficiently long (more than 25 min), and if labelled RNA has appeared in the nuclear sap before the addition of inhibitor.

Salivary glands were isolated from fourth instar larvae of the dipteran *Chironomus tentans*¹³. Four animals were used in each experiment. Four salivary glands were explanted into 50 μ l of modified Cannons medium¹⁴⁻¹⁵ supplied with tritiated cytidine and tritiated uridine (Amersham) at specific activities of 25–30 Ci mmol⁻¹. After prelabelling for 25 or 45 min, actinomycin D (Calbiochem, Los Angeles) was added. The sister glands were used as control glands and the incubations were carried out at 18° C. Fixation, microdissection, extraction of RNA and electrophoresis in 1% agarose were performed as described elsewhere¹⁶⁻¹⁸. After the electrophoretic run, the gel slabs were treated with cold TCA to aid the removal of non-specific label¹⁹. The analyses of nuclear and cytoplasmic RNA from glands incubated with actinomycin D showed that labelling was inhibited by more than 98% (ref. 16). After 90 min of normal labelling with tritiated nucleosides, the chromosomal RNA acquired nearly maximal labelling, and a large amount of label appeared in the cytoplasm. A total length (pulse + chase) of 90 min was therefore considered as a suitable period for incubation.

The electrophoretic analyses of chromosomal RNA (Fig. 1a), nuclear sap RNA (Fig. 1b) and cytoplasmic RNA (Fig. 1c) after 25 min of labelling and after 25 min of labelling plus 65 min of synthesis inhibition with actinomycin D are presented in Fig. 1. The profile of chromosomal RNA after 25 min of incorporation displays the usual bimodal distribution

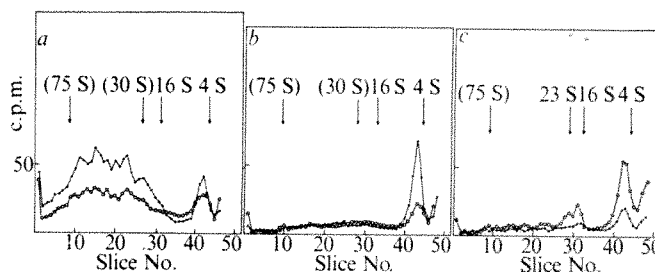


Fig. 1 Electrophoretic separations of, *a*, chromosomal RNA, *b*, nuclear sap RNA and, *c*, cytoplasmic RNA after 25 min of labelling and after 25 min labelling + 65 min of synthesis inhibition. Four glands from four different animals were placed in an incubation droplet of 50 μ l containing 100 μ Ci tritiated cytidine and 100 μ Ci tritiated uridine, and their sister glands were placed as controls in another 50 μ l of the same medium. The glands were incubated at 18° C for 25 min. Next, actinomycin D was introduced to one of the droplets at a concentration of 10 μ g ml⁻¹ and the glands were incubated for another 65 min, while the incubation of the control glands was stopped. After incubation the glands were fixed, and the cell components from five cells per gland were isolated by micro-dissection. The labelled RNA from each sample was released by pronase-sodium dodecyl sulphate treatment, and the electrophoresis was carried out in 1% agarose gel. To remove actinomycin D and RNase-resistant radioactivity the gels were treated with cold TCA¹⁹. At the end of the run the whole gel slab was placed in a 250 ml beaker containing 5% TCA at 4° C, and washed for 60 min. The treatment was repeated in a new volume of TCA and the gel was finally washed twice for 40 min in 1 l of distilled water at 4° C. Before radioactivity measurement, the gel was sliced and the slices were transferred to Packard counting vials, each containing 10 ml of toluene scintillator (1 l of which contains 30 ml solouene (Packard), 5.5 g of Permablend (Packard) and 20 ml methoxyethanol), incubated for 3 h at 60° C and finally counted in a Packard liquid scintillation spectrometer (Model 3380). *E. coli* RNA was used as marker (23S, 16S and 4S). The position of 75S and 30S were determined in parallel analyses of BR 2 RNA nucleolar RNA respectively. ●, Normal cells; ○, cells treated with actinomycin D.

with a peak in the 4S range and with label heterogeneously distributed in the 16–100S range. The subsequent treatment with actinomycin D for 65 min reduced the labelling of chromosomal RNA to 50–60% of the control values rather uniformly in the whole spectrum. The electrophoretic pattern of 25 min-labelled nuclear sap and cytoplasmic RNA shows only trace amounts of label in the high molecular weight ranges, but there is a rather distinct peak in the 4S region. During the ensuing 65 min of actinomycin D chase, no significant quantity of labelled heterogeneous RNA was released from the chromosomes into the nuclear sap. It is, of course, not possible to exclude that a release of heterogeneous RNA to nuclear sap may take place, but the product must be degraded instantaneously after its detachment. The radioactivity profile of cytoplasmic RNA displays an enhanced labelling in the 4S region as an expression of 4S RNA transport from the nucleus to the cytoplasm²¹. There is also a slightly increased radioactivity level in the position of the rRNA components, but there is no significant increase in labelling in the range above 28S. Thus, in spite of the fact that a considerable reduction of labelled chromosomal heterogeneous RNA takes place during actinomycin D chase, there is no corresponding increase of label either in nuclear sap or in the cytoplasm. Practically no rapidly labelled high molecular weight RNA moves out from the nucleus to the cytoplasm if labelling for 25 min is combined with 65 min actinomycin D chase.

It is yet not understood whether nascent RNA chains blocked by actinomycin D are detached and then broken down or if they are broken down without leaving the transcription sites. Electron micrographs of nucleolar DNA, containing nascent preribosomal RNA in 'Christmas tree' formation, show a random loss of incomplete RNA molecules during actinomycin D treatment irrespective of the

length of molecules (Mr F. Trendelenburg and W. W. Franke, unpublished.) A random and nonphysiological release of unfinished RNA chains may be the interpretation of the electrophoretic analyses of heterogeneous chromosomal RNA showing a parallel decay of labelling in the whole spectrum after actinomycin D chase. A labelling period of 25 min before the addition of the drug is sufficiently long, however, for completion of a substantial amount of 4S RNA, and during the subsequent actinomycin D treatment, transport of labelled 4S RNA from nucleus to cytoplasm can be demonstrated.

The electrophoretic analyses of labelled RNA from chromosomes (Fig. 2a), nuclear sap (Fig. 2b) and cytoplasm (Fig. 2c) after 45 min of labelling and after 45 min of labelling plus 45 min of actinomycin D chase are shown in Fig. 2. The pattern of labelled chromosomal RNA after 45 min of labelling shows a bimodal distribution similar to that after 25 min of incubation (Fig. 2a). Whereas the labelling of chromosomal RNA during 45 min of actinomycin D chase decayed 30–35%, the relative distribution of the heterogeneous RNA remained roughly the same as in the control profile. The emergence of a distinct radioactivity peak around the 30S region in the drug-treated glands can be explained by accumulation of preribosomal RNA of nucleolar origin on the chromosomes¹⁵. This appearance of preribosomal 30S RNA on the chromosomes seems to be potentiated by treatment with actinomycin D. The electrophoretic pattern of nuclear sap RNA after 45 min of labelling contains, besides a distinct 4S RNA peak, label distributed rather heterogeneously in the 16–100S range, with a small peak in the 75S region (Fig. 2b). The material distributed between 16–30S is likely to be of ribosomal origin¹⁵. When a normal 45 min pulse is followed by synthesis inhibition for 45 min, the radioactivity profile of nuclear sap RNA exhibits a decrease (30–35%) compared with the normal profile, except for rRNA in the 16–30S range, which is slightly increased. The reduction of label is most pronounced in the 4S and 35–40S regions. The electrophoretic analysis of labelled cytoplasmic RNA shows distinct peaks in the 4S and 18S regions and a small one in the 28S region (Fig. 2c). There is also a minor but significant peak in the 75S region¹⁷. 75S RNA as well as rRNA appears in the cytoplasm somewhat earlier than has been previously described¹⁷. After 45 min of actinomycin D chase the

electrophoretic pattern displays a generally increased labelling in the whole spectrum, although the increase is less in the highest molecular weight range. Distinct peaks can be seen in the 4S region and in the position of the ribosomal 18S and 28S RNA components. An additional rather discrete radioactivity component emerges in the 35–40S range during actinomycin D chase. It can be seen that the reduction of labelled RNA during actinomycin D chase is largest in this range of the profile of nuclear sap RNA. This cytoplasmic 35–40S RNA has characteristic labelling kinetics, and thus this peak is not an artefact due to actinomycin D treatment. A more extensive description of this RNA fraction will be presented elsewhere.

In spite of the lack of release of prelabelled heterogeneous RNA from the chromosomes during synthesis inhibition with actinomycin D (Fig. 1), radioactivity is increased in the high molecular weight range of cytoplasmic RNA (Fig. 2). This means that prelabelled nonribosomal RNA is being transported from the nuclear sap. In fact, more than 80% of the increase in radioactivity associated with the nonribosomal cytoplasmic RNA in the 16–100S range can be accounted for by the decrease of label in the same range of the nuclear sap profile. Only 30–35% of the prelabelled nuclear sap RNA, however, disappears during 45 min of actinomycin D chase.

I have found that after 25 min incorporation of radioactive precursors into *Chironomus tentans* salivary glands, the chromosomal RNA is labelled, but no appreciable amount of label appears in the nuclear sap and cytoplasm except in the 4S region. During a subsequent actinomycin D chase for 65 min the labelling of heterogeneous chromosomal RNA decreases by 40–50% but the decrease is not accompanied by an increase of label in the nuclear sap and cytoplasm except in the 4S range. A labelling period of 45 min is sufficiently long to allow a substantial amount of labelled heterogeneous chromosomal RNA to enter the nuclear sap and cytoplasm. If a labelling period of 45 min is followed by a 45 min actinomycin D chase, an export of nonribosomal high molecular weight RNA from the nucleus to the cytoplasm can be demonstrated.

One could explain the findings of the present work as follows; first, the labelled heterogeneous chromosomal RNA consists mainly of nascent chains or of nascent and finished RNA chains, all bound to the DNA template and, second, the appearance of finished RNA molecules (processed or unprocessed) in nuclear sap before the addition of actinomycin D is a prerequisite for transport of labelled RNA from nucleus to cytoplasm. Although some qualitative aspects of RNA transfer from the nucleus to the cytoplasm can be studied by the pulse-chase technique including actinomycin D, it is definitely not a method of choice for examining turnover rate, processing of rapidly labelled RNA and for quantitation of heterogeneous RNA transfer from nucleus to cytoplasm. This is because actinomycin D interferes with the completion and release of heterogeneous chromosomal RNA, and the contribution of nonreleasable label to the total labelled heterogeneous nuclear RNA cannot be determined in most cell systems.

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¹ Kersten, W., Kersten, H., and Rauen, H. M., *Nature*, **187**, 60–61 (1960).

² Goldberg, I. H., Rabinowitz, M., and Reich, E., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 2094–2101 (1962).

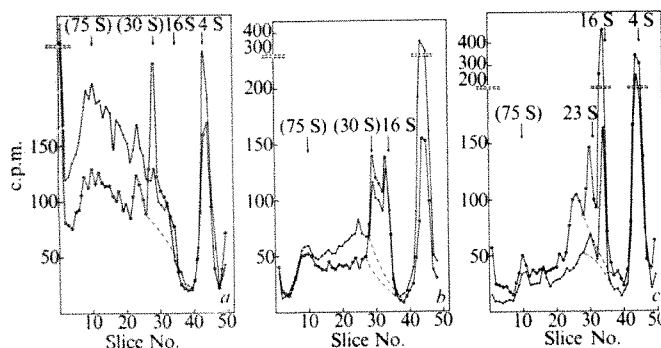


Fig. 2 Electrophoretic separation of, a, chromosomal RNA, b, nuclear sap RNA and, c, cytoplasmic RNA after 45 min labelling and after 45 min labelling+45 min of synthesis inhibition. Four glands from four different animals were placed in 50 μ l of incubation medium containing 100 μ Ci tritiated cytidine and 100 μ Ci tritiated uridine, and their sister glands were placed in another 50 μ l of the same medium. The glands were incubated at 18° C for 45 min. Next, actinomycin D was introduced to one of the droplets at a concentration of 10 μ g ml⁻¹ and the glands were incubated for another 45 min, while the incubation of control glands was interrupted. Cell components from five cells per gland were dissected. ●, Normal cells; ○, cells treated with actinomycin D.

- ³ Harbers, E., and Müller, W., *Biochem. biophys. Res. Commun.*, **7**, 107-110 (1962).
- ⁴ Tamaoki, T., and Müller, G. C., *Biochem. biophys. Res. Commun.*, **9**, 451-454 (1962).
- ⁵ Harris, H., *Nature*, **198**, 184-185 (1963).
- ⁶ Bruns, G. P., Fischer, S., and Lowy, B. A., *Biochim. biophys. Acta.*, **95**, 280-290 (1965).
- ⁷ Scherrer, K., Marcaud, L., Zajdela, F., London, I. M., and Gros, F., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1571-1578 (1966).
- ⁸ Attardi, G., Parnas, H., Hwang, M.-I. H., and Attardi, B., *J. molec. Biol.*, **20**, 145-182 (1966).
- ⁹ Penman, S., Vesco, C., and Penman, M., *J. molec. Biol.*, **34**, 49-69 (1968).
- ¹⁰ Endo, Y., Tominaga, H., and Natori, Y., *Biochem. biophys. Acta.*, **240**, 215-217 (1971).
- ¹¹ Singer, R. H., and Penman, S., *J. molec. Biol.*, **78**, 321-334 (1973).
- ¹² Scholtissek, C., *Eur. J. Biochem.*, **28**, 70-73 (1972).
- ¹³ Beermann, W., *Chromosoma*, **5**, 139-198 (1952).
- ¹⁴ Cannon, G. B., *Science*, **146**, 1063 (1964).
- ¹⁵ Ringborg, U., and Rydlander, L., *J. Cell Biol.*, **51**, 355-368 (1971).
- ¹⁶ Egyházi, E., Daneholt, B., Edström, J.-E., Lambert, B., and Ringborg, U., *J. molec. Biol.*, **44**, 517-532 (1969).
- ¹⁷ Daneholt, B., and Hosick, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 442-446 (1973).
- ¹⁸ Egyházi, E., *J. molec. Biol.*, **84**, 173-183 (1974).
- ¹⁹ Edström, J. E., and Tanguay, R., *J. molec. Biol.*, **84**, 569-583 (1974).
- ²⁰ Daneholt, B., Edström, J.-E., Egyházi, E., Lambert, B., and Ringborg, U., *Chromosoma*, **28**, 399-417 (1969).
- ²¹ Egyházi, E., and Edström, J.-E., *Biochem. biophys. Res. Commun.*, **46**, 1551-1556 (1972).

Defective DNA repair in Fanconi's anaemia

FIBROBLASTS from patients with Fanconi's anaemia (FA) show increased susceptibility to transformation *in vitro* by SV40 virus¹, as well as spontaneous chromosome breakage². Chromosomal instability has also been observed in cultured peripheral blood lymphocytes and in bone marrow cells³. More recently, Sasaki and Tonomura⁴ demonstrated an increased susceptibility of lymphocytes from patients with FA to chromosome breakage by DNA cross linking agents. These results were interpreted as an indication that FA cells are defective in some DNA repair mechanism.

The studies we report here demonstrate that DNA repair by fibroblasts from a patient with FA is defective and suggest that the abnormality is due to a defect in the excision of DNA lesions. This is probably the result of a deficiency of a specific exonuclease.

The first step in the removal of ultraviolet-induced thymidine dimers or any other DNA damage which does not directly produce breaks in the DNA is the production of a single-strand scission by an endonuclease⁵. This can be demonstrated in normal cells by observing the shift in sedimentation of DNA, 90 min after irradiation with ultraviolet light, from the normal distribution to one in which the small DNA fragments sediment at a much slower rate (Fig. 1a). Figure 1b shows the results of incubating the cells for 10 h after they have been irradiated. A fraction of the DNA shifted back toward the normal sedimentation peak, suggesting that repair was taking place.

There was also a reduction in the S value of DNA in FA cells as a result of ultraviolet irradiation. Figure 1a shows that 90 min after FA cells were subjected to ultraviolet irradiation of 250 ergs mm⁻², the major peak disappeared and the DNA sedimented at the lower sedimentation rate characteristic of the light DNA occurring in FA cells in the absence of ultraviolet irradiation. Incubation of FA cells for 10 h after ultraviolet irradiation (Fig. 1b) did not result in any shift back towards the normal sedimentation pattern. These results suggest that the cells can produce single strand breaks after ultraviolet irradiation,

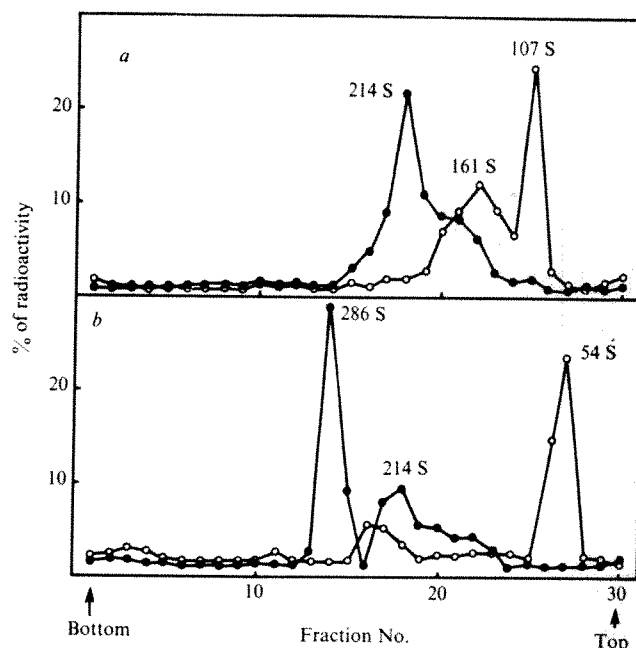


Fig. 1 Alkaline sucrose gradient sedimentation of labelled DNA from normal (●) and Fanconi's anaemia (○) fibroblasts (American Type Culture Collection No. HG261) after ultraviolet irradiation: *a*, 90 min after 250 ergs mm⁻²; *b*, 10 h after 250 ergs mm⁻². Cells were grown as a monolayer in minimal Eagle's medium with Earle's balanced salt solution supplemented with 10% foetal calf serum (inactivated), kanamycin (100 µg ml⁻¹), and non-essential amino acids. Two days after seeding the medium was replaced by medium containing ³H-thymidine (0.2 µCi ml⁻¹, specific activity 50 Ci mmol⁻¹, Amersham/Searle), and the cultures were grown for 18-22 h at 37° C in an atmosphere of 5% CO₂-95% air. The monolayer cells were washed three times with calcium and magnesium-free phosphate-buffered saline (PBS) and scraped or trypsinised from the flasks. The cell suspension was washed again and diluted to 5 × 10⁵ cells per ml with PBS. The sucrose solution was made with nuclease-free crystal sucrose (Schwarz/Mann) in double distilled water containing 0.9 M NaCl, N NaOH and 1 mM EDTA-disodium, pH 12.2. Linear sucrose gradients in polyallomer centrifuge tubes (36 ml) with a concentration of 5% sucrose at the top and 20% at the bottom were generated by a Beckman density gradient former. At the top of each gradient 0.3 ml of 0.5 N NaOH were overlaid and 0.3 ml of cell suspension (5 × 10⁵ cells per ml labelled with ³H-thymidine in PBS) was gently pipetted in. The tubes were kept for 2 h at room temperature and at 4° C for 16 h. The gradients were centrifuged at 23,000 r.p.m. for 2 h at 10° C in an SW 27 rotor of a Spinco Model L3-50 (Beckman) ultracentrifuge. Fractionation of the gradients was performed with a hypodermic needle pierced through the bottom of the tube and fractions were collected with a peristaltic pump through an automated fraction collector. Each fraction was precipitated by addition of 5% trichloroacetic acid, collected on an MF Millipore filter. Radioactivity on the dried filters was determined by transfer to 10 ml of a toluene based scintillation cocktail which was counted in a Beckman LS 250 liquid scintillation spectrometer.

tion, indicating the presence of an endonuclease, but cannot complete the repair process.

After cells have been irradiated with ultraviolet light and produce a single strand break (nick) near the induced pyrimidine dimers, the next step in the repair process involves the unwinding of the two strands in the neighbourhood of the nick and the polymerisation of a new strand of DNA complementary to the intact member of the DNA double helix⁵. This polymerisation can be demonstrated by unscheduled DNA synthesis detected autoradiographically by the incorporation of ³H-thymidine into the cellular DNA or, more specifically, by repair replication as assayed by Cleaver⁶. Unscheduled DNA synthesis in FA cells is comparable with that which occurs in normal cells (Fig. 2).

The incorporation of ^3H -thymidine, as indicated by the number of grains over the nuclei of the irradiated cells, increased as a function of the dose of irradiation and there was no distinguishable difference between the relative number of grains in FA cells and normal cells.

Unscheduled DNA synthesis was induced by treating FA cells with 4-nitroquinoline-N-oxide (4NQO), a carcinogen known to induce unscheduled DNA synthesis and replication repair synthesis in human cells (our unpublished studies). This agent induced no unscheduled DNA synthesis in the cells of patients with xeroderma pigmentosum^{7,8}, suggesting that it does not produce single-strand breaks directly. The fact that it induced unscheduled synthesis in FA cells suggests that they can make single-strand scissions after treatment with 4NQO as well as after irradiation with ultraviolet light.

To complete the repair of ultraviolet-induced DNA damage, it is necessary for the damaged strand to be excised so that the newly synthesised strand can be linked by phosphodiester bonds to the end of the remaining portion of the damaged strand. This is generally considered to be the function of a specific exonuclease. Exonucleases which seem to be specific for ultraviolet-induced damage have been isolated from cells⁹. The excision of thymidine dimers can be demonstrated by measuring the dimer content of irradiated DNA immediately after irradiation and again after irradiated cells have been incubated for a repair period. After the irradiation the number of dimers detectable in the DNA diminishes⁵. Figure 3 shows the results of an experiment in which normal cells were exposed to various doses of ultraviolet light and the number of dimers was determined in the DNA immediately and after a 24 h incubation. The number of thymidine dimers was a function of the ultraviolet dose over the range examined and after 24 h a large fraction of the dimers had been removed from the DNA of the normal cells.

When FA cells were subjected to the same doses of ultraviolet irradiation, the number of dimers measured immediately was essentially the same as seen in normal cells (Fig. 3). During a 24 h postirradiation period, however, FA cells could remove only a small fraction of the induced dimers. This difference was not apparent at ultraviolet doses below 150 ergs mm^{-2} , possibly because the FA cells could remove during the 24 h incubation,

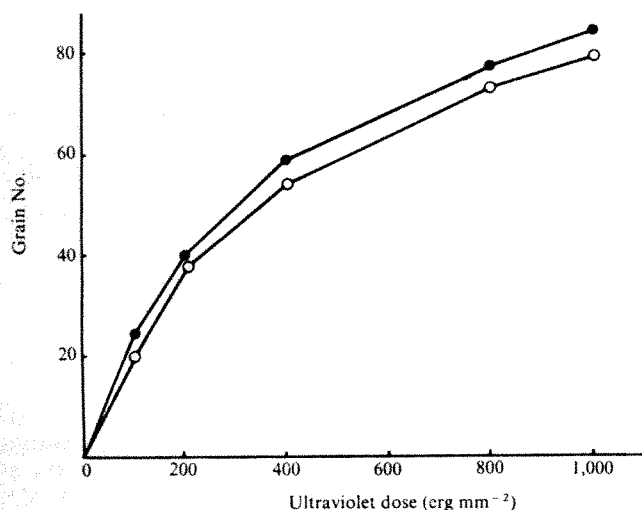


Fig. 2 Effect of ultraviolet irradiation on grain count of normal fibroblasts (●) and FA fibroblasts (○). Cells on coverslips were rinsed with PBS and pulsed with ^3H -thymidine ($2 \mu\text{Ci ml}^{-1}$, 50 Ci mmol^{-1}) for 1 h to identify cells in S phase. After ultraviolet irradiation at various doses, cells were exposed to ^3H -thymidine again for 2 h. Autoradiographs were prepared and the average grain count over lightly labelled cells was determined after a 2-week exposure.

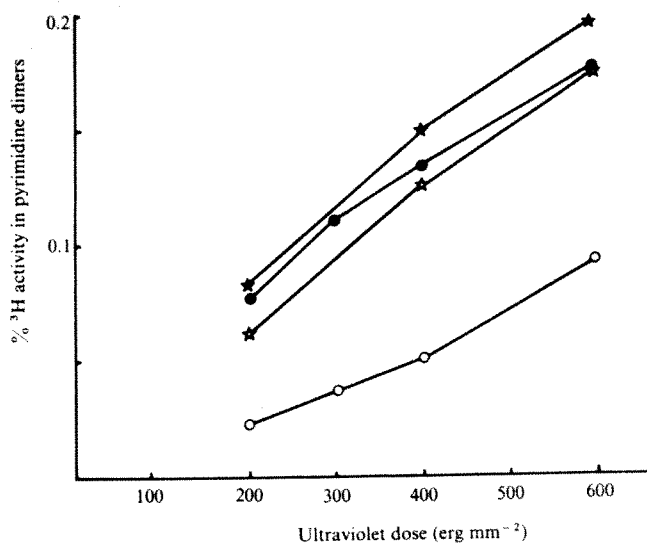


Fig. 3 Comparison of excision of thymine-containing pyrimidine dimers from ultraviolet-irradiated, ^3H -thymidine-labelled normal human fibroblasts and fibroblasts from a patient with FA: normal human fibroblasts immediately after irradiation (●) and 24 h after irradiation (○); FA cells immediately after irradiation (★) and 24 h after irradiation (☆). Cells growing in a glass Petri dish were labelled with ^3H -thymidine ($5 \mu\text{Ci ml}^{-1}$, 50 Ci mmol^{-1}). Cultures were then rinsed with PBS and irradiated with ultraviolet light at a dose rate of $10 \text{ ergs mm}^{-2} \text{ s}^{-1}$. After irradiation some cultures were fixed immediately with perchloric acid in the cold. Others were rinsed with PBS and allowed to grow for 24 h and then fixed. 5×10^6 cells were scraped into a Pyrex tube ($7.5 \times 100 \text{ mm}$) and precipitated with 5% trichloroacetic acid. The precipitate was washed, dried and hydrolysed in 0.2 ml of 98% formic acid by heating at 175°C for 2 h. The hydrolysates were spotted on a sheet of Whatman No. 1 paper for two-dimensional chromatography and the percentage of radioactivity was measured. This method cannot distinguish the difference between normal and FA with an ultraviolet dose below 150 ergs mm^{-2} . Either FA cells demonstrate normal excision at a low dose of ultraviolet light or the method is not sufficiently sensitive to detect it.

the relatively small number of dimers produced by lower doses of ultraviolet light. This indicates that although the FA cells could produce the endonucleolytic scission as the first step of the repair process and could polymerise nucleotides to repair or replace the damaged strand of DNA, they were deficient in the ability to remove the strand of DNA bearing the abnormal thymine dimers.

We have demonstrated that FA cells possess most of the functions required for the repair of ultraviolet-induced DNA damage. They can produce single strand scissions in response to pyrimidine dimer induction and they can polymerise nucleotides into a new complementary strand to replace that damaged portion of the DNA molecule. However, they seem to be deficient in an exonuclease function which removes the damaged strand of DNA after the endonucleolytic strand scission has been made. It is interesting that the FA cells studied seem capable of rejoining X-ray-induced strand breaks, suggesting that ligase activity is present (our unpublished data).

Regan *et al.* have stated that FA cells can normally excise ultraviolet-induced pyrimidine dimers¹⁰. The reason for the discrepancy between their results and ours is not clear, but may relate to their use of lower doses of ultraviolet irradiation. Our studies have demonstrated that a dose of at least 150 ergs mm^{-2} is necessary to demonstrate a difference between normal and FA cells. Thus, although FA cells do not seem to be as sensitive to ultraviolet light as xeroderma pigmentosum cells, they are more sensitive than normal cells. In fact, our results

indicate that exonuclease activity is not completely lacking. This may also explain the lack of ultraviolet-induced skin cancer in these patients.

Another possibility is that different cell lines were studied. Our studies have all been performed on one strain of fibroblasts (American Type Culture Collection) originally obtained from a patient with FA. Therefore we cannot yet conclude that the findings are characteristic of all FA cells. Nevertheless, they are interesting since they demonstrate a failure of DNA repair at a site not previously described.

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Received February 11; revised April 15, 1974.

- ¹ Todaro, G. J., Green, H., and Swift, M. R., *Science*, **153**, 1252-1254 (1966).
- ² Schroeder, R. M., Anschütz, F., and Knopp, A., *Human-genetik*, **1**, 194-196 (1964).
- ³ Swift, M. R., and Hirschhorn, K., *Ann. intern. Med.*, **65**, 496-503 (1966).
- ⁴ Sasaki, M. S., and Tonomura, A., *Cancer Res.*, **33**, 1829-1836 (1973).
- ⁵ Cleaver, J. E., *J. invest. Dermat.*, **54**, 181-195 (1970).
- ⁶ Cleaver, J. E., *Nature*, **218**, 652-656 (1968).
- ⁷ Jacobs, A. J., O'Brien, R. L., Parker, J. W., and Paolilli, P., *Mutat. Res.*, **16**, 420-424 (1972).
- ⁸ Stich, H. F., and San, R. H. C., *Mutat. Res.*, **13**, 279-282 (1971).
- ⁹ Lindahl, T., Gally, J. A., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 597-603 (1969).
- ¹⁰ Regan, J. D., Setlow, R. B., Carrier, W. L., and Lee, W. H., in *Proc. fourth int. Cong. Radiat. Res., Symp. Biol. Med.* North Holland, Amsterdam, (in the press).

Introduction of mouse L cell nucleus into heterologous mammalian cells

HARRIS¹ first achieved the introduction of a chick erythrocyte nucleus into other nucleated mammalian cells, without any detectable incorporation of chick erythrocyte cytoplasm, using inactivated Sendai virus. Since then chick erythrocyte nuclei have provided a way of investigating the genetic regulation of nuclear synthetic activities in the presence of a genetically disparate cytoplasm and nucleus^{2,3}. Similar studies with nuclei from other mammalian cells have been presented, however, by the lack of a method for obtaining nuclei suitable for introduction into another type of cells.

When Carter⁴ first reported the enucleation of mammalian cells by the drug cytochalasin B, a metabolic product of the fungus *Helminthosporium dematioides*, he predicted that the separated nuclei and the enucleated cytoplasm might be used in nuclear exchange experiments. Since then the fusion of enucleated cells of one type with nucleated cells of another type has been achieved^{5,6}. During our earlier electron microscopic studies^{7,8} on the enucleation of mouse L cells with cyclochalasin B we observed that the nucleus ejected from the cell carried along with it a very thin rim of cytoplasm within the plasma mem-

brane. As the fusion of cells induced by the inactivated Sendai virus is believed to involve the fusion of two plasma membranes it seemed possible to fuse the nuclei obtained from cytochalasin B-treated cells with other types of cells. Here we report the introduction of mouse L cell nuclei, prepared by treating the cells with cytochalasin B, into nucleated human HeLa cells with the aid of inactivated Sendai virus.

Mouse L cells (clone 929) used for obtaining the nuclei were grown on the surface of plastic Petri dishes as monolayers in Eagle's medium containing 15% calf serum, 100 units ml⁻¹ of penicillin and 100 µg ml⁻¹ of streptomycin (GM). The cultures were prelabelled with tritiated thymidine; before use, 100 µCi of ³H-thymidine was added to the cultures every 6 h for a total of 42 h. This resulted in the labelling of almost every cell. Such prelabelled sheets of L cells were treated with cytochalasin B to bring about enucleation^{4,9}. The method used was a modification of the technique described by Prescott *et al.*⁹.

Briefly, it consisted of inserting plastic Petri dishes carrying adherent cell sheets into stainless steel centrifuge tubes. The tubes contained BM supplement with 1 µg ml⁻¹ of amphotericin B and 5 µg ml⁻¹ cytochalasin B (Serva, Feinbiochemica, Heidelberg) predissolved in dimethylsulphoxide (DMSO) at a concentration of 1 µg ml⁻¹. Centrifugation at room temperature for 45 min at 10,000g resulted in almost complete enucleation. The Petri dishes were inverted on the bottom of the test tubes (cell side facing down) so that the plane of cell layer was at an angle of 37° ± 2° to the line of centrifugal force.

The addition of amphotericin B to the reaction mixture facilitated the cytochalasin B-induced enucleation process (unpublished results). The sediment at the bottom of the centrifuge tubes consisted mainly of L cell nuclei. Figure 1 shows the enucleation phenomenon. Observed under the electron microscope the extruded nuclei reveal the presence of a thin rim of cytoplasm around them containing ribosomes, very rarely one or two mitochondria and a few fragments of endoplasmic reticulum. It was itself enclosed by a plasma membrane (Fig. 2).

The L cell nuclei were introduced into human HeLa cells maintained in GM. These cells (10⁶) were seeded in Leighton tubes containing glass coverslips and incubated at 37° C. After 48 h, the culture medium was removed and the cell sheet was layered with a thin suspension of L cell nuclei and 500 haemagglutinin units of Sendai virus (inactivated with 0.06% β-propiolactone¹⁰). The cultures were

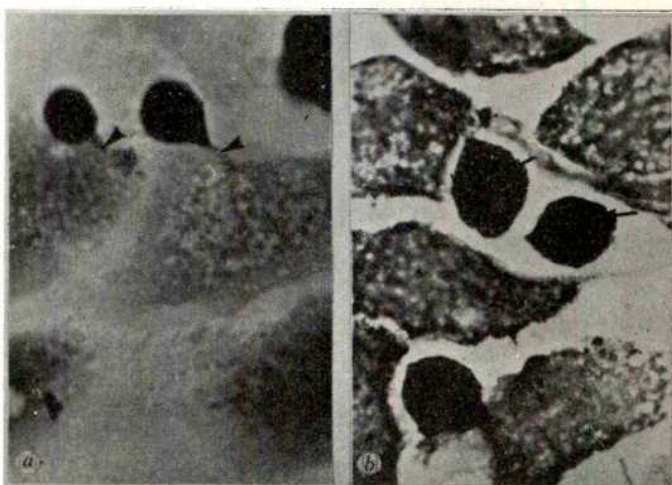


Fig. 1 L cell enucleation by cytochalasin B. a, during extrusion from the cells, nuclei are connected to the cells by thin cytoplasmic processes (arrows); b, Nuclei ejected from the cell cytoplasm show prominent nucleoli (arrows). Giemsa stain (×528).

kept in the cold for 10 min followed by incubation at 37° C for 1 h. Fresh GM was added after washing off the excess nuclei and inactivated virus. Some of the coverslips were processed immediately for autoradiography but other coverslip cultures were incubated further and examined at regular intervals.

The presence of labelled L cell nuclei near the unlabelled HeLa cell nucleus (Fig. 3b) provided unequivocal evidence of the introduction of the L cell nuclei into HeLa cells. Approximately 10% of the cells were binucleate heterokaryons (Fig. 3a), the remaining being multinucleate heterokaryons, homokaryons or mononucleate HeLa cells. (Heterokaryons and somatic cell hybrids are the terms generally reserved for cells arising following the fusion of two or more genetically different types of cytoplasm and nuclei and as such have been used here.)

The presence of L cell membrane properties on the cell surface of heterokaryons provided evidence for the effective functioning of the introduced L cell nucleus. Mouse species-specific antigens were detected by the mixed haemadsorption technique^{11,12}. L cells carry on their surface a type C virus-induced antigen and this was detected on heterokaryons by indirect membrane immunofluorescence using anti-Moloney virus serum as described by Fenyo *et al.*¹³. HeLa cells showed complete absence of these surface properties.

The multinucleate heterokaryons and homokaryons gradually disappeared from the culture within 1–2 weeks, leaving behind either mononucleate hybrid cells or HeLa cells. The recognition of hybrid cells presented no problem as their cytoplasm was not well spread and the nucleus was frequently larger than the HeLa cell nucleus. These hybrids continue to express some of the L cell surface traits. A detailed account of the studies on the karyotypic analysis of these hybrids and their antigenic properties will be reported elsewhere.

The possible contamination of these hybrids with a small amount of L cell cytoplasm, which remains attached to the L cell nucleus, cannot be excluded. It seems unlikely, however, that such a small amount of cytoplasm in the hybrid will have any controlling effect on the expression of nuclear functions as it would be rapidly diluted out in the hybrid progeny cells.

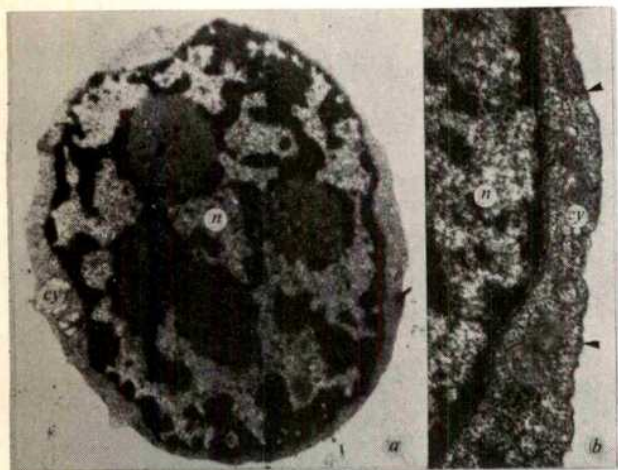


Fig. 2 Electron micrographs of the ejected L cell nuclei. *a*, Ejected L cell nucleus, *n*, surrounded by a thin fragment of the cell cytoplasm, *cy*, containing some of the cytoplasmic organelles ($\times 8,200$); *b*, Enlarged portion of the ejected L cell nucleus showing a distinct plasma membrane (arrows) surrounding the cytoplasmic fragment, *cy*, which in turn encloses the nucleus, *n* ($\times 24,000$).

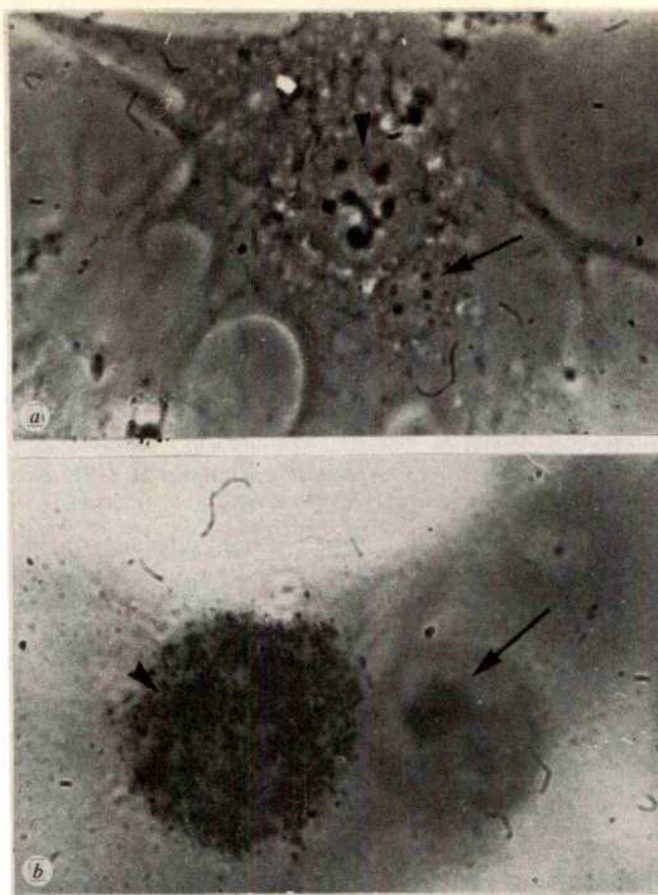


Fig. 3 *a*, Phase contrast photomicrograph of a HeLa cell containing the introduced large L cell nucleus (arrow). Note the relatively small HeLa cell nucleus (long arrow) ($\times 1,125$); *b*, Autoradiograph of a heterokaryon containing unlabelled HeLa cell nucleus (long arrow) and the labelled L cell nucleus (arrow) within HeLa cell cytoplasm. The L cells were grown in ³H-thymidine before the nuclei were removed and introduced into HeLa cells ($\times 1,418$).

Our demonstration of the introduction of L cell nuclei into HeLa cells makes possible the introduction of isolated mammalian cell nuclei into enucleated cells, thereby allowing true nuclear exchange experiments which have been previously impossible with mammalian cells.

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Received February 25, 1974.

- ¹ Harris, H., *Nature*, **206**, 583 (1965).
- ² Harris, H., *Cell Fusion, The Dunham Lectures* (Harvard University Press, Cambridge, Massachusetts, 1970).
- ³ Schwartz, A. G., Cook, P. R., and Harris, H., *Nature new Biol.*, **230**, 5 (1971).
- ⁴ Carter, S. B., *Nature*, **213**, 261 (1967).
- ⁵ Poste, G., *Nature*, **229**, 123 (1971).
- ⁶ Ladda, R. L., and Estensen, R. D., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1528 (1970).
- ⁷ Sethi, K. K., Pelster, B., Piekarski, G., and Brandis, H., *Nature new Biol.*, **243**, 255 (1973).
- ⁸ Sethi, K. K., Pelster, B., and Brandis, H., *J. gen. Virol.*, **21**, 435 (1973).
- ⁹ Prescott, D. M., Myerson, D., and Wallace, J., *Expl Cell Res.*, **71**, 480 (1970).
- ¹⁰ Neff, J. M., and Edders, J. F., *Proc. Soc. exp. Biol. Med.*, **129**, 854 (1968).
- ¹¹ Poste, G., and Reeve, P., *Nature new Biol.*, **229**, 123 (1971).
- ¹² Watkins, J. F., and Grace, D. M., *J. Cell Sci.*, **2**, 193 (1967).
- ¹³ Fevno, E. M., Girundner, G., Klein, G., Klein, E., and Harris, H., *Expl Cell Res.*, **68**, 323 (1971).

Effect of β_2 microglobulin antibody on effector function of T-cell mediated cytotoxicity

A MEMBRANE fragment of human lymphoid cells obtained by papain digestion has been found to be composed of two subunits which differ in molecular weight^{1,2}. The heavy molecular weight subunit (33,000 daltons) carries the HL-A serologically defined (SD) antigens while the light molecular weight subunit (11,000 daltons) carries antigenic determinants reacting with antiserum directed against β_2 microglobulin (β_2m)³⁻⁶. Treatment of human lymphocytes with antisera directed against the HL-A SD antigens results in capping of these antigens⁷; addition of β_2m antibody to lymphocytes will likewise cause capping of the HL-A SD antigens^{8,9}. Furthermore, activation of lymphocytes in mixed leukocyte culture (MLC) can be inhibited by β_2m antibody^{9,10}. These data suggested to the investigators^{9,10} that the HL-A subunit and/or the β_2m subunit may be part of the same receptor complex of the T lymphocyte. This concept is supported by the recent studies of β_2m demonstrating extensive homology between β_2m and the constant regions of the heavy chain (and the light chain) of immunoglobulin IgG^{11,12}. Consequently β_2m was thought to have a recognition function similar to that of immunoglobulin.

Lymphocytes stimulated by allogeneic cells in MLC specifically destroy target cells autologous to the stimulating cells¹³. Effector cells generated in MLC are thought to be thymus-derived (T) cells¹⁴. We wish to report here that preincubation of MLC-generated effector cells with β_2m antibody has no effect on their ability to specifically recognise and destroy target cells autologous to the sensitising cells. Preincubation of target cells with anti β_2m antibody resulted in enhancement of cytotoxicity due to an antibody-dependent cell-mediated cytotoxicity.

The purification of lymphocytes, generation of effector cells in MLC and preparation of ⁵¹Cr-labelled target cells has been previously described^{13,15}. Anti- β_2m antiserum was prepared in New Zealand rabbits¹⁶. All sera were decanted by heating at 56°C for 30 min. The anti- β_2m was rendered specific by absorption with lyophilised human serum, lyophilised urine of normal individuals and erythrocyte membranes. The antiserum gave a single precipitin line on Ouchterlony immunodiffusion plates and immunoelectrophoresis with pure β_2m and with sera from patients containing large amounts of β_2m . Addition of purified β_2m to the antiserum completely eliminated the enhancement of cytotoxicity when the target cells were

preincubated with β_2m antibody. For preincubation of effector or target cells, a volume of 50 μ l of undiluted β_2m antiserum or normal rabbit serum was added to 0.1 ml of lymphocytes (2×10^6) and incubated at 4°C for 1 h. The cells were centrifuged (400g) for 10 min, washed three times in tissue culture media 199 (TC-199) and used immediately in the cytotoxicity reaction. The target cells were labelled with ⁵¹Cr before incubation with the β_2m antiserum.

The cytotoxicity reaction was conducted in microculture plates with 1×10^6 effector cells and 1×10^4 target cells contained in a total volume of 0.2 ml TC-199 supplemented with 20% human frozen pooled plasma. The plate was incubated for 4 h at 37°C in an atmosphere of 5% CO₂ and 95% air. Then the plate was centrifuged, an aliquot removed and ⁵¹Cr release monitored as previously described¹⁵. All reactions were done in triplicate.

As demonstrated in Table 1, lymphocytes of individual A were sensitised in MLC to the histocompatibility antigens of individuals B or C. The degree of stimulation was measured by ³H-thymidine incorporation. The allogeneic combinations AB or AC produced good stimulation while the autologous combination (AA) produced relatively little ³H-thymidine incorporation. After 6 d, ⁵¹Cr labelled lymphoblasts were added to the responder cells (now termed effector cells). Significant ⁵¹Cr release was obtained when the target and stimulator cells were autologous (AB/B). When the stimulator and target were allogeneic (AC/B) no ⁵¹Cr release was demonstrated. In addition, when the responder-stimulator combination was autologous (AA/B) or when the target and responder cells were autologous (AB/A) no ⁵¹Cr release was obtained. Preincubation of the sensitised effector cells with the β_2m antiserum had no significant effect on the ability of the cells to specifically destroy target cells. Preincubation of the target cells with the same concentration of anti- β_2m resulted in an enhancement of the cytotoxicity, which was due to an antibody-dependent cell-mediated type of cytotoxicity first described by Moller¹⁷. In this type of cytotoxicity unsensitised lymphocytes perform equally well as effector cells. As demonstrated in Table 1, unsensitised lymphocytes (A) used as effector cells against the target cell (B) produced excellent ⁵¹Cr release. The β_2m antiserum was used in the experiments reported here at a final dilution of one to three. Complete inhibition of MLC was produced with this same antiserum at a dilution 1 to 50¹⁸.

Antisera directed against either β_2m or the SD antigens of the HL-A complex inhibit the MLC. In addition, β_2m and the SD antigens have been shown to be associated on the lymphocyte membrane. These facts, in addition to the homology between β_2m and the constant domains of IgG, have led several investi-

Table 1 Effect of β_2 microglobulin antibody on effector and target cells

Responder/stimulator	MLC Mean c.p.m. \pm s.d.	Target	Preincubation serum (cells preincubated)	⁵¹ Cr Release*		
				Exp. value mean \pm s.d.	Exp. value minus spontaneous release	⁵¹ Cr Release
AB	31,873 \pm 2,461	B	normal rabbit (target and effector)	3,547 \pm 231	1,980	46.7
AA	795 \pm 382	B		1,591 \pm 84	24	0.6
AB	31,873 \pm 2,461	A		1,533 \pm 125	-34	-0.8
AC	41,954 \pm 3,412	B		1,727 \pm 107	160	3.8
AB	31,873 \pm 2,461	B	anti- β_2m (effector)	3,641 \pm 54	2,074	48.9
AB	31,873 \pm 2,461	B	anti- β_2m (target)	2,874 \pm 73	2,156	95.4
AB	31,873 \pm 2,461	B	anti- β_2m antibodies with β_2m (target)	1,899 \pm 171	1,181	52.3
A	(unsensitised)	B	anti- β_2m (target)	2,861 \pm 56	2,143	94.8

* Spontaneous release (c.p.m. \pm s.d.) for B (Preincubated normal rabbit serum): 1,567 \pm 21. Spontaneous release for B (preincubated anti- β_2m): 718 \pm 34. Frozen thawed c.p.m. for B (preincubated normal rabbit serum): 5,804 \pm 156. Frozen thawed B (preincubated anti- β_2m): 2,978 \pm 71.

$$^{51}\text{Cr release} = \left[\frac{\text{Expected value} - \text{spontaneous release}}{\text{Frozen thawed} - \text{spontaneous release}} \right] \times 100$$

gators to suggest that the β_2m subunit and the alloantigenic subunit are closely associated with the lymphocyte receptor^{9,10}. Convincing evidence has been put forward demonstrating that the genetic loci responsible for stimulation in MLC are separate from the HL-A loci coding for the SD antigens²¹⁻²⁵. Evidence from several laboratories^{19,20} have suggested that effector cells generated in MLC recognise only SD antigens. Therefore, the receptor sites on the T cells responsible for cell destruction are presumably directed toward the SD antigens. Our data suggest that the receptor sites responsible for the recognition do not seem to be associated with β_2m . In addition, Poulik *et al.* have demonstrated that there is no association between β_2m and surface immunoglobulin on the membrane of human B cells⁸. One cannot rule out the possibility that the receptor which recognises the gene product of the MLC loci is of a different type (that is, associated with β_2m) than that responsible for cell destruction.

The relationship between β_2m and the antigen on the target cell responsible for cell mediated cytotoxicity is unclear because of the interference by the antibody-dependent cell-mediated cytotoxicity. This problem can be eliminated by using F(ab)₂ of the β_2m antibody in the cytotoxicity reaction. Experiments toward this end are in progress.

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- ¹ Miyakawa, Y., Tanigaki, N., Yagi, Y., and Pressman, D., *Immunology*, **24**, 67 (1973).
- ² Cresswell, P., Turner, M. J., and Strominger, J. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1603 (1973).
- ³ Nakamuro, K., Tanigaki, N., and Pressman, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2863 (1973).
- ⁴ Peterson, P. A., Rask, L., and Lindholm, J. B., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁵ Grey, H. M., Kubo, R. T., Colon, S. M., and Poulik, M. D., Cresswell, P., Springer, T., Turner, M., and Strominger, J. L., *J. exp. Med.*, **138**, 1608 (1973).
- ⁶ Tanigaki, N., Nakamuro, K., Appella, E., Poulik, M. D., and Pressman, D., *Biochem. biophys. Res. Commun.*, **55**, 1234 (1973).
- ⁷ Bernoco, D., Cullen, S., Scudeler, G., Trinchieri, G., and Ceppellini, R., *Histocompatibility Testing*, 1972 (edit. by Dausset, J., and Colombani, J.), 527 (Munksgaard, Copenhagen, 1973).
- ⁸ Poulik, M. D., Bernoco, M., Bernoco, D., and Ceppellini, R., *Science*, **182**, 1354 (1973).
- ⁹ Solheim, B. G., and Thorsby, E., *Tissue Antigens*, **4**, 1974.
- ¹⁰ Bach, M. L., Huang, S., Hong, R., and Poulik, M. D., *Science*, **182**, 1352 (1973).
- ¹¹ Smithies, O., and Poulik, M. D., *Science*, **175**, 187 (1972).
- ¹² Peterson, P. A., Cunningham, B. A., Berggard, I., and Edelman, G. E., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1967 (1972).
- ¹³ Lightbody, J. J., Bernoco, O., Miggiano, V. C., and Ceppellini, R., *G. Batt. Immun.*, **64**, 273 (1971).
- ¹⁴ Mosier, D., and Cnator, H., *Eur. J. Immun.*, **1**, 459 (1971).
- ¹⁵ Lightbody, J., and Bach, F. H., *Annls. Inst. Pasteur, Paris*, **124**, 311 (1973).
- ¹⁶ Poulik, M. D., and Bloom, A. D., *J. Immun.*, **110**, 1430 (1973).
- ¹⁷ Moller, E., *Science*, **147**, 873 (1965).
- ¹⁸ Lightbody, J., and Poulik, M. D., *Fedn Proc.* (in the press).
- ¹⁹ Eijssvoegel, V. P., du Bois, M. J. G. J., Melief, C. J. M., *et al.*, *Histocompatibility Testing*, 1972 (edit. by Dausset, J., and Colombani, J.), 501 (Copenhagen, Munksgaard, 1973).
- ²⁰ Trinchieri, G., Bernoco, D., Curtoni, S. E., *et al.*, *Histocompatibility Testing*, 1972 (edit. by Dausset, J., and Colombani, J.), 509 (Copenhagen, Munksgaard, 1973).

- ²¹ Yunis, E. J., and Amos, D. B., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3031 (1971).
- ²² Bach, F. H., and Amos, D. B., *Science*, **156**, 1506 (1967).
- ²³ Plate, J. M., Ward, F. E., and Amos, D. B., in *Histocompatibility Testing*, 1970 (edit. by Terasaki, P. I.), 531 (Munksgaard, Copenhagen, 1970).
- ²⁴ Dupont, B., Good, R., Hansen, G., Jersild, C., Nielsen, L. S., Park, B., Svejgaard, A., Thomsen, M., and Yunis, E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 52 (1974).
- ²⁵ Eijssvoegel, V. P., Konig, L., de Groat-Kooy, L., Huismans, L., van Rood, J. J., van Leeuwen, A., and du Toit, E. D., *Transplantn. Proc.*, **4**, 199 (1972).

New alloantigen genetically linked to the major histocompatibility locus of the mouse

THE mouse *H-2* locus and its genetic homologue, the *HL-A* locus in man, are much more complex than originally envisaged, and are now referred to as the major histocompatibility complex (MHC)¹. Transplant rejection is more closely correlated with mixed lymphocyte reaction (MLR) than with tissue typing²⁻⁶ and in addition to an *MLR* region in the MHC other regions are of obvious importance in the context of recognition, for example the immune responsiveness regions (*Ir*)^{7,8}. The only gene products of the MHC so far characterised are those recognised serologically by 'tissue typing' antisera, that is, *H-2* antigens and their homologues in other species. These products are not the only substances involved in the fate of allografts; indeed sensitisation against an allograft seems to require MLR differences although serologically detected components have an, as yet undefined, role in the effector arc of rejection⁹⁻¹¹. Though there are suggestions that MHC gene products other than *H-2* (*HL-A*, and so on) may be primarily involved in graft rejection¹², the only *in vivo* studies pointing strongly in that direction were carried out in the rat enhancement model¹³. In these studies rat heart allografts were passively enhanced by alloantisera from which Ag-B (the *H-2* homologue in rats) antibodies had been removed by absorption with appropriate red blood cells (RBC), which suggests a role for antibodies directed against other products of the MHC.

MLR and graft-versus-host reactions may occur in the absence of serological differences at the MHC³⁻⁵ and since genetically engineered situations have led to the detection of new T- and new B-cell associated antigens in the mouse system¹⁴⁻¹⁷, great importance attaches to the definition of other products controlled by the MHC. Here we describe some properties of a gene product found on thymus-derived lymphocytes and determined by a gene near the *K-end* of the MHC.

In a complement-dependent cytotoxicity test, an anti-serum (kxb anti-d) prepared in (B10.Br×C57BL/6)_{F1} mice by immunisation with DBA/2 spleen cells showed, after absorption with B10.D2 or BALB/c (*H-2^b*) RBC, only 25-30% ⁵¹Cr release with B10.D2 lymph node target cells. This target cell is used to provide a congenic system; hence genetic markers not related to the MHC remain undetected. A membrane suspension from BALB/c spleen cells was solubilised by controlled papain digestion, fractionated on DEAE Sephadex-A50, and six peaks eluted stepwise at (1) 0.05 M, (2) 0.1 M, (3) 0.15 M, (4) 0.2 M, (5) 0.25 M and (6) 0.5 M NaCl were collected¹⁸. Fractions 3 and 4 contained the *H-2* activity (*H*-antigens). These six fractions when monitored with the RBC absorbed antiserum, showed a totally different pattern: only fraction 5, eluted with 0.3 M NaCl showed specific inhibition of immune cytotoxicity (160 ID₅₀ units mg⁻¹). An included fraction corresponding

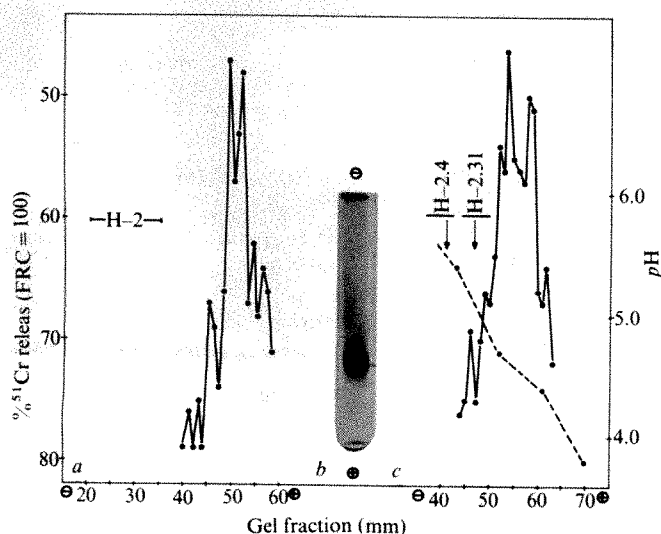


Fig. 1 Polyacrylamide gel electrophoresis. *a*, Serological profile of a highly purified antigen fraction. Gel slices (1 mm) were eluted, dialysed and freeze dried, and the material was tested for alloantigenic activity by inhibition of complement-dependent lysis (^{51}Cr release). For this purpose an H-2 alloantiserum was first absorbed with red blood cells from H-2^d mice¹³. Inhibition of immune cytotoxicity (O—O). The position where H-antigens would be found in the same system¹⁹ is indicated by the horizontal bar. *b*, Staining pattern of the new alloantigen; migration is from - to +. The discontinuous system used in the present work has been described in detail previously^{18,19}. *c*, Isotachopheresis of the same fraction, performed as detailed elsewhere^{18,19}. pH measurements were carried out in the gel eluates (1 mm fractions). The position (pH) where H-2 antigens would appear but which were removed by previous DEAE chromatography, is indicated by arrows. A specificity check was performed in parallel (not shown) using a RBC-absorbed B10.D2 anti-B10.A antiserum with B.10A target cells. Inhibition of immune cytotoxicity (O—O); pH (●—●). FRC=full release control, indicating 100% ^{51}Cr release.

to an apparent molecular weight of 36,000 (1,700 ID₅₀ units mg^{-1}) was isolated by Sephadex G-100 chromatography. On SDS electrophoresis this fraction migrated with an average M_r of 34,000. After elution from polyacrylamide gel electrophoresis, a serologically active peak was obtained at a relative migration value of about 0.67, corresponding to a single band in a stained gel run in parallel. For comparison, H antigens migrated in the same system with R_F values of about 0.4. A further purification on DEAE Sephadex-A50 provided an active fraction with an electrophoretic and serological pattern as depicted in Fig. 1*a* and *b*. Fig. 1*c* shows the pI (pH 4.6) of this substance compared to about pH 5.0 (H-2.31) and 5.4 (H-2.4) for H antigens. Furthermore this material contains 0.57 mol SH per mol protein as determined by DTNB (ref. 20).

Previous results indicated that desorption of papain-solubilised H-antigens reacting with H-2.34 antisera occurred later than the main H-2 fraction on ion exchange chromatography²¹. The reactivity of the RBC-absorbed H-2 antiserum with the new antigen was, however, unimpaired after the antiserum had been further absorbed with DBA-1 (H-2^a) lymph node cells, thus eliminating H-2.34. A genetic linkage to the MHC is clear since the antiserum used was obtained from (B10.Br \times C57BL/6)_{F1} mice providing congenicity with the B10.D2 target cells.

When tested for *D-end* activity, two antisera ($K^kD^d \times K^bD^b$) anti K^dD^d (Fig. 2*a*) and ($K^kD^d \times K^{bc}D^{bc}$) anti K^dD^d (Fig. 2*b*) showed no residual titre using K^kD^d or K^dD^d target cells respectively. This indicates that the residual cytotoxicity observed in Fig. 2*b* for the H-2.31 antiserum and in Fig. 2*c* is a *K-end* reaction and that the antigenic product is controlled by gene(s) located near the *K-end* of the MHC.

It is of particular significance in this context that the *Ir*

region is in the close vicinity of the *K-end* of the MHC⁸. *Ir* gene products have been considered to be candidates for T-cell recognition structures⁷. We would like to propose the term 'R-antigen' for these substances in contrast to

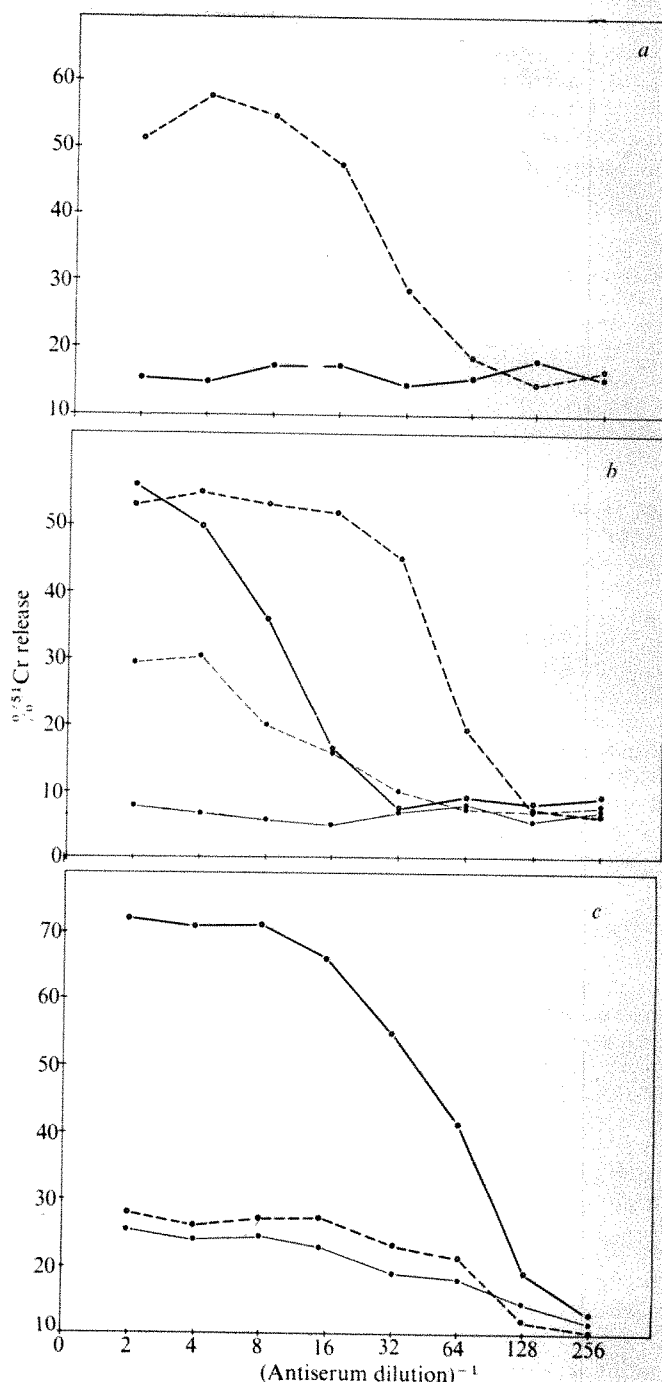


Fig. 2 An antiserum (H-2.4, 10, 13, 31, 34, 40, 41, 42, 43, 44) was prepared by immunisation of $K^dD^d \times K^bD^b$ F_1 hybrids with (K^dD^d) mice. Titres obtained in cytotoxicity test (^{51}Cr release) using *a*, B10.A (K^dD^d) target cells with the H-2 antiserum described above (AS 73/5) after absorption with DBA/1 (K^dD^d) lymph node cells (which removes H-2.13, 34), (O—O) and with AS 73/5 absorbed with B10.D2 (K^dD^d) red blood cells (removing the remaining H-2 specificities), (●—●); *b*, B10.D2 target cells with H-2.4 (—) and H-2.31 (---) antisera before (O) and after (●) RBC absorption. *c*, Serological pattern of AS 73/5 tested against B10.D2 target cells before (O—O) and after (●—●) absorption with B10.D2 or BALB/c RBC and after further absorption with DBA/1 lymph node cells (●—●).

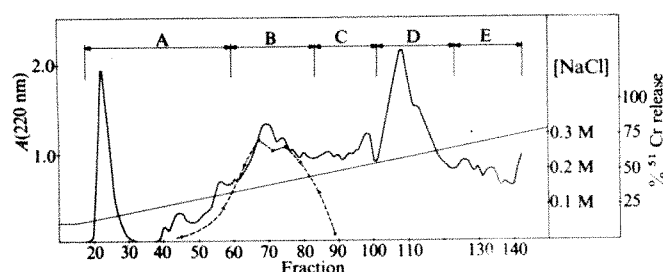


Fig. 3 DE-52 chromatography pattern of a thymus cell membrane preparation solubilised with papain (1:100, 1 h, 37° C) and previously run on a P-300 Biogel column. The DEAE column was developed with a linear NaCl gradient (0.0 M–0.3 M) in a total volume of 750 ml 0.05 M Tris-HCl buffer, pH 8.0. Fractions of 5 ml were collected. The H-2 serological activity (●—●) was measured by inhibition of complement-mediated cytotoxicity (^{51}Cr release) and is shown to be confined to the region of pool B. Pools (A to E) were made as indicated. Note the magnitude of pool D. Using an H-2^d thymus preparation the specific activity (ID₅₀ units) obtained with the RBC absorbed antiserum for pool D was 2.5 times (380 U mg⁻¹) the activity recovered from comparable spleen material prepared under identical conditions.

the H antigens mentioned previously. An association of the present antigen with T cells comes from two sources. First, target lymph node cells treated with anti- θ serum, which gave a 50% kill, did not lead to the two-fold titre increase expected if the antiserum were B-cell reactive. Second, when thymocyte membranes were extracted and fractionated by ion exchange chromatography as described previously²², the major component present (pool D) had identical physicochemical and serological properties to the antigen isolated from spleen cells (Fig. 1b and Fig. 3). This despite the fact that under normal conditions thymocytes are not a target for complement-mediated lysis by this antiserum. Thymocytes therefore contain the antigen (tentatively called R1.1) which is exposed, however, only on extrathymic cells. Recently several new specificities not attributable to *D*- or *K*-end gene products have been described (see refs 14, 16). Whereas these seem to be B-cell features, the antigenic products we describe here has T-cell affinities. Like other MHC gene products, the biological significance of this new substance remains to be determined.

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- ¹ Yunis, E. J., and Amos, D. B., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3031–3035 (1971).
- ² Demant, P., *Transplant. Rev.*, **15**, 164–202 (1973).
- ³ Klein, J., and Park, J. M., *J. exp. Med.*, **137**, 1213–1225 (1973).
- ⁴ Livnat, S., Klein, J., and Bach, F. H., *Nature new Biol.*, **243**, 42–44 (1973).
- ⁵ Bach, F. H., Widmer, M. B., Bach, M. L., and Klein, J., *J. exp. Med.*, **136**, 1430–1444 (1972).
- ⁶ Rychlikova, M., Demant, P., Ivanyi, P., *Nature new Biol.*, **230**, 271–272 (1971).
- ⁷ Benacerraf, B., and McDevitt, H. O., *Science*, **175**, 273–279 (1972).
- ⁸ McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpfling, J. H., and Snell, G. D., *J. exp. Med.*, **135**, 1259–1278 (1972).
- ⁹ Eijssvoogel, V. P. et al., *Histocompatibility Testing*, 501–508

- (Munksgaard, Copenhagen, 1972).
- ¹⁰ Trinchieri, G., Bernoco, D., Curtioni, S. E., Miggianno, V. C., and Ceppellini, R., *Histocompatibility Testing*, 509–519 (Munksgaard, Copenhagen, 1972).
- ¹¹ Möller, E., *Tissue Antigens*, **3**, 235–240 (1973).
- ¹² Edidin, M., and Henney, C. S., *Nature new Biol.*, **246**, 47–49 (1973).
- ¹³ Davies, D. A. L., and Alkins, B. J., *Nature*, **247**, 294–297 (1974).
- ¹⁴ David, C. S., Shreffler, D. C., and Frelinger, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2509–2514 (1973).
- ¹⁵ Götze, D., Reisfeld, R. A., and Klein, J., *J. exp. Med.*, **138**, 1003–1008 (1973).
- ¹⁶ Sachs, D. H., and Cone, J. L., *J. exp. Med.*, **138**, 1289–1304 (1973).
- ¹⁷ Hauptfeld, V., Klein, D., and Klein, J., *Science*, **181**, 167–168 (1973).
- ¹⁸ Hess, M., and Davies, D. A. L., *Eur. J. Biochem.*, **41**, 1–13 (1974).
- ¹⁹ Hess, M., and Smith, W., *Eur. J. Biochem.*, **43**, 471–477 (1974).
- ²⁰ Robyt, J. F., Ackerman, R. J., Chittenden, C. G., *Archs. Biochem. Biophys.*, **147**, 262–269 (1971).
- ²¹ Davies, D. A. L., *Transplantation*, **8**, 51–70 (1969).
- ²² Davies, D. A. L., *Transplantation*, **5**, 31–42 (1967).

Generation of antistreptolysin O activity in contaminated sera

THE ability to lyse erythrocytes of reduced streptolysin O, produced by strains of Lancefield's group A streptococci apparently results from attachment to membrane cholesterol. Cholesterol in aqueous emulsion will inhibit streptolysin activity at concentrations of about 1.0 $\mu\text{g ml}^{-1}$ (ref. 1). Sera contaminated with bacteria can occasionally give false positive tests for anti-streptolysin antibody but the underlying mechanism has never been defined. We have excluded the possibility that bacterial contamination leads to release of free cholesterol from either ester-bound or protein-bound cholesterol by showing that (1) cholesterol added to contaminated sera showing positive antistreptolysin activity is bound to the same extent as in normal serum, and (2) that ultrafiltrates of such contaminated sera do not contain free cholesterol. Therefore it seemed likely that bacteria in serum might release proteolytic enzymes which would cleave the streptolysin molecule. But the studies reported here indicate that the system is more complex.

Organisms were subcultured from single colonies on agar plates into 1.0 ml amounts of a horse serum-nutrient broth mixture 2:3 v/v. After incubation at 37° C for various periods, cultures were serially diluted in phosphate-buffered saline (0.15 M; pH 6.5) and antistreptolysin activity measured². Results were expressed as that dilution of culture which gave 50% lysis of added human erythrocytes in the presence of 1.0 IU of streptolysin. Table 1 shows the distribution of positive activity in a number of common genera of microorganisms.

Most strains of *Pseudomonas* were positive (90%). Coagulase positive staphylococci showed an 86% positive rate as distinct from 32% for *Staphylococcus albus*. Strains of Enterobacteriaceae such as *Escherichia coli* and *Proteus* species were uniformly negative as were bacillus species, *Haemophilus*, streptococci, pneumococci and *Candida* species. One positive strain of *Neisseria catarrhalis* was detected and one positive strain of *Clostridium histolyticum*. Staphylococcal strains gave positive results with both aerobic and anaerobic cultures. In all cases positive activity appeared slowly after 36–48 h and was maximal after 4–6 d incubation.

Investigation showed that serum is essential, broth cultures themselves having no antistreptolysin activity. Concentrations of serum less than 2.0% gave poor results,

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WALLACH, Nolan R. Harmonic Analysis on Homogeneous Spaces. (Pure and Applied Mathematics: a Series of Monographs and Textbooks.) Pp.xv+361. ISBN-0-8247-6010-7. (New York: Marcel Dekker, Inc., 1973.) \$25.75.

Astronomy

ALLEN, C. W. Astrophysical Quantities. Third edition. Pp.x+310. ISBN-0-485-11150-0. (London: The Athlone Press, University of London, 1973. Distributed by Tiptree Book Services, Ltd., Tiptree, Essex.) £6.25 net.

BROWN, Peter Lancaster. Comets, Meteorites and Men. Pp.255+51 photographs. ISBN-0-7091-3916-0. (London: Robert Hale and Company, 1973.) £3.20 net.

JOHN, Laurie (edited by). Cosmology Now. Pp.168+8 plates. (London: British Broadcasting Corporation, 1973.) £2.75.

KAPLAN, S. A., and TSYTOVICH, V. N. Plasma Astrophysics. (International Series of Monographs in Natural Philosophy, Vol. 59.) Pp.xiii+302. ISBN-0-08-01790-7. (Oxford and New York: Pergamon Press, 1973.) £9.50.

THE AMERICAN EPHEMERIS AND NAUTICAL ALMANAC for the year 1975. Pp.viii+568. (Washington, DC: Nautical Almanac Office, United States Naval Observatory; London: Her Majesty's Nautical Almanac Office, Royal Greenwich Observatory, 1973. For sale by US Government Printing Office, Washington, DC.; and HMSO, London.) \$8.30.

Physics

BARFORD, N. C. Mechanics. Pp.xv+391. ISBN-0-471-04840-2. (London and New York: John Wiley and Sons, 1973.) n.p.

BELINFANTE, F. J. A Survey of Hidden-Variables Theories. (International Series of Monographs in Natural Philosophy, Vol. 55.) Pp.xix+354. (Oxford and New York: Pergamon Press, 1973.) £8.

BLAKELY, J. M. Introduction to the Properties of Crystal Surfaces. (International Series on Materials Science and Technology, Vol. 12.) Pp.xi+261. ISBN-0-08-017641-0. (Oxford and New York: Pergamon Press, 1973.) £3.50.

CONN, G. K. T., and FOWLER, G. N. Essays in Physics, Vol. 5. Pp.224. ISBN-0-12-184805-1. (London and New York: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £3; \$8.50.

ELLIOTT, R. J., and GIBSON, A. F. An Introduction to Solid State Physics and its Applications. (A Title in the Nature-Macmillan Physics Series.) Pp.xx+490. ISBN-333-11023-4. (London and Basingstoke: The Macmillan Press, Ltd., 1974.) £5.95 net.

FELD, Michael S., JAVAN, Ali, and KURNIT, Norman A. (edited by). Fundamental and Applied Laser Physics. (Proceedings of the Esfahan Symposium, August 29 to September 5, 1971.) Pp.xiii+952. ISBN-0-471-25701-X. (New York and London: Wiley-Interscience, John Wiley and Sons, 1973.) £14.

GOMRAS, P., and KISHI, D. Wave Mechanics and its Applications. (International Series of Monographs in Natural Philosophy, Vol. 58.) Pp.xii+238. ISBN-0-08-016979-1. (Oxford and New York: Pergamon Press, 1973.) £3.50.

GREFF, Mino. (edited by). Solid State Surface Science. Vol. 3. Pp.xi+222. ISBN-0-8247-6017-4. (New York: Marcel Dekker, 1973.) \$24.50.

HAKEN, H., and WAGNER, M. (edited by). Cooperative Phenomena. Pp.xv+459. ISBN-3-540-06203-3. (Berlin and New York: Springer-Verlag, 1973.) 132 DM; \$59.40.

ICHIMARU, S. Basic Principles of Plasma Physics: a Statistical Approach. (Frontier in Physics.) Pp.xviii+324. (Reading, Mass., and London: W. A. Benjamin, Inc., 1973.) \$19.50 cloth; \$12.50 paper.

JARZEBSKI, Z. M. Oxide Semiconductors. Translated from the Polish by B. Grzybowski-Swierkosz. Translation edited by Brian Randall Pamplin. Pp.xi+285. ISBN-0-08-016968-6. (Oxford and New York: Pergamon Press; Warsaw: Wydawnictwa Naukowo-Techniczne, 1973.) £6.

JORTNER, J., and KESTNER, N. R. (edited by). Electrons in Fluids: The Nature of Metal-Ammonia Solutions. Pp.xii+493. ISBN-3-540-06310-2. (Berlin and New York: Springer-Verlag, 1973.) 107.50 DM; \$44.10.

KELLY, A. Strong Solids. Second edition. (Monographs on the Physics and Chemistry of Materials.) Pp.xiii+285. (Oxford: Clarendon Press; London: Oxford University Press, 1973.) £7 net.

KILMISTER, C. W. General Theory of Relativity. (The Commonwealth and International Library: Selected Readings in Physics.) Pp.ix+365. ISBN-0-08-017645-3. (Oxford and New York: Pergamon Press, 1973.) £3.50 hard cover; £2 Flexicover.

LEVIN, F. S., and FESHBACH, H. Reaction Dynamics. (Documents on Modern Physics.) Pp.viii+216. (New York, London and Paris: Gordon and Breach, Science Publishers, 1973.) £6.65 cloth; £3.50 paper.

MASON, Warren P., and THURSTON, R. N. (edited by). Physical Acoustics: Principles and Methods, Vol. 10. Pp.xii+403. ISBN-0-12-477910-7. (New York and London: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$39.50; £18.95.

MCCLELAND, B. J. Statistical Thermodynamics. (Studies in Chemical Physics.) Pp.xvi+334. ISBN-412-20780-X. (London: Chapman and Hall and Science Paperbacks; New York: Halsted Press, a Division of John Wiley and Sons, Inc., 1973.) £4 paperback; £7 hardback.

MISNER, Charles W., THORNE, Kip S., and WHEELER, John Archibald. Gravitation. Pp.xvii+1279. ISBN-0-7167-0344-0. (San Francisco and Reading: W. H. Freeman and Company, 1973.) £19.20 clothbound; £8.60 paperback.

MLADJENOVIC, M. Radioisotope and Radiation Physics: an Introduction. Translated by Sonja Subotic. Pp.xii+242. (New York and London: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$19.

MORGAN, D. V. (edited by). Channeling: Theory, Observation and Applications. Pp.xv+486. ISBN-0-471-61510-2. (London and New York: Interscience, John Wiley and Sons, 1973.) £11.75.

NUCLEAR LEVEL SCHEMES A=45 through A=257 from Nuclear Data Sheets. Edited by Nuclear Data Group. ISBN-0-12-355650-3. (New York and London: Academic Press, Inc., a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$23.50.

SCHATZMAN, Evry (edited by). Cergèse Lectures in Physics, Vol. 6. Pp. vii+731. (New York, London and Paris: Gordon and Breach, Science Publishers, 1973.) £20.60.

SEEGER, Karlheinz. Semiconductor Physics. (Springer Study Edition.) Pp.xv+514. ISBN-0-387-81186-9. (Wien and New York: Springer-Verlag, 1973.) 430 schillings; 60 DM.

SEEGER, Raymond J. Benjamin Franklin: New World Physicist. (The Commonwealth and International Library: Selected Readings in Physics.) Pp.xii+190. ISBN-0-08-017648-8. (Oxford and New York: Pergamon Press, 1973.) £2.50.

STEINER, Karl-Heinz. Interactions Between Electromagnetic Fields and Matter. (Vieweg Tracts in Pure and Applied Physics, Vol. 1.) Pp.xvi+351. ISBN-3-528-08313-1. (Oxford and New York: Pergamon Press; Braunschweig: Vieweg und Sohn, 1973.) 92 DM.

VANDENBOSCH, Robert, and HUIZENGA, John R. Nuclear Fission. Pp.xii+422. ISBN-0-12-710850-5. (New York and London: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$29.50.

WHALLEY, E., JONES, S. J., and GOLD, L. W. (edited by). Physics and Chemistry of Ice. (Papers presented at the Symposium held in Ottawa, Canada, 14-18 August 1972.) Pp.xiii+403. (Ottawa: Royal Society of Canada, 1973.) n.p.

WHITTLE, R. M., and YARWOOD, J. Experimental Physics for Students. Pp.370. ISBN-412-09770-2. (London: Chapman and Hall, 1973.) n.p.

Chemistry

ADDISON, C. C. (senior reporter). Inorganic Chemistry of the Main-group Elements, Vol. 1. (A Review of the Literature Published between July 1971 and September 1972. A Specialist Periodical Report.) Pp.xii+444. ISBN-0-85186-752-9. (London: The Chemical Society, 1973.) £9.

AVERY, H. E. Basic Reaction Kinetics and Mechanisms. Pp.x+174. ISBN-333-15381-2. (London and Basingstoke: The Macmillan Press, Ltd., 1974.) £2.50 paperback; £5 hardcover.

BARD, Allen J. (edited by). Encyclopedia of Electrochemistry of the Elements, Vol. 1. Pp.xii+495. ISBN-0-8247-6093-X. (New York: Marcel Dekker, Inc., 1973.) \$60.

BELL, R. P. The Proton in Chemistry. Second edition. Pp.310. ISBN-0-412-11820-3. (London: Chapman and Hall, 1973.) £6.30 net.

BEVAN, S. C., GREGG, S. J., and PARKYNS, N. D. (edited by). Progress in Vacuum Microbalance Techniques, Vol. 2. (Proceedings of the Tenth Conference on Vacuum Microbalance Techniques, Brunel University, Uxbridge, England, June 1972.) Pp.xiv+252. ISBN-0-85501-075-4. (London: Heyden and Son; Rheine/Westf: Heyden und Sohn, GmbH., 1973.) £9; DM 74.

BLUMENFELD, L. A., VOEVODSKI, V. V., and SEMENOV, A. G. Electron Spin Resonance in Chemistry. (Monographs on Electron Spin Resonance.) Pp.xii+322. ISBN-0-85274-108-1. (London: Adam Hilger, Ltd., 1973.) £10 net.

BOSCHKE, F. (managing editor). Topics in Current Chemistry/Fortschritte der Chemischen Forschung, Vol. 43: New Concepts III. Pp.iv+115. ISBN-3-540-06400-1. (Berlin and New York: Springer-Verlag, 1973.) DM 48; \$19.70.

BUTLER, George B., O'DRISCOLL, Kenneth F., and SHEN, Mitchel (edited by). Review in Macromolecular Chemistry, Vol. 10. Pp.v+361. ISBN-0-8247-6174-X. (New York: Marcel Dekker, Inc., 1973.) \$25.50.

CASTLE, Raymond N. (edited by). Pyridazines. (The Chemistry of Heterocyclic Compounds: a Series of Monographs, Vol. 28.) Pp.xii+905. ISBN-0-171-38213-2. (New York and London: Interscience, John Wiley and Sons, 1973.) £40.

FRIED, Ilana. The Chemistry of Electrode Processes. Pp.x+225. ISBN-0-12-267650-5. (London and New York: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £4.90; \$13.75.

GEISSMAN, T. A. (senior reporter). Biosynthesis, Vol. 2. (A Review of the Literature published during 1972. A Specialist Periodical Report.) Pp.ix+308. ISBN-0-85186-513-5. (London: The Chemical Society, 1973.) £8.

GREENWOOD, N. N. (senior reporter). Spectroscopic Properties of Inorganic and Organometallic Compounds, Vol. 6. (A Review of the Literature Published during 1972.) Pp.xv+663. ISBN-0-85186-053-2. (London: The Chemical Society, 1973.) £11.

GUIOCHON, G., and POMMIER, C. Gas Chromatography in Inorganics and Organometallics. Translated by STS, Inc., Ann Arbor, Michigan. Pp.viii+322. ISBN-0-250-97522. (Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1973.) £10.

HARBORNE, J. B. *Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis*. Pp.x+278. ISBN-0-12-10540-3. (London: Chapman and Hall; New York: Halsted Press, a Division of John Wiley and Sons, Inc., 1973.) £4.80 net.

HAWS, E. J., HILL, R. R., and MOWTHORPE, D. J. *The Interpretation of Proton Magnetic Resonance Spectra: a Programmed Introduction*. Pp.xii+180. ISBN-0-85501-063-0. (London, New York and Rheine: Heyden and Sons, Ltd., 1973.) £2.50; \$6.90; DM 20.50.

HUYSER, Earl S. (edited by). *Methods in Free-Radical Chemistry*, Vol. 4. Pp.ix+192. ISBN-0-8247-6089-1. (New York: Marcel Dekker, 1973.) \$22.50.

JOHNSON, B. F. G. (senior reporter). *Inorganic Chemistry of the Transition Elements*, Vol. 2. (A Review of the Literature Published between October 1971 and September 1972. A Specialist Periodical Report.) Pp.xvi+501. (London: The Chemical Society, 1973.) £9.50.

JONES, J. R. *The Ionisation of Carbon Acids*. Pp.vii+236. ISBN-0-12-389750-5. (London and New York: Academic Press, 1973.) £4.80; \$13.50.

KARGER, Barry L., SNYDER, Lloyd R., and HORVATH, Csaba. *An Introduction to Separation Science*. Pp.xix+586. ISBN-0-471-45860-0. (New York and London: Wiley-Interscience, John Wiley and Sons, 1973.) £9.75.

KROGER, F. A. *The Chemistry of Imperfect Crystals*, Vol. 1: Preparation, Purification, Crystal Growth and Phase Theory. 2nd revised edition. Pp.xiii+313. ISBN-0-7204-0281-6. (Amsterdam and London: North-Holland Publishing Company; New York: American Elsevier Publishing Company, Inc., 1973.) Dfl. 70; \$28.

MAY, Clayton A., and TANAKA, Yoshio (edited by). *Epoxy Resins: Chemistry and Technology*. Pp.xii+801. ISBN-0-8247-1446-6. (New York: Marcel Dekker, Inc., 1973.) \$59.50.

NAEGELE, John A. (edited by). *Air Pollution Damage to Vegetation*. (A Symposium sponsored by the Division of Agricultural and Food Chemistry at the 161st Meeting of the American Chemical Society, Los Angeles, Calif., March 31-April 1, 1971.) (Advances in Chemistry Series, No. 122.) Pp.xiii+137. ISBN-842-0183-8. (Washington, DC: American Chemical Society, 1973.) \$9.95 cloth; \$5.45 paper.

ONGLEY, P. A. (edited by). *Organic: Practical Handbook of Organic Chemistry*. English translation by B. J. Hazzard. Pp.xx+747. ISBN-0-08-012789-4. (Oxford and New York: Pergamon Press, 1973.) £8.

PRATESI, P. (symposium editor). *Medicinal Chemistry—III*. (Main Lectures presented at the Third International Symposium on Medicinal Chemistry, held in Milan, Italy, 13-15 September 1972.) (International Union of Pure and Applied Chemistry: Organic Chemistry Division.) Pp. 353-522. ISBN-0-408-70538-8. (London: Butterworth and Co. (Publishers), Ltd., 1973.) £3.50.

PRATESI, P. (symposium editor). *Medicinal Chemistry—Special Contributions—Milan 1972*. (Presented at the 3rd International Symposium on Medicinal Chemistry.) Pp. 151. ISBN-0-408-70542-6. (London: Butterworth and Co. (Publishers), Ltd., 1973.) £4.90.

PARKER, W. (edited by). *Alcyclic Compounds*. (Organic Chemistry, Series One, Volume 5. MTP International Review of Science. (Consultant Editor: D. H. Hey, FRSC.) Pp.317. (London: Butterworth and Co. (Publishers), Ltd.; Baltimore, Md.: University Park Press, 1973.) £10 net; \$24.50.

PATAI, Saul. *The Chemistry of the Carbon-Halogen Bond*. Part 1: Pp. xiii+1-608. Part 2: Pp.xiii+609-1215. ISBN-0-471-66943-1. (London and New York: Interscience, John Wiley and Sons, 1973.) £22.

SAFE, S. and HUTZINGER, O. *Mass Spectrometry of Pesticides and Pollutants*. Pp. 220. ISBN-0-87819-023-6. (Cleveland, Ohio: CRC Press Inc., 1973.) n.p.

SCOTT, Arthur F. (edited by). *Survey of Progress in Chemistry*, Vol. 6. Pp.xii+340. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$29.50; £4.15.

SLACK, A. V., and JAMES, G. Russell (edited by). *Ammonia*, Part 1. (Fertilizer Science and Technology Series, Vol. 2.) Pp.xiii+414. ISBN-0-8247-1630-2. (New York: Marcel Dekker, Inc., 1973.) \$39.50.

VAN SOMEREN, Ernest H. S., and BIRKS, F. T. *Spectrochemical Abstracts*, Vol. xviii, 1971-72. Pp.136. ISBN-0-85274-115-4. (London: Adam Hilger, Ltd., 1973.) £6 net.

STREITWIESER, jun., Andrew, and TAFT, Robert W. (edited by). *Progress in Physical Organic Chemistry*, Vol. 10. Pp.vii+506. ISBN-0-471-83356-8. (New York and London: Interscience, John Wiley and Sons, 1973.) £14.25.

WALKER, Philip L., and THROWER, Peter A. (edited by). *Chemistry and Physics of Carbon*, Vol. 11. Pp.x+329. ISBN-0-8247-6051-4. (New York: Marcel Dekker, Inc., 1973.) \$28.50.

ZWEIG, Gunter (edited by). *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. 7: Thin-Layer and Liquid Chromatography and Analyses of Pesticides of International Importance. Edited by J. Sherma and G. Zweig. Pp.xvii+729. ISBN-0-12-784307-8. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$45; £21.15.

Technology

BILLINTON, Roy, RINGLEE, Robert J., and WOOD, Allen J. *Power-System Reliability Calculations*. (Monographs in Modern Electrical Technology.) Pp.173. (Cambridge, Mass. and London: The MIT Press, 1973.) \$12.95.

BROWN, Paul B., MAXFIELD, Bruce W., and MORAFF, Howard. *Electronics for Neurobiologists*. Pp.xi+543. ISBN-0-262-02094-7. (Cambridge, Mass. and London: The MIT Press, 1973.) £9.

DYM, Clive L. *Introduction to the Theory of Shells*. (Structures and Solid Body Mechanics.) Pp.xi+159. ISBN-0-08-017785-9. (Oxford and New York: Pergamon Press, 1974.) £4.50 hard cover; £2.85 flexi cover.

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HATSOPOULOS, G. N., and GYFTOPOULOS, E. P. *Thermionic Energy Conversion*, Vol. 1: Processes and Devices. Pp.xi+265. ISBN-0-262-08059-1. (Cambridge, Mass. and London: The MIT Press, 1973.) \$17.95.

MANDELL, Gordon K., CAPORASO George J., and BENGEN, William P. *Topics in Advanced Model Rocketry*. Pp.xviii+631. ISBN-0-262-02096-3. (Cambridge, Mass. and London: The MIT Press, 1973.) \$25.

PASSAGLIA, E. (edited by). *Measurement of Physical Properties*. Part 2: Magnetic Properties and Mössbauer Effect. (Techniques of Metals Research, Vol. VI, Part 2.) Pp.xi+529. ISBN-0-471-12231-9. (New York and London: Interscience, John Wiley and Sons, 1973.) £21.25.

SCHULTZ, Jerold. *Polymer Materials Science*. (Prentice-Hall International series in the Physical and Chemical Engineering Sciences.) Pp.xv+524. ISBN-0-13-687038-4. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1974.) \$20.

SHARPE, R. S. (edited by). *Research Techniques in Nondestructive Testing*, Vol. 2. Pp.xii+454. ISBN-0-12-639052-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £9.20; \$25.75.

THOMAS, A. F., and ABBEY, F. *Calculational Methods for Interacting Arrays of Fissile Material*. (International Series of Monographs in Nuclear Energy, Vol. 108.) Pp.xi+127. ISBN-0-08-017660-7. (Oxford and New York: Pergamon Press, 1973.) £4.50.

THOMPSON, J. M. T., and HUNT, G. W. *A General Theory of Elastic Stability*. Pp.322. (London and New York: John Wiley and Sons, 1973.) £7.50.

TOULOUKIAN, Y. S., GERRITSEN, J. Koolhaas, and SHAFER, W. H. (edited by). *Thermophysical Properties Research Literature Retrieval Guide*. Supplement 1, 1964-1970. Vol. 3: Alloys, Intermetallic Compounds, and Cermetes. Vol. 5: Mixtures and Solutions. Vol. 6: Coatings, Systems, and Composites. (Washington and London: IFI/Plenum, 1973.) \$316.25.

WICJMAN, B. A. *Algol 60 Compilation and Assessment*. (APIC Studies on Data Processing, No. 10.) Pp.vii+320. ISBN-0-12-748250-4. (London and New York: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £5.50; \$15.50.

Geography

SPENCER, Joseph E. *Oriental Asia: Themes Towards a Geography*. (Foundations of World Geography.) Pp.x+146. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1973.) \$7.50 cloth.

WHITBY, M. C., ROBINS, D. L. J., TANSEY, A. W., and WILLIS, K. G. *Rural Resource Development*. Pp.243. (London: Methuen and Co., Ltd., 1974.) £2.50 cloth; £1.30 paper.

WOOD, C. M., LEE, N., LAKER, J. A., and SAUNDERS, P. J. W. *The Geography of Pollution: a Study of Greater Manchester* Pp.x+150. ISBN-0-7190-0564-7. (Manchester: Manchester University Press, 1974.) £2 net.

Earth sciences

BATH, Markus. *Introduction to Seismology*. Pp.395. ISBN-3-7643-0634-3. (Basel and Stuttgart: Birkhauser Verlag, 1973.) Sfr. 42.

BOWEN, David. *Britain's Weather: Its Workings, Lore and Forecasting*. Pp.310. (Newton Abbot: David and Charles, 1973.) £1.50.

COATES, Donald R. (edited by). *Environmental Geomorphology and Landscape Conservation*. Vol. 3: Non-Urban. (Benchmark Papers in Geology.) Pp.xi+483. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1973.) n.p.

FRASER, Ronald (compiled by). *Oceanography of the South Pacific 1972*. (Papers presented at an International Symposium held in Wellington, New Zealand, 9 to 15 February, 1972.) Pp.xii+524. (Wellington, NZ: New Zealand National Commission for Unesco, 1973.) n.p.

FRENZEL, Burkhard. *Climatic Fluctuations of the Ice Age*. Translated by A. E. M. Nairn. Pp.xxiv+306. ISBN-0-8295-0226-2. (Cleveland and London: The Press of Case Western Reserve University, 1973.) \$2.50.

FURON, Raymond. *Éléments de Paléoclimatologie*. (Sciences de la Terre.) Pp.216. (Paris: Librairie Vuibert, 1972.) n.p.

GOLDBERG, Edward D. (edited by). *North Sea Science*. (NATO North Sea Science Conference, Aviemore, Scotland, 15-20 November 1971.) Pp.xvii+500. ISBN-0-262-07056-1. (Cambridge, Mass. and London: The MIT Press, 1973.) \$18.95.

JONES, O. A., and ENDEAN, B. (edited by). *Biology and Geology of Coral Reefs*. Vol. 1: Geology. Pp.xvi+410. (New York and London: Academic Press, Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$28.

KERVAN, Louis. *Preuves en Géologie et Physique de Transmutations à Faible Énergie*. Pp.186. ISBN-2-224-00053-7. (Paris: Librairie Maloine, S. A. Editeur, 1973.) n.p.

LOMNITZ, Cinna. *Global Tectonics and Earthquake Risk*. (Developments in Geotectonics, Vol. 5.) Pp.xiv+320. ISBN-0-444-41076-7. (Amsterdam, London and New York: Elsevier Scientific Publishing Company, 1974.) Dfl.82; \$32.80.

NEWELL, Reginald E., KIDSON, John W., VINCENT, Dayton G., and BOER, George J. *The General Circulation of the Tropical Atmosphere and Interactions with Extratropical Latitudes*, Vol. 1. Pp.xiii+258. ISBN-0-262-14012-8. (Cambridge, Mass. and London: The MIT Press, 1973.) \$25.

NIXON, Peter H. (edited by). *Lesotho Kimberlites*. Pp.xii+350+70 plates. (Maseru, Lesotho: Lesotho National Development Corporation, 1973.) R13; \$19.

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PITCHER, Max G. (edited by). *Arctic Geology*. (Proceedings of the Second International Symposium on Arctic Geology, held February 1-4, 1971, at San Francisco, California.) (Mémorial 19.) Pp.xvii+747. (Tulsa, Oklahoma: The American Association of Petroleum Geologists, 1973.) \$30.

RIEKE, III, Herman H., and CHILINGARIAN, George V. *Compaction of Argillaceous Sediments*. (Developments in Sedimentology, Vol. 16.) Pp.xiv+424. ISBN-0-444-41054-6. (Amsterdam, London and New York: Elsevier Scientific Publishing Company, 1974.) Dfl.85; \$29.80.

TIRATSOO, E. N. *Oilfields of the World*. Pp.viii+376. ISBN-0-901360-06-6. (Beaconsfield: Scientific Press, Ltd., 1973.) £8.50 net.

WALTHAM, A. C. (compiled and edited by). *Assisted by Sweeting, M. M. The Limestones and Caves of North-West England. (The Limestones and Caves of Britain.)* Pp.477 (54 plates). ISBN-0-7153-6181-3. (Newton Abbot: David and Charles, 1974. Published for The British Cave Research Association.) £6.95 net.

Biological sciences

BIRCH, G. G., and GREEN, L. F. (edited by). *Molecular Structure and Function of Food Carbohydrate*. Pp.xi+308. ISBN-0-85334-582-1. (London: Applied Science Publishers, Ltd., 1973.) £10.

BIRKS, B. J. B., and WEST, R. G. (edited by). *Quaternary Plant Ecology*. (The 14th Symposium of The British Ecological Society. University of Cambridge, 28-30 March 1972.) Pp.ix+326. ISBN-0-632-09120-7. (Oxford and London: Blackwell Scientific Publications, 1973.) £13.

BOURNF, G. H., and DANIELLI, J. F. (edited by). *Assisted by JEON, K. W. International Review of Cytology*, Vol. 36. Pp.vii+349. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$24; £11.30.

CALVIN, Melvin, and PRYOR, William A. (with introductions by). *Organic Chemistry of Life*. (Readings from *Scientific American*.) Pp.xii+452. (San Francisco: W. H. Freeman and Company, 1973.) \$12 cloth; \$6.95 paper.

CASIDA, John E. (edited by). *Pyrethrum: The Natural Insecticide*. Pp.xvii+329. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$16; £7.70.

CAMPRELL, P. N., and DICKENS, F. (edited by). *Essays in Biochemistry*, Vol. 9. Pp.viii+180. ISBN-0-12-158109-8. (London and New York: Academic Press, 1973. Published for The Biochemical Society.) £2.40; \$6.75.

CBE Style Manual. Third edition. Prepared by the Committee on Form and Style of the Council of Biology Editors. Pp.xii+297. (Washington, DC: American Institute of Biological Sciences, 1972. Published for the Council of Biology Editors.) n.p.

CHAVIN, Walter (edited by). Responses of Fish to Environmental Changes. Pp.x+459. ISBN-0-398-02743-9. (Springfield, Ill.: Charles C. Thomas, Publisher, 1973.) \$19.75.

CHERRY, Joe H. Molecular Biology of Plants: a Text-Manual. Pp.ix+204. ISBN-0-231-03642-6. (New York and London: Columbia University Press, 1973.) £6.

CHIARELLI, A. B., and CAPANNA, E. (edited by). Cytotaxonomy and Vertebrate Evolution. Pp.xv+783. ISBN-0-12-172535-9. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £14.50; \$41.

COOK, G. M. W., and STODDART, R. W. Surface Carbohydrates of the Eukaryotic Cell. Pp.xiii+345. ISBN-0-12-186850-8. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £7; \$19.50.

DAVIS, Bernard D., DULBECCO, Renato, EISEN, Herman N., GINSBERG, Harold S., WOOD, W. Barry, and McCARTY, Maclyn. Microbiology, including Immunology and Molecular Genetics. Second edition. Pp.xv+1562. ISBN-06-140683-X. (Hagerstown, Md. and London: Medical Department, Harper and Row, Publishers, 1973.) \$27.50.

DENTON, Thomas E. Fish Chromosome Methodology. Pp.vii+166. ISBN-0-398-02831-1. (Springfield, Ill.: Charles C. Thomas, Publisher, 1973.) n.p.

DODGE, John D. The Fine Structure of Algal Cells. Pp.xii+261. ISBN-0-12-219150-1. (London and New York: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £6.

DWEK, Raymond A. Nuclear Magnetic Resonance (N.M.R.) in Biochemistry: Applications to Enzyme Systems. Pp.xviii+395. ISBN-0-19-854614-9. (Oxford: Clarendon Press; London: Oxford University Press, 1973.) £8 net.

GIESE, Arthur C. (edited by). Photophysiology: Current Topics in Photochemistry and Photochemistry, Vol. 8. Pp.xviii+269. (New York and London: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$22.50.

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GRELL, Karl G. Protozoology. Pp.viii+554. ISBN-3-540-06239-4. (Berlin and New York: Springer-Verlag, 1973.) 107 DM; \$43.90.

HOCKING, Brian. Biology: The Ultimate Science. (Transworld Student Library.) Pp.142. ISBN-0-552-40014-9. (London: Transworld Publishers, Ltd., in association with Richard Sadler, Ltd., 1973.) 75p.

HOUSE, C. R. Water Transport in Cells and Tissues. (Monographs of the Physiological Society, No. 24.) Pp.vi+562. ISBN-0-7131-4215-4. (London: Edward Arnold (Publishers), Ltd., 1974.) £10.50 net.

KAESTNER, Alfred. Lehrbuch der Speziellen Zoologie. Band 1: Wirbellose. 3. Teil. Insects: B. Spezieller Teil. Pp. 273-907. (Jena: Veb Gustav Fischer Verlag, 1973.) n.p.

KAGEN, Lawrence J. Myoglobin: Biochemical, Physiological, and Clinical Aspects. (Columbia Series in Molecular Biology.) Pp.xii+151. ISBN-0-231-03421-0. (New York and London: Columbia University Press, 1973.) £5.

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LASKIN, Allen I., and LECHEVALIER, Hubert A. (edited by). Handbook of Microbiology. Vol. 2: Microbial Composition. Pp.1060. ISBN-0-87819-582-3. (Cleveland, Ohio: CRC Press, a Division of the Chemical Rubber Co.; Oxford: Blackwell Scientific Publications, 1973.) £17.50.

LEACH, Sydney J. (edited by). Physical Principles and Techniques of Protein Chemistry, Part C. (Molecular Biology: an International Series of Monographs and Textbooks.) Pp.xiv+621. ISBN-0-12-440103-1. (New York and London: Academic Press; a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$44; £19.

LOVENBERG, Walter (edited by). Iron-Sulfur Proteins. Vol. 1: Biological Properties. (Molecular Biology: an International Series of Monographs and Textbooks.) Pp.xiii+385. ISBN-0-12-456001-6. (New York and London: Academic Press, a subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$33.

LUCAS, Alfred M., and STETTENHEIM, Peter R. Avian Anatomy-Integument. Part 1: Pp.iv+1-340. Part 2: Pp.x+341-750. (Agriculture Handbook 362.) (US Department of Agriculture, in co-operation with Michigan Agriculture Experiment Stations, Michigan State University.) (Washington, DC: US Government Printing Office, 1972.) \$13 per 2 part set.

MARGULIS, Lynn (edited by). Proceedings of the Fourth Conference on Origins of Life: Chemistry and Radio-chemistry. Pp.xvii+291. ISBN-3-540-06066-9. (Berlin and New York: Springer-Verlag, 1973.) 54.20 DM; \$24.50.

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MILLER, Stanley L., and ORGEL, Leslie E. The Origins of Life on the Earth. (Concepts of Modern Biology Series.) Pp.x+229. (Englewood Cliffs, N.J.: Prentice-Hall, Inc., 1973.) n.p.

NELSON, Bryan. Azraq: Desert Oasis. Pp.xix+435+90 photographs. ISBN-0-7139-0475-5. (London: Allen Lane, 1973.) £6.

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QUERNER, Hans, et al. New Perspectives in Evolution. (Transworld Student Library.) Pp.160. ISBN-0-552-40012-2. (London: Transworld Publishers, Ltd., in association with Richard Sadler, Ltd., 1973.) 80p.

ROMOSER, William S. The Science of Entomology. Pp.x+449. ISBN-0-02-403420-7. (New York: Macmillan Publishing Co., Inc.; London: Collier-Macmillan, Publishers, 1973.) £4.50.

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Applied biological sciences

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BORGSTROM, Bengt, DAHLQVIST, Arne, and HAMBRAEUS, Leif. (edited by). Intestinal Enzyme Deficiencies and their Nutritional Implications. (Symposia of the Swedish Nutrition Foundation, XI.) Pp.149. (Stockholm: Almqvist and Wiksell, 1973.) Sw. kr. 50.

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GREAVES, Melvyn F., OWEN, John J. T. and RAFF, Martin C. T. and B. Lymphocytes: Origin, Properties and Roles in Immune Response (Imperial Cancer Research Fund Tumour Immunology Unit and Medical Research Council Neuroimmunology Project, Department of Zoology, University College, London, U.K.) Pp.vii+316. ISBN-90219-2047-6. (Amsterdam: Excerpta Medica; New York: American Elsevier Publishing Co., Inc., 1973.) Dfl. 86; \$33.10.

GROSS, J. F., KAUFMANN, R., and WETTERER, E. (edited by). Modern Techniques in Physiological Sciences. Pp.536. ISBN-0-12-304450-2. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £7; \$19.75.

HAMILTON, Max. Lectures on the Methodology of Clinical Research. Pp.x+197. ISBN-0-443-01080-3. (Edinburgh and London: Churchill Livingstone, 1974.) £5 net.

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HOFMANN, R. R. The Ruminant Stomach: Stomach Structure and Feeding Habits of East African Game Ruminants. (East African Monographs in Biology, Vol. 2.) Pp.ix+354. (Nairobi: East African Literature Bureau, 1973.) £6.50 soft cover; £10 hard cover.

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IMMUNOPOTENTIATION (Ciba Foundation Symposium 18, New Series.) Pp.ix+355. ISBN-90-219-4019-1. (Amsterdam, London and New York: Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers, 1973.) Dfl. 47.50; \$18.30.

KAHN, Raymond H., and LANDS, William E. M. (edited by). Prostaglandins and Cyclic AMP: Biological Actions and Clinical Applications. (Proceedings of a Symposium held at the Towsley Center for Continuing Medical Education, The University of Michigan Medical Center, Ann Arbor, Michigan, November 13-15, 1972.) Pp.xvii+306. ISBN-0-12-394450-3. (New York and London: Academic Press, Inc., a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$11.50.

KAKULAS, B. A. (edited by). Basic Research in Myology. Part 1. (Proceedings of the Second International Congress on Muscle Diseases, Perth, Australia, 22-26 November 1971.) Pp.xii+698. ISBN-90-219-0163-3. (Amsterdam: Excerpta Medica; New York: American Elsevier Company, Inc., 1973.) Dfl. 157; \$62.80.

KAKULAS, B. A. (edited by). Clinical Studies in Myology. Part 2. (Proceedings of the Second International Congress on Muscle Diseases, Perth, Australia, 22-26 November, 1971.) Pp.xii+724. ISBN-90-219-0246-X. (Amsterdam: Excerpta Medica; New York: American Elsevier Company, Inc., 1973.) Dfl. 157; \$62.80.

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LAZARITD, J., ALANT, O., and KOTHE, W. Chirurgie bei Diabetes. Pp.222. (Budapest: Akademiai Kiado, 1973.) £4.

MATHE, G., and TUBIANA, M. (guest editors). The Natural History, Diagnosis and Treatment of Hodgkin's Disease I. (Series Haematologica, Vol. VI, 1, 1973.) Pp.115. ISBN-87-16-01361-1. (Copenhagen: Munksgaard, 1973.)

MATHE, G., and TUBIANA, M. (guest editors). The Natural History, Diagnosis and Treatment of Hodgkin's Disease II. (Series Haematologica, Vol. VI, 2, 1973.) Pp.117-269. ISBN-87-16-01363-8. (Copenhagen: Munksgaard, 1973.) D.kr. 166 the two parts.

McKELVEY, Jr., John J. Man Against Tsetse: Struggle for Africa. Pp.xii+306. ISBN-0-8014-0768-0. (Ithaca and London: Cornell University Press, 1973.) £5.95.

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MEDICAL RESEARCH SYSTEMS IN EUROPE. (A joint Wellcome Trust-Ciba Foundation Symposium. Ciba Foundation Symposium, 21, New Series.) Pp.viii+335. ISBN-90-219-4022-1. (Amsterdam, London and New York: Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers, 1973.) Dfl. 43; \$17.20.

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MOEN, Aaron N. Wildlife Ecology: an Analytical Approach. (Series of Books in Agricultural Science.) Pp.xviii+457. ISBN-0-7167-0826-4. (San Francisco and Reading: W. H. Freeman and Company, 1973.) £7.60.

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SUSS, R., KINZEL, V., and SCRIBNER, J. D. Cancer: Experiments and Concepts. Translated from the German by J. D. Scribner. Pp.xxi+285. ISBN-3-540-90042-X. (Berlin and New York: Springer-Verlag, 1973.) 26.50DM; \$12.

TAYLOR, Robert B. Feeling Alive After 65: The Complete Medical Guide for Senior Citizens and Their Families. Pp.xv+232. ISBN-0-87000-226-0. (New Rochelle, NY: Arlington House Publishers, 1973.) \$7.95.

THE HUMAN LENS IN RELATION TO CATARACT. (Ciba Foundation Symposium 19, New Series.) Pp.x+324. ISBN-90-219-4020-5. (Amsterdam, London and New York: Elsevier/Excerpta Medica/North-Holland, Associated Scientific Publishers, 1973.) Dfl. 43.50; \$16.70.

Psychology : psychiatry

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REESE, Hayne W. (edited by). Advances in Child Development and Behavior. Vol. 8. Pp.xiv+298. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$27.50; £8.40.

SCOTT, John Paul, and SENAY, Edward C. (edited by). Separation and Depression: Clinical and Research Aspects. (A Symposium presented at the Chicago Meeting of the American Association for the Advancement of Science, 27 December 1970.) Pp.viii+256. (Washington, DC: American Association for the Advancement of Science, 1973.) \$19.95.

SZASZ, Thomas S. The Ethics of Psychoanalysis: The Theory and Method of Autonomous Psychotherapy. Pp.xiv+226. ISBN-0-07100-7759-9. (London: Routledge and Kegan Paul, 1974.) £4 net.

ULLMAN, Montague, KRIPPNER, Stanley and VAUGHAN, Alan. Dream Telepathy. Pp.xiii+300+ 21 plates. ISBN-85500-012-0. (London: Turnstone Books, 1973.) £2.95.

Sociology : education

BUTCHER, H. J., and PONT, H. B. (edited by). Educational Research in Britain 3. Pp.xi+420. ISBN-0-340-16792-0. (London: University of London Press, Ltd., 1973.) £4.60 net.

COLE, Jonathan R., and COLE, Stephen. Social Stratification in Science. Pp.xiv+283. ISBN-0-226-11338-8. (Chicago and London: The University of Chicago Press, 1973.) £5.65.

GILL, Owen. Whitegate: an Approved School of Transition. (Social Research Series.) Pp.139. ISBN-0-8523-4523. (Liverpool: Liverpool University Press, 1974.) £4 net.

HARMS, Ernest (edited by). Drugs and Youth: The Challenge of Today. Pp.xi+237. ISBN-0-08-017063-3. (Oxford and New York: Pergamon Press, 1973.) £5.

LAYTON, David. Science for the People: The Origins of the School Science Curriculum in England. Pp.226. ISBN-0-04-507001-6. (London: George Allen and Unwin, Ltd., 1973.) £3.55 net.

MILGRAM, Stanley, and SHOTLAND, R. Lance. Television and Anti-social Behavior: Field Experiments. Pp.xii+183. ISBN-0-12-496350-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.)

WHITLEY, Richard (edited by). Social Processes of Scientific Development. Pp.ix+286. ISBN-0-7100-7705-X. (London and Boston, Mass.: Routledge and Kegan Paul, 1974.) £5.95 net.

General

ALLOTT, Robin. The Physical Foundation of Language: Exploration of a Hypothesis. Vol. 1: Pp.96. Vol. 2: Pp.113. (Seaford, Sussex: Robin Allott, 29 Headland Avenue, 1973.)

ARMEN, Jean-Claude. Gazette-Boy. Translated from the French by Stephen Hardman. Pp.127. (London, Sydney and Toronto: The Bodley Head, 1974.) £2 net.

BAINES, John D. The Environment. (Past-into-Present Series.) Pp.96. ISBN-0-7134-1795-1. (London: B. T. Batsford, Ltd., 1973.) £1.50 net.

BARKER, Ronald, and ESCARPIT, Robert. (edited by). The Book Hunger. Pp.155. (Paris: Unesco; London: Harrap, 1973.) £2.

BENTHALL, Jonathan (edited with an introduction by). The Limits of Human Nature. (Essays based on a course of lectures given at the Institute of Contemporary Arts, London.) Pp.xiv+282. (London: Allen Lane, 1973.) £4 cased; £2.50 paper.

BENVENUTO, Edoardo. Materialismo e Pensiero Scientifico. Pp.viii+307. (Milano: Tamburini Editore, 1974.) L.6000.

BERENDZFN, Richard (edited by). Life Beyond Earth and the Mind of Man. (A Symposium held at Boston University, November 20, 1972.) Pp.vii+106. (Washington, DC: National Aeronautics and Space Administration, 1973. For sale by US Government Printing Office, Washington.) \$1.25.

BIOGRAPHICAL MEMOIRS OF FELLOWS OF THE ROYAL SOCIETY. Vol. 19. Pp.694+25 photographs. (London: The Royal Society, 1973.) n.p.

BODFCHTEL, Johann, and GIERLOFF-EMDEN, Hans-Gunter. The Earth From Space. Translated by Hildegard Mayhew and Lotte Evans. Pp.176. ISBN-0-7153-6076-0. (Newton Abbot: David and Charles, 1974.) £6.50 net.

BRANDON-COX, Hugh. Summer of a Million Wings: Arctic Quest for the Sea Eagle. Pp.184 (26 plates). ISBN-0-7153-6355-7. (Newton Abbot: David and Charles, 1974.) £3.50 net.

CATT, Ivor. Computer Worship. Pp.149. ISBN-0-273-00243-0. (London: Pitman Publishing, 1973.) £1.80 net.

activity increasing with increasing amounts of serum to an optimum of 40–50%. Beyond this, activity was often reduced, but it seems that this may be related to a reduction in the number of organisms in the cultures. The findings suggest that certain organisms are stimulated by a serum substrate to form an inducible enzyme which converts the substrate to an activated form capable of destroying or of inhibiting the action of streptolysin O. The addition of positive serum-broth filtrates to aliquots of fresh normal serum results in generation of new activity in the added serum. As well as lacking the active principle, broth filtrates do not contain enzyme as shown by their inability to generate activity when added to fresh serum. Preliminary tests have located the active serum fraction in the alpha-1 globulin region but it has not yet been possible to separate the enzyme from serum components. The enzyme, serum substrate and active factor seem to be heat stable at 60° C for at least 2 h. Horse, sheep, human and rabbit sera all gave positive results although sheep serum was slightly less effective.

Table 1 Distribution of antistreptolysin activity in microorganisms. Cultures were tested daily for up to 28 d

Organism	No. tested	No. and percentage showing antistreptolysin activity at 1/100 or greater dilution of culture	Reciprocal of mean dilution showing 50% haemolysis
<i>Staph. pyogenes</i>	69	59 (86)	1,600
<i>Staph. albus</i>	25	8 (32)	1,600
B-haemolytic streptococci	14	0 (0)	—
Pneumococci	17	0 (0)	—
<i>Haemophilus sp.</i>	10	0 (0)	—
<i>Neisseriae sp.</i>	12	1 (8.3)	200
<i>Bacillus sp.</i>	16	0 (0)	—
<i>Proteus sp.</i>	25	0 (0)	—
<i>E. coli</i>	58	0 (0)	—
<i>Pseudomonas sp.</i>	92	83 (90)	1,200
<i>Clostridium sp.</i>	13	1 (7.6)	800
<i>Bacteroides</i>	8	0 (0)	—
<i>Candida sp.</i>	19	0 (0)	—

There is evidence that normal serum contains an inhibitor of the active serum factor produced as above, since active serum-broth filtrates of 4 d cultures added to aliquots of fresh serum gave levels of activity after 5–6 h which were much below that expected from the dilution factor. In each case this was followed, after 2–3 d incubation at 37° C, by a rise in the active serum factor to a level some four to five times greater than in the original 4 d cultures. But addition of young broth cultures of positive organisms to filtrates containing high levels of active factor did not reduce these on further incubation. This suggests that in the original 4 d cultures the growing organisms had inactivated a certain percentage of the serum precursor either directly or by activation of some inhibitor system, but that the activated factor itself was not further affected by growing organisms.

Apart from the intrinsic interest of this serum system which may be more complex than outlined, the distribution of positive and negative strains raises problems which clearly may be related to taxonomy as well as providing a possible epidemiological marker. We are investigating whether this property of certain microorganisms relates in any way to other established biochemical activities, and trying to define more clearly the nature of the reacting components, and to show whether the same mechanism operates for different species. Preliminary studies seem to exclude a role for plasminogen activation to plasmin since plasminogen activated with streptokinase does not seem to affect streptolysin activity. Other possible pathways include activation of a kallikreinogen-kallikrein system by the bacterial enzyme or

activation of some complement intermediate compounds.

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¹ Watson, K. C., Rose, T. P., and Kerr, E. J. C., *J. clin. Path.*, **25**, 855–891 (1972).

² Rantz, L. A., and Randall, E., *Proc. Soc. exp. Biol. Med.*, **59**, 22–25 (1945).

Antibodies recognise specific structures of triple-helical polynucleotides built on poly(A) or poly(dA)

ANTIGENIC determinants of nucleic acids may comprise individual bases or base sequences, as in denatured DNA, or specific conformational features, as in helical forms¹. Experimentally induced antibodies have shown selective reactivity toward double-stranded RNA^{2–6}, RNA-DNA hybrids⁴, or distinct features of poly(G)·poly(C)⁷ or poly(dG)·poly(dC)⁴. Some of these antibodies can be used to identify double-helical RNA in virus-infected cells^{8,9} and to quantitate it in RNA extracted from such cells¹⁰, or to distinguish between double-stranded RNA and RNA-DNA hybrids in mixtures of enzyme reaction products¹¹. Triple-helical poly(A)·2poly(U) can also be distinguished from double-helical forms^{2,9}. Triple-helical regions can form where continuous purine sequences occur¹², and they might be involved as intermediates or as recognition sites in transcription^{13,14}. Thus antibodies that recognise triple-helical nucleic acids are important in the definition of conformation-dependent antigenic determinants, as a model for protein recognition of specific nucleic acid sites, and they may identify such sites in naturally occurring nucleic acids. We describe here such antibodies that can differentiate three-stranded structures built on poly(A) from others built on poly(dA).

Sera were obtained from rabbits immunised with methylated bovine serum albumin complexes¹⁵ of copolymers formed by mixing: poly(A)+2poly(U), or poly(dA)+poly(U) or poly(dA)+poly(dT)+poly(U). The sera were tested with single, double and triple-stranded polynucleotides. Sera that reacted with free poly(A) or denatured DNA were passed through columns of DNA bound to Sepharose¹⁶. These absorbed sera, and all others used did not react with native or denatured DNA or with poly(A), poly(U), poly(I), poly(C), poly(dA) or with ribosomal or transfer RNA or double-stranded reovirus RNA, poly(I)·poly(C), poly(dA)·poly(dT) or poly(A)·poly(dT).

Since poly(A) and poly(U) may combine to form either a double-stranded or triple-stranded helix^{17,18}, the specificity of the anti-poly(A)·2poly(U) sera for the triple-helical form was tested when homopolymers were mixed in varying proportions. For reaction with these sera, the maximal amount of effective antigen was present when the ratio in the mixture was 2poly(U):1poly(A) (Fig. 1). When equal amounts of homopolymers were mixed, with the double-stranded form predominant, only 5–10% as much reactive antigen was present. The increase in the amount of effective antigen when the poly(U) was increased from 50% to 67% reflected the transition from a double-stranded to a triple-stranded structure^{17,18}, and was the opposite pattern to that

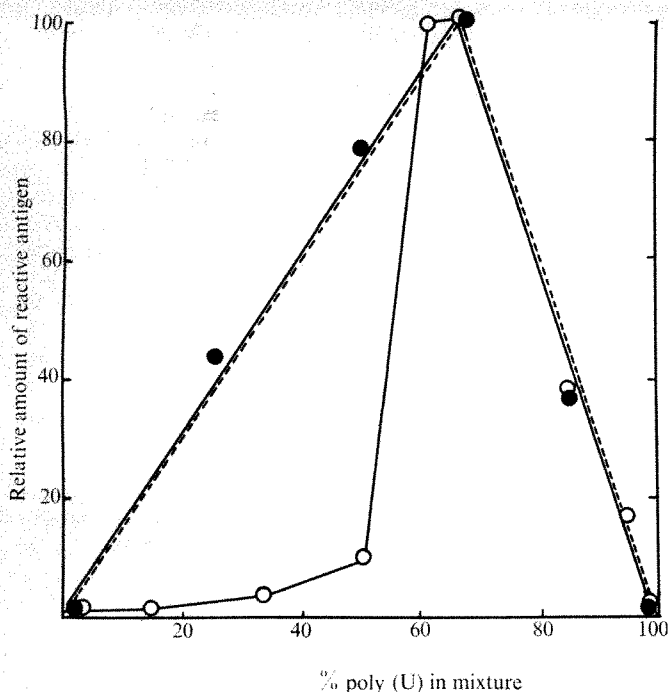


Fig. 1 Specificity of antisera for triple-stranded polymers, measured by formation of reactive antigens in varying mixtures of poly(U) with poly(A) for anti-poly(A)·2poly(U) antiserum (○) and of poly(U) with poly(dA) for anti-poly(dA)·2poly(U) antiserum (●). Homopolymers (Miles) were mixed at concentrations of 1×10^{-5} M nucleotide in 0.14 M NaCl, 0.01 M Tris, pH 7.4, with 5 mM $MgSO_4$ and 1.5 mM $CaCl_2$. Each mixture was diluted and assayed in quantitative micro-complement fixation as described before²³, but in the above Tris-saline buffer, in a total volume of 1.4 ml per reaction mixture. A complement fixation curve was obtained for each polynucleotide mixture. Relative amounts of reactive antigen were calculated from the total polynucleotide required to reach a given point on the complement fixation curve, such as 50% complement fixation in the antibody excess region. Anti-poly(A)·2poly(U) serum was used at a 1/2,000 dilution and anti-poly(dA)·2poly(U) serum at a 1/1,000 dilution. The broken line represents the maximal theoretical value expected if only triple-helical copolymer is formed.

observed when antibodies to a double-stranded form were used with the same mixtures¹. Further, the anti-poly(A)·2poly(U) serum did not react, even at a 1/100 serum dilution, with double-helical poly(I)·poly(C) or reovirus RNA, while it reacted at a 1/5,000 dilution with the homologous poly(A)·2poly(U). Its reaction with homologous antigen was not inhibited by a hundred-fold excess of native DNA, poly(dA)·poly(dT), poly(A)·poly(dT) or poly(I)·poly(C). Thus this serum was specific for the triple-helical polyribonucleotide.

Antisera were obtained from rabbits immunised with a copolymer made by mixing equal amounts of poly(dA) and poly(U). In this case the original serum reacted, at a 1/1,500 dilution with homologous antigen and at a 1/100 dilution with poly(A) and denatured DNA. After it was passed through a DNA-Sepharose column, it no longer reacted with poly(A) or denatured DNA, but was still fully reactive with homologous antigen.

Mixtures of poly(dA) and poly(U) form only a triple-stranded helix¹⁹, so that even if they are mixed in equal amounts, some poly(dA)·2poly(U) and free poly(dA) are formed. This may have been the case with the immunogen, and could explain the separate antibody population that reacted with poly(A) and denatured DNA and was removed by absorption.

When poly(dA) and poly(U) were mixed in varying proportions, the maximum amount of reactive antigen was again

formed when the ratio was 2poly(U):1poly(dA). However, the pattern differed from that seen with the anti-poly(A)·2poly(U) serum, since in this case there was a continuous increase of reactive antigen as poly(U) was added to poly(dA) (Fig. 1) rather than a sudden increase when the poly(U) was increased above 50%. This supports the conclusion that only the triple-stranded structure is formed.

This serum did not react with the double-helical structures listed earlier, and its reaction with homologous antigen was not inhibited by native DNA, poly(dA)·poly(dT), poly(A)·poly(dT) or poly(I)·poly(C).

Antisera to poly(dA)·poly(dT)·poly(U) were also specific for the triple-stranded form reported to occur with this mixture of homopolymers^{19,20}. The sera did not react with the homopolymer components alone or with poly(dA)·poly(dT) or any of the other single or double-stranded polymers listed earlier.

The specificities of these sera were examined further in tests of reciprocal cross-reactivities among the triple-helical polynucleotides. Even at a 1/100 serum dilution, antiserum specific for the exclusively polyribonucleotide copolymer poly(A)·2poly(U) was not cross reactive with poly(dA)·2poly(U) or with poly(dA)·poly(dT)·poly(U); it was effective with homologous antigen at a 1/1,500 serum dilution.

Antisera to the two forms containing poly(dA) were mutually cross-reactive, giving virtually identical complement fixation curves with either antigen (Fig. 2), but neither cross-reacted with the poly(A)-containing triple-helix. The apparent cross-reactivity of poly(dA)·2poly(U) and poly(dA)·poly(dT)·poly(U) was further examined to determine whether when poly(U) was added to poly(dA)·poly(dT), it simply displaced the poly(dT) to form poly(A)·2poly(U). Specific precipitates were formed with anti-poly(dA)·2poly(U) serum and with either of the poly(dA)·2poly(U) or poly(dA)·poly(dT)·poly(U) antigens. The latter precipitate contained all three nucleotides (Fig. 3). Thus the antiserum did precipitate a polymer containing all three components and the two triple-helices must have similar conformations.

The cross-reaction described above indicated that the presence of the poly(dA) controlled the overall configuration of the two similar polymers, while the presence of poly(A) determined the different conformation of the poly(A)·2poly(U). This role of polypurine was tested further by

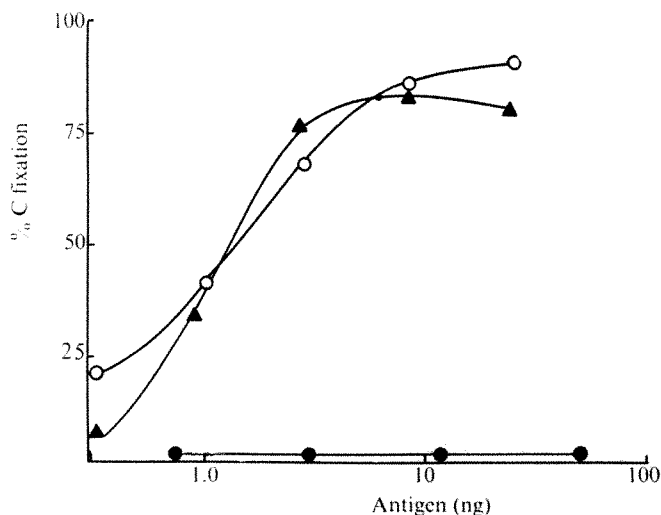


Fig. 2 Cross-reactions of triple-helical polynucleotides with anti-poly(dA)·2poly(U) antiserum. Quantitative complement fixation reactions of poly(dA)·2poly(U) (○) and poly(dA)·poly(dT)·poly(U) (▲) with a 1/1,000 dilution of serum and the lack of reactivity of poly(A)·2poly(U) (●) with a 1/100 dilution.

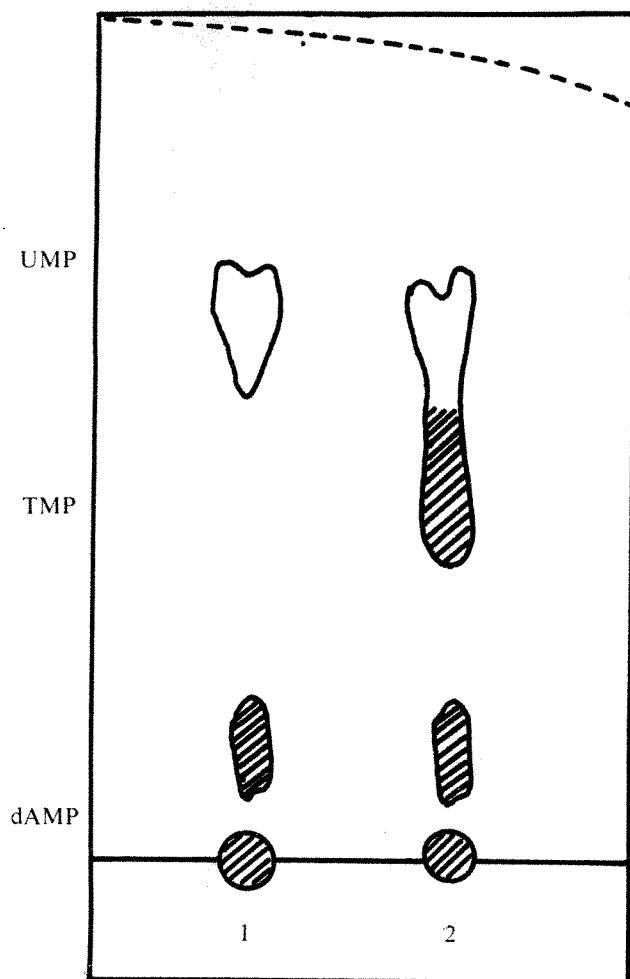


Fig. 3 Analysis of precipitates formed with anti-poly (dA)-2poly(U) antiserum and poly(dA)-2poly(U) or poly(dA)-poly(dT)-poly(U). Poly(dA)-2poly(U) was formed by mixing 4.5×10^{-8} mol of poly(dA) nucleotide with 9×10^{-8} mol of poly(U) nucleotide in 0.3 ml containing 0.3 M NaCl, 0.01 M phosphate, pH 7.4. The mixture was heated at 100°C and slowly cooled to room temperature and then to 4°C . Poly(dA)-poly(dT) was prepared in the same way with a mixture of 4×10^{-8} mol of nucleotide for each homopolymer. An identical amount of poly(U) was then added in 0.15 ml of saline-phosphate at 37°C , and the mixture was cooled to 4°C . Anti-poly(dA)-2poly(U) antiserum (1 ml) was added to each of the two polymer mixtures, and these were incubated for 20 h at 4°C . Both precipitates were collected by centrifugation in the cold, drained, washed with 0.5 ml of saline-phosphate, centrifuged again and resuspended in 0.2 ml of water. The suspension was incubated with $5\mu\text{g}$ of pancreatic RNase for 3 h at 42°C and most of the precipitate dissolved. Pancreatic DNase ($10\mu\text{g}$) and venom phosphodiesterase ($50\mu\text{g}$) were added and the mixtures were incubated for a further 2.5 h at 37°C . The mixture was lyophilised and redissolved in 0.025 ml of water. Of this, 0.01 ml was spotted on an Eastman thin-layer chromatogram with a fluorescent indicator background, and chromatographed in a propanol-ammonium sulphate-phosphate solvent system²⁴. Nucleotides were localised under ultraviolet light, and deoxyribose was localised by a cysteine-sulphuric acid spray²⁵, in comparison with known nucleotide standards that were run in parallel with the hydrolysate. Sample 1 was the hydrolysed precipitate formed with poly(dA)-2poly(U) and sample 2 that formed with poly(dA)-poly(dT)-poly(U). The hatched areas indicate the presence of both ultraviolet-detectable and acid cysteine-reactive material. Most of the latter was in the position of dAMP or, in sample 2, of dTMP as well. Some remained at the origin; in control experiments, protein stained in this way and stayed at the origin.

adding poly(U) to the hybrid poly(A)·poly(dT), which did not react alone with antibody to triple-stranded poly(A)·2poly(U). As poly(U) was added, new complexes formed and reacted strongly with the serum (Fig. 4a). At the same time, the formation of the new complexes was reflected in a reduction in double-stranded antigen available to react with specific anti-hybrid antibody (Fig. 4b). If the third strand were situated in the major groove of the original double helix, as with poly(A)·2poly(U)²¹, it may sterically block some of the double-stranded determinants while forming the triple helix. Alternatively, the addition of the third strand may have altered the conformation of the original double helix. In either case, the effect was not total, as 20–30% of the poly(A)·poly(dT) determinants remained accessible to the anti-hybrid antibodies, as judged from the degree of lateral shift in the complement fixation curve (Fig. 4).

The new complexes of poly(A)·poly(dT)·poly(U) did not react with antisera to either of the poly(dA)-containing helices. Thus the two triple-helical polynucleotides formed with poly(A) were immunologically similar to each other but distinct from the triple-helical polymers formed with poly(dA), while the latter two were very similar to each other.

The structure of the antigenic determinants cannot be defined precisely, but the backbones of two or three strands probably contribute to one determinant, and they must be in the proper orientation with respect to each other, because antibodies reactive with poly(dA)·poly(dT)·poly(U) did not react with poly(dA)·poly(dT) or poly(A)·poly(dT)·poly(U). Similarly, antibodies to double-helical poly(A)·poly(dT) may fail to react with other helices containing one of these chains, such as poly(A)·poly(U) or poly(dA)·poly(dT). Again it seems that portions of both helices, in proper orientation, form one determinant. With several antigen systems, the maximal size of the corresponding antibody combining site is about $7 \times 12 \times 35\text{\AA}$ (ref. 22). This could encompass a full helical turn or the full width of the

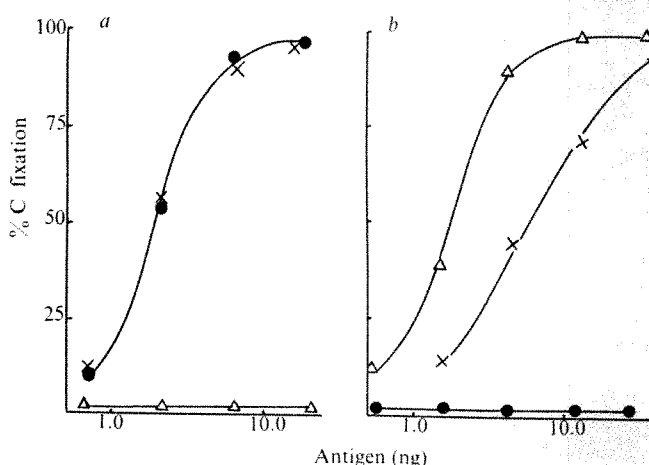


Fig. 4 Complement fixation cross reactivity of poly(A)-2poly(U) and poly(A)-poly(dT)-poly(U) with: (a) anti-poly(A)-2poly(U) antiserum and (b) anti-poly(A)-poly(dT) antiserum. The poly(A)-2poly(U) (●) was prepared by mixing 1 volume of poly(A) with 2 volumes of poly(U), with both polymers at a concentration of 0.4×10^{-7} mol of nucleotide per ml in 0.15 M NaCl, 0.01 M phosphate, pH 7.4, at 37°C . Triple-helical complexes (X) were formed by the addition of 1.2×10^{-8} mol of poly(U) nucleotide to 1.6×10^{-8} mol of poly(A)-poly(dT) (△) nucleotide, in 0.025 ml, in 0.4 M NaCl; the mixture was incubated with complement fixation buffer. The anti-poly(A)-2poly(U) antiserum was used at 1/1,000 dilution for reaction with triple-stranded forms and at 1/100 was not reactive with poly(A)-poly(dT). The anti-poly(A)-poly(dT) antiserum was used at 1/1,000 dilution.

helix, including portions of three adjacent strands, in a shallow cavity or groove on the antibody molecule.

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- ¹ Stollar, B. D., in *The Antigens*, 1, 1-85 (edit. by Sela, M.) (Academic Press, New York, 1973).
- ² Nahon, E., Michelson, A. M., and Lacour, F., *Biochim. biophys. Acta*, **149**, 127-139 (1967).
- ³ Schwartz, E. F., and Stollar, B. D., *Biochem. biophys. Res. Commun.*, **35**, 115-120 (1969).
- ⁴ Stollar, B. D., *Science*, **169**, 609-611 (1970).
- ⁵ Plescia, O. J., Strampp, A., and Kwiatkowski, Z., *Fedn. Proc.*, **28**, 695 (1969).
- ⁶ Talal, N., Steinberg, A. D., and Daley, G. G., *J. clin. Invest.*, **50**, 1248-1252 (1971).
- ⁷ Nahon-Merlin, E., Michelson, A. M., Verger, C., and Lacour, F., *J. Immunol.*, **107**, 222-226 (1971).
- ⁸ Silverstein, S. C., and Schur, P. H., *Virology*, **41**, 564-566 (1970).
- ⁹ Stollar, B. D., and Stollar, V., *Virology*, **42**, 276-280 (1970).
- ¹⁰ Stollar, V., and Stollar, B. D., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 993-1000 (1970).
- ¹¹ Colby, C., Stollar, B. D., and Simon, M. I., *Nature new biol.*, **229**, 172-174 (1971).
- ¹² Morgan, A. R., and Wells, R. D., *J. molec. Biol.*, **37**, 63-80 (1968).
- ¹³ Zubay, G., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 456-461 (1962).
- ¹⁴ Miller, J. H., and Sobell, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1201-1205 (1966).
- ¹⁵ Plescia, O. J., Braun, W., and Palczuk, N. C., *Proc. natn. Acad. Sci. U.S.A.*, **52**, 279-285 (1964).
- ¹⁶ Poonian, M. S., Schlabach, A. J., and Weissbach, A., *Biochemistry*, **10**, 424-427 (1971).
- ¹⁷ Felsenfeld, G., Davies, D. R., and Rich, A., *J. Am. chem. Soc.*, **79**, 2023-2024 (1957).
- ¹⁸ Stevens, C. L., and Felsenfeld, G., *Biopolymers*, **2**, 293-314 (1964).
- ¹⁹ Riley, M., Maling, B., and Chamberlin, M. J., *J. molec. Biol.*, **20**, 359-385 (1966).
- ²⁰ Rich, A., *Proc. natn. Acad. Sci. U.S.A.*, **46**, 1044-1053 (1960).
- ²¹ Arnott, S., and Bond, P. J., *Nature new biol.*, **244**, 99-101 (1973).
- ²² Kabat, E. A., *J. Immunol.*, **97**, 1-11 (1966).
- ²³ Wasserman, E., and Levine, L., *J. Immunol.*, **87**, 290-295 (1961).
- ²⁴ Wahba, A. J., and Friedkin, M., *J. biol. Chem.*, **237**, 3794-3801 (1962).
- ²⁵ Buchanan, J. G., *Nature*, **168**, 1091 (1951).

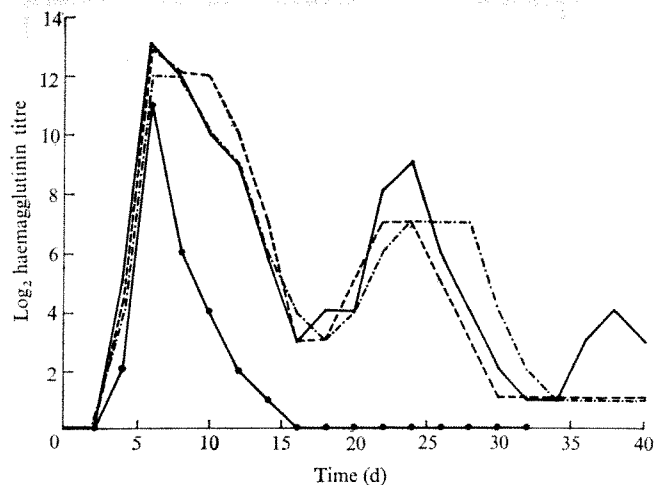


Fig. 1 Anti-sheep RBC response in chickens treated with CoF. Plot of haemagglutinin levels (\log_2 titre) following an intravenous injection of 10^{10} sheep erythrocytes. ●—● The average haemagglutinin titres for a group of 10 normal birds. The response reaches maximum at 6 d and thereafter rapidly declines, remaining at or near zero after 16 d. —○—○ Haemagglutinin titres of individual birds treated with cobra venom factor (CoF). The first haemagglutinin response of all birds is slightly increased in comparison with controls at the peak and delayed in the decline phase; all birds also show a further peak (maximum at day 24-25). In the case of one bird a further (third) peak developed (maximum at day 38).

antibody product and is thought to operate by the process of segregation of B cells into germinal centres².

We have investigated the O-agglutinin response to an intravenous injection of 10^9 heated *Salmonella adelaide* O organisms (Fig. 2). The agglutinin response kinetically resembles that to HSA or sheep erythrocytes, but the initial peak and decline is followed by at least four further peaks of declining magnitude. The class of immunoglobulin involved was investigated by treatment of serum samples with 0.3 M 2-mercaptoethanol for 1 h; the results showed that all agglutinin titres were reduced near to zero. Thus, most antibody was 19S immunoglobulin. The occurrence of 7S chicken agglutinins for *S. adelaide* O antigen was further investigated by use of radiolabelled antibodies prepared against chicken 7S and 19S immunoglobulin. Such antisera, shown to be specific for H chains by standard immunoelectrophoresis, were added to washed agglutinates of *S. adelaide* (heated O organisms) prepared in antigen excess, with 25 μ l of neat chicken serum. After 30 min interaction the uncombined radiolabelled immunoglobulin was removed by washing in physiological (0.15 M) NaCl, and the radioactivity remaining attached to the agglutinates was counted. The counts expressing amounts of 19S antibody are shown in Fig. 3 (range of four peak levels 40,000-82,000 c.p.m.). While estimates of antibody of 19S Ig class in serum samples reproduced the pattern of the previously described agglutination tests, counts of absorbed anti-7S Ig were low (range 100-2,000 c.p.m.) confirming the virtual absence of 7S antibody.

The difference between the curve of agglutinins of the serum response to *S. adelaide* O antigen and the antibody responses to HSA and sheep erythrocytes, is attributed to deficient 7S antibody production in the bird in response to an immunogenic stimulus to the lipopolysaccharide O antigen (see also data from the mouse³). It is hypothesised that normal negative feedback of the primary response requires switch over from 19S to 7S immunoglobulin, without which a single injection of antigen causes a series of cyclical fluctuations in 19S antibody.

Antibody responses to bacterial polysaccharides are unique in several ways. Thus, they depend exclusively on the B-cell

Effect of host decompensation on homeostasis of antibody production in fowl

THE primary response to human serum albumin (HSA) in the 6 to 10-week-old fowl is characterised by an early and accelerating rise in antibody level to a peak at about 9 d followed by an abrupt decline¹. Comparison of the kinetics of this decline from the peak gave a close correspondence between it and the curve of decline of passively transfused labelled homologous 7S immunoglobulin. It seems clear that antibody production has been switched off before the peak and little ensues thereafter. The primary haemagglutinin response to sheep erythrocytes has a similar shape but the peak is earlier (Fig. 1)². The *in vivo* suppressive effect is attributed to the negative feedback inhibition of the 7S

system⁴. Lipopolysaccharide O antigens interact with complement and activate the bypass mechanism of the complement cascade⁵.

This leads to the postulate that the switch over from 19S to 7S biosynthesis of antibody may be a complement-dependent process which is selectively inactivated by lipopolysaccharide antigens. Experiments were undertaken in birds which were immunised with a normal immunogenic stimulus (sheep erythrocytes) during a period of treatment with cobra venom factor sufficient to reduce *in vivo* levels of complement. Cobra venom factor (CoF) was prepared by a modification of previous methods^{6,7}.

One unit of anticomplementary activity was taken as the amount required to reduce the haemolytic capacity of chicken serum by 50%. The preparation of CoF reduced complement levels in the above test 160 fold. It was injected (150 U kg^{-1}) every other day according to the schedule recommended in ref. 8.

The response to a single injection of 10^{10} sheep erythrocytes now took the form shown in Fig. 1. The peak of the first rise of serum agglutinins is elevated (from average titre of 2^{10} to 2^{12-13}). The decline phase is somewhat shifted to the right and in all three cases there is a second rise and fall of serum agglutinins which reaches a peak on day 24 at 2^{7-9} titre. In the case of one bird this cycle is repeated once more, the titre reaching 2^7 on day 38. Another consequence was that virtually all antibody was in the form of 19S immunoglobulin without 7S immunoglobulin. The treatment of sera with 0.3 M 2-mercaptoethanol always reduced to near zero the agglutination titre of serum samples of the three treated with CoF shown in Fig. 1. In control birds, agglutinin titres in the period 5–15 d were also considerably reduced by 0.3 M 2-mercaptoethanol. All the reduced sera were treated to determine the presence or absence of incomplete haemagglutinins by addition of rabbit anti-chicken 7S immunoglobulin. In control birds this caused an increase of the peak titre from 2^7 to 2^{10} , the level declining thereafter. The sera of CoF-treated birds failed to show any appreciable increase following addition of rabbit anti-chicken 7S immunoglobulin.

Treatment with CoF, therefore, acts to impede the normal homeostatic mechanisms which terminate the antibody response to sheep erythrocytes. As this effect was accompanied by a defect of the normal switch from 19S to the 7S immunoglobulin form of antibody, an analogy can be drawn with the situation obtaining in the bird's response to *S. adelaide* O organisms. Here, too, the switch from 19S to 7S antibody fails to occur, and presumably this relates to some distinctive property of this type of antigen. Similarly, it was found that the antibody response to the H antigen of *S. adelaide* failed to terminate after the first peak of antibody (at 5–7 d) but underwent repeated cycles of antibody production of slowly diminishing magnitude.

Pepys^{9,10} has found that treatment of the mouse with CoF results in decrease and/or delay in the antibody responses to two thymus-dependent antigens (sheep erythrocytes and alum-precipitated ovalbumin) but no change in the responses

to two other thymus-independent antigens (pneumococcal polysaccharide type III and polyvinyl pyrrolidone). According to Pepys, the possibility that the CoF effect is due to antigenic competition is unlikely as the use of CoF in previous recipients of CoF cancelled the effect—circumstances in which antigenic competition would have been augmented. It was proposed that complement plays a role in T and B cell cooperation.

Our results can be interpreted as a conversion by CoF of the normal response of a thymus-dependent antigen (sheep erythrocytes) into one with the characteristics typical of a thymus-independent antigen. Both the O (lipopolysaccharide) and H (flagellar) antigens of *S. adelaide* are typical thymus-independent antigens^{11–13} in other animals.

A possible hypothesis to account for our present findings is that lipopolysaccharide and other thymus-independent antigens fail to switch 19S to 7S antibody production and fail to secure lasting homeostasis of the response since they activate the alternative pathway and lead to $\text{C3} \rightarrow \text{C3b} \rightarrow \text{C3b}$ (inactive) conversion⁹.

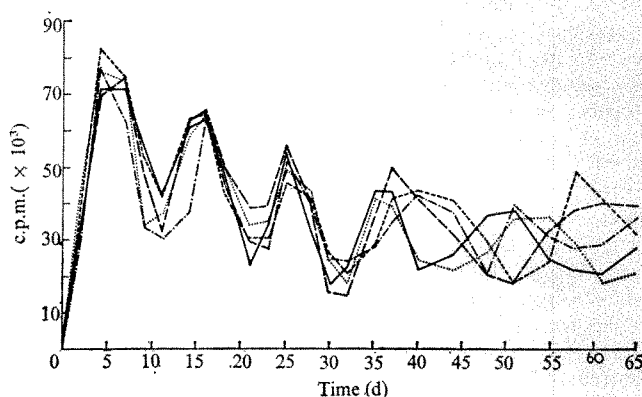


Fig. 3. Plot of antibody levels estimated by radio-immunoassay for individual birds injected with 10^9 *S. adelaide* O organisms. The response consists of a regular series of separated peaks, each followed by a phase of decline. At least five peaks are recognisable for each bird. Each plot represents the response of one bird.

Finally, it becomes necessary to investigate whether a T-independent response owes its characteristics (recycling of antibody production and lack of 7S response) to a positive anticomplementary action which causes an ineffective switch from 19S to 7S antibody production and defective homeostasis secondarily to a lack of avid 7S antibody. The consequences of this hypothesis can readily be tested and we hope to report the results of such investigations at a later date.

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¹ White, R. G., French, V. I., and Stark, J. M., *J. med. Microbiol.*, **3**, 65 (1970).

² White, R. G., in *Immunopotential* (edit. by Wolstenholme, G. E. W., and Knight, J.), 47 (Ciba Foundation Symposium No. 18, Elsevier, Amsterdam, 1973).

³ Britton, S., and Möller, G., *J. Immun.*, **100**, 1326 (1968).

⁴ Andersson, B., and Blumgren, H., *Cell Immun.*, **2**, 411 (1971).

⁵ Lachmann, P. J., and Nichol, P., *Lancet*, **i**, 465 (1973).

⁶ Müller-Eberhard, H. J., *Fedn Proc.*, **26**, 744 (1967).

⁷ Müller-Eberhard, H. J., and Fjellström, K. E., *J. Immun.*, **107**, 1666 (1971).

⁸ Cochrane, C. G., Müller-Eberhard, H. J., and Aikin, B. S., *J. Immun.*, **105**, 55 (1970).

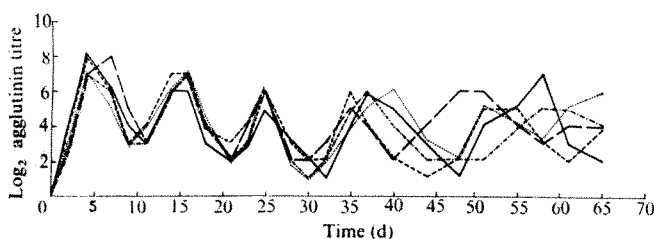


Fig. 2. Plot of agglutinin levels (\log_2 titre) for individual adult birds injected with 10^9 *S. adelaide* O. The initial response has a peak at 4–7 d followed by a rapid decline. At least four further cycles of antibody response are shown by all birds. Each plot represents the response of one bird.

- ⁹ Pepys, M. B., *Nature new Biol.*, **237**, 157 (1972).
¹⁰ Pepys, M. B., thesis, Cambridge University (1973).
¹¹ Humphrey, J. H., Parrott, D. M. V., and East, J., *Immunology*, **7**, 419 (1964).
¹² Andersson, B., and Blumgren, H., *Cell Immun.* **2**, 411 (1971).
¹³ Feldmann, M., *J. exp. Med.*, **135**, 735 (1972).

Vaccinia virus cytotoxin(s)

THE mechanisms whereby cytopathic viruses damage or kill susceptible cells are not fully understood. Some experiments with pox viruses involving partially inactivated virus or various metabolic inhibitors suggest a correlation between the production of virus proteins and the appearance of cytopathic effects without necessarily establishing a casual relationship between the two events^{1,2}. Other work suggests that inhibition of host protein synthesis, and by extension induction of cytopathic effects by vaccinia virus, is independent of virus-induced protein synthesis but is some function of the virus particle itself³. Here, we report direct experiments in which vaccinia-specific materials were isolated from infected HeLa cells, rendered free from infective virus and shown to be toxic to susceptible uninfected HeLa cells under appropriate test conditions. To our knowledge this is the first documented example of an observable cell-killing effect of vaccinia specific protein(s). It differs from that of the penton capsomere (toxin) of adenovirus which affects cell membranes from the outside causing them to detach from glass⁴, in that we believe it acts intracellularly.

HeLa cells were infected with vaccinia virus and collected 48 h after infection; soluble virus-free preparations (HVSA) containing both viral and hostspecific precipitinogens were prepared⁵. Preliminary work confirmed previous observations^{1,6} that HVSA had no effect on its own when added to cultures of HeLa cells either in suspension or in monolayers. This meant that either no virus-induced toxic substances existed, or, that they did and were not being taken up by the cells. To test the latter possibility a suitable biological system was sought in which cells could be induced to take up macromolecules in a biologically active state. Various membrane modifiers or uptake inducers⁷⁻¹¹, were tried but none proved more effective or easy to handle than hypertonic salt solutions.

For the test system, HeLa cells were assigned to four groups: group 1 was treated with calf serum medium, group 2 with calf serum medium and MgSO₄ (at the maximum concentration which resulted in $\geq 95\%$ cell survival; see Fig. 1a), group 3 with uninfected cell extract and MgSO₄ and, group 4 with HVSA and MgSO₄. Several hours after treatment, some of the HVSA-treated cells were dead as judged by vital staining; after overnight incubation when dead cells had detached and disintegrated, viable cells were stained and counted. The results of 23 experiments (Fig. 1b) show that there is in HVSA, something which is lethal to approximately 30% of HeLa cells present in the test population.

To determine the specificity of this cytotoxic mechanism HVSA was fractionated, using -S-S- linked immunosorbents^{12,13} derived from sera⁹ raised to uninfected HeLa cells or sera from rabbits infected with rabbit-grown vaccinia virus, to yield HeLa- and vaccinia-specific fractions, each overtly uncontaminated by the other in immunodiffusion tests. HVSA was also fractionated on DEAE-cellulose to yield a toxic fraction (Fig. 2) which contained several demonstrable vaccinia antigens in immunodiffusion tests. Only vaccinia-specific fractions were toxic; uninfected cell

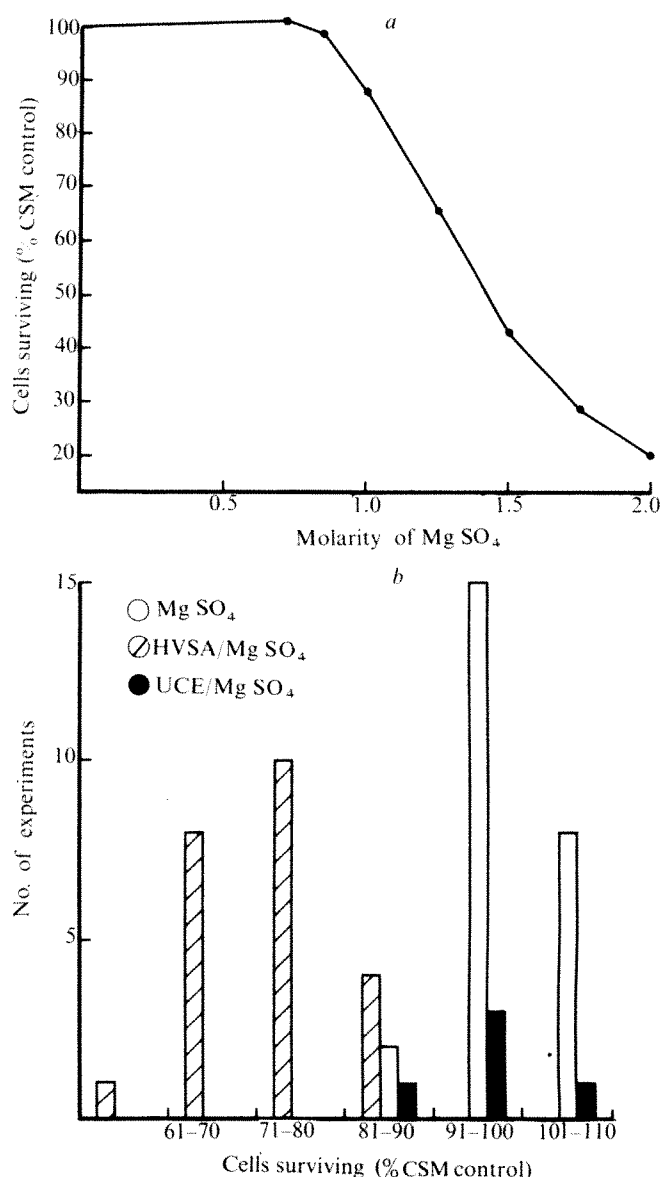


Fig. 1 Survival of HeLa cells treated with MgSO₄, infected cell extract (HVSA)/MgSO₄ and uninfected cell extract (UCE)/MgSO₄, expressed as a percentage of a calf serum medium (CSM)-treated control. Cells from 4 to 6-d-old monolayers were collected with EDTA, counted, diluted in CSM and continuously stirred. Samples (0.2 ml) were pipetted into the wells of a microtitre plate (Flow, M29-ART) so as to give 300-400 cells per well, and the plates centrifuged at 150g for 10 min. Plates were incubated overnight (37°C, 5% CO₂ in air). CSM was removed by inverting the plate, subjecting it to a sharp wrist flick, and removing the residual fluid by placing the plate on sterile filter paper. The cells were treated as follows: *a*, Varying concentrations of MgSO₄ in CSM were added and the cells subjected to the manipulative/counting procedures described in *b*. The object was to determine the highest concentration of MgSO₄ which $\geq 95\%$ of the cells would tolerate, that is, cells would go on to divide upon being returned to CSM. In this case it was 0.85 M MgSO₄, and this was used in the test as described below. Every line (or subline) of HeLa cells used was examined for MgSO₄ sensitivity. This only varied significantly when HeLa cells of a different source or cells obtained by certain selection pressures are used. When one line of normal cells is used the variation from day to day was minimal. *b*, For the test, the following mixtures were added: 0.85 M MgSO₄ in CSM, HVSA (or UCE) mixed with an equal volume of 1.7 M MgSO₄ in CSM, or CSM alone as a control. The cells were treated for 15 min at 37°C, washed twice in 0.2 ml volumes of CSM by the method described above and incubated in 0.2 ml of CSM overnight. Twenty-four hours after treatment the cells were

washed in phosphate buffer (PBS, pH 7.0, I , 0.26), stained for 15 min in freshly filtered 1% crystal violet in PBS Dulbecco A, washed in PBS, stained for 5 min in Lugol's iodine, and washed in PBS again. The wells were filled with PBS and covered with a piece of glass to avoid a refracting meniscus and the cells were observed using the $\times 4$ objective of a Prior Inverted microscope. Cells in the four corners of each well were counted representing about 80% of the area of the well; counts of about 500 cells per well were obtained with a standard deviation of 36 for CSM-treated controls. Routinely 8 or 12 wells were used for the CSM or $MgSO_4$ controls and four wells (to conserve materials) for the HVSA and UCE samples. The majority of tests resulted in approximately 70% of the cells surviving the combined $MgSO_4$ +HVSA treatment, where the control for $MgSO_4$ -treated cells was $99 \pm 6\%$ of the CSM control. Histogram shows data from 23 such experiments involving HSVA survival, in brackets of 10%, the frequency with which a given survival of cells was observed. The data for $MgSO_4$ only treatment is derived from the same experiments and is given to show the mildness of this treatment. A similar spread of results for CSM treated cells is obtained when these are measured as a percentage of the $MgSO_4$ -treated control.

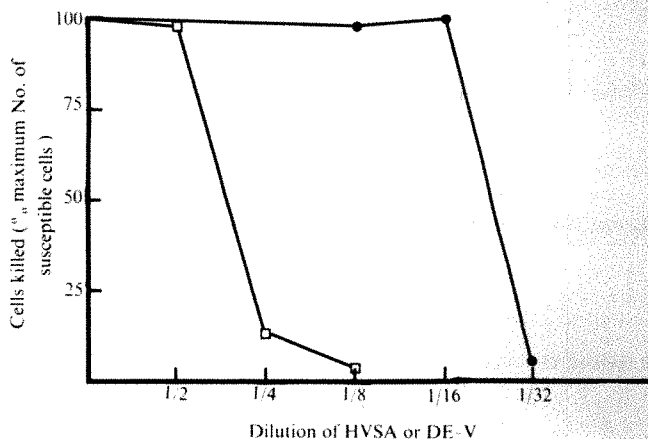


Fig. 2 Titration of toxicity of crude HVSA and partially purified vaccinia-specific antigens. HVSA (1.0 ml) was adsorbed to DEAE-cellulose (phosphate buffer pH 6.8, I , 0.0175) and eluted with stepwise increments of NaCl. The only toxic fraction (DE-V) was that eluted by 1.0 M NaCl and this was concentrated to 1.0 ml and titrated. Titres were expressed as the highest dilution causing death of the maximum number of susceptible cells in the system. Representative results are shown above. Initial concentrations of protein for HVSA □, and DE-V ●, were 20 and 2 mg ml⁻¹ respectively, representing a 10-fold purification. The total toxic activity expressed by DE-V was thus eight times greater than that of crude HSVA per unit volume and 80 times greater per mg protein.

extracts and HeLa materials from infected cells were not toxic in this test. The possibility that some virus-specific component allowed the cells to take up a toxic dose of $MgSO_4$ during the (technically convenient) simultaneous exposure of cells to $MgSO_4$ and HVSA (or vaccinia-specific fractions) was examined by pretreating cells with $MgSO_4$, removing $MgSO_4$, and adding HVSA; the latter still exhibited toxicity, thereby eliminating this possibility. The only biochemical activity associated with HVSA that we have demonstrated so far is the ability to release lysosomal enzymes¹⁴ from rabbit liver lysosome preparations. The level of pre-released β -glucuronidase (the marker enzyme chosen for assay) in uninfected cell extract was twice that in HVSA (due presumably to the loss of enzymes into the medium from the necrotic cells from which HVSA is prepared), but only HVSA caused the release of more enzyme (20–70% of maximum releasable by Triton-X100) from intact lysosomes.

Attempts to titrate toxicity showed that the activities of neat HVSA and 1/2 dilutions of HVSA were approximately the same as judged by the number of cells killed (approximately 30% in each case) and at higher dilutions the activity dropped rapidly to zero. This seemingly low activity may reflect the presence of inhibitors in HSVA. For example, comparative titration of HVSA (20 mg protein ml⁻¹) and the DEAE-cellulose toxic fraction (2 mg protein ml⁻¹) showed the latter to be eight times more active per unit volume or 80-fold more active per mg protein (Fig. 2). All these estimates of toxicity are conservative because activity dropped rapidly to zero. This seemingly low activity apparently stores indefinitely at -20° or -70° C, was slowly inactivated at 37° C, but in the presence of 0.85 M $MgSO_4$, all toxic activity was abolished after 15 min incubation at 37° C.

Preliminary experiments have been carried out in which HVSA was treated with a solid-phase protease (trypsin linked to Enzacyl AA, Koch-Light). This resulted in a loss of toxicity which, together with the fact that immunosorbents specific for vaccinia antigens removed the toxicity from HVSA, suggests that the active factor(s) is a protein and not infectious nuclei acid¹⁵ or double-stranded RNA¹⁶.

Various approaches have been tried, with crude HVSA, to discover why only 30% of the cells were killed. Presumably there is a subpopulation of HeLa cells with the ability to take up and/or be killed by the toxic factor(s) present in HVSA, an attribute which could be dependent on either

phenotypic or genotypic heterogeneity. First, synchronous cultures of HeLa cells were prepared by mitotic selection and hydroxyurea treatment¹⁷ and used in the test system at four different time points spanning one growth cycle. No difference was detected in the susceptibility of cells to HVSA with respect to different phases of cell growth; in each case only 30% of the cells died. Second, attempts were made to select cells which exhibited a more uniform response to $MgSO_4$ stress when the latter is applied to cells in monolayers. This would be reflected in a steeper drop in the viability of cells beyond some critical concentration of $MgSO_4$. To date we have not succeeded in selecting a population possessing this kind of sensitivity as a stable characteristic. The third experiment was carried out with cells derived from the survivors of a toxicity test and again only 30% of the cells died. It is technically very difficult to carry out a quantitative test on the survivors. These experiments suggest that susceptibility to the cytotoxic factor depends on a hitherto undefined transient physiological state which allows some of the cells, on treatment with $MgSO_4$, to take up a lethal dose of cytotoxin.

In summary, we present evidence for the existence of vaccinia-specific protein(s) which under defined conditions can cause the death of susceptible HeLa cells. Formal proof that the toxic action is an intracellular as opposed to a membrane-mediated one is difficult to obtain. But no toxicity is induced by HSVA *per se* in contrast to, for example, adenovirus penton which interacts with cell membranes causing them to detach from glass surfaces⁴.

Finally we would not extrapolate from these results to suggest that pox-virus infections are accompanied by a toxæmia in the classical bacterial sense—no such evidence has ever been adduced—but we think that an examination of virus-specific products isolable from infected animal tissues would be worthwhile. This might lead to the recognition of virus-induced factors, which are toxic to the cells in which they are made, and which contribute to the overall complex mechanism of viral pathogenicity.

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- ¹ Appleyard, G., Westwood, J. C. N., and Zwartouw, H. T., *Virology*, **18**, 159-169 (1962).
- ² Bablanian, R., *Symp. Soc. gen. Microbiol.*, **22**, 359-381 (1972).
- ³ Moss, B., *J. Virol.*, **2**, 1028-1037 (1968).
- ⁴ See Ref. 2 for references.
- ⁵ Birkbeck, T. H., and Stephen, J., *J. gen. Virol.*, **8**, 133-142 (1970).
- ⁶ Singh, L. M., *Indian J. Exp. Biol.*, **10**, 344-346 (1972).
- ⁷ Pagano, J. S., McCutchan, J. H., and Vaheri, A., *J. Virol.*, **1**, 891-7 (1967).
- ⁸ Ryser, H. J.-P., *J. Cell Biol.*, **32**, 737-750 (1967).
- ⁹ Ryser, H. J.-P., *Science*, **159**, 390-396 (1968).
- ¹⁰ Morfe, A., Popa, L., Burducea, O., and Portocala, R., *Acta Virol.*, **13**, 169-178 (1969).
- ¹¹ Lucy, J. A., *Nature*, **227**, 815-817 (1970).
- ¹² Paton, C. R., and Allison, A. C., *Nature*, **227**, 707-708 (1970).
- ¹³ Crook, N. E., Stephen, J., and Smith, H., *Immunochemistry*, **9**, 945-960 (1972).
- ¹⁴ Nicklin, M. G., and Stephen, J., *Immunochemistry*, **11**, 35-44 (1974).
- ¹⁵ Thacore, H., and Wolff, D. A., *Nature*, **218**, 1063-1064 (1968).
- ¹⁶ Pagano, J. S., *Prog. med. Virol.*, **12**, 1-48 (1970).
- ¹⁷ Cordell-Stewart, B., and Taylor, H. W., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1326-1330 (1971).
- ¹⁸ Nias, A. H. W., and Fox, M., *Cell Tiss. Kinet.*, **4**, 375-398 (1971).

Effects of dopamine-like drugs on rat striatal adenylyl cyclase have implications for CNS dopamine receptor topography

THE involvement of central dopaminergic neurones in the pathogenesis of several extrapyramidal movement disorders is well documented^{1,2}. Moreover it has been suggested that dopaminergic mechanisms may also be involved in the physiological mechanisms underlying psychoses³. For these reasons there is considerable neuropharmacological interest in the interaction between anti-Parkinsonian and neuroleptic drugs and central dopaminergic systems. Recently it has been shown that homogenates of tissues containing dopaminergic synapses respond to low concentrations of dopamine by an increased production of cyclic AMP. These areas include the bovine superior cervical ganglion⁴, the rat and bovine retina⁵, rat basal ganglia⁶, olfactory tubercle, nucleus accumbens⁷ and cortex⁸. The effects of dopamine in some of these systems are mimicked by dopamine receptor stimulating drugs such as apomorphine^{6,7} and 1-(3,4-dihydroxybenzyl)-4-(2-pyrimidyl) piperazine (S584)⁹ and antagonised by neuroleptic drugs^{6,10-12}. It seems, therefore, that these systems represent valid models of CNS dopamine receptors and it has been suggested that they may even comprise the dopamine receptor itself⁶. We have studied the dopamine-sensitive adenylyl cyclase of the rat striatum in order to define some of the structural requirements for dopamine receptor agonists.

In accordance with previous reports¹⁰, we found that addition of low concentrations of dopamine to rat striatal homogenates increased cyclic AMP accumulation during a

brief incubation *in vitro*. Half maximal stimulation was obtained at approximately 3 μ M dopamine. Experimental details are shown in Fig. 1. The effects of various compounds related to dopamine and apomorphine were also investigated. Homogenates were incubated with drugs at concentrations between 10⁻⁶ and 10⁻³ M. These results are summarised in Tables 1 and 2.

Dopamine and its N-methyl derivative epinine had similar activities as agonists. Further methyl group substitution of the amine function as in the N,N-dimethyl and the quaternary methyl dopamine derivatives led to compounds that were less active agonists.

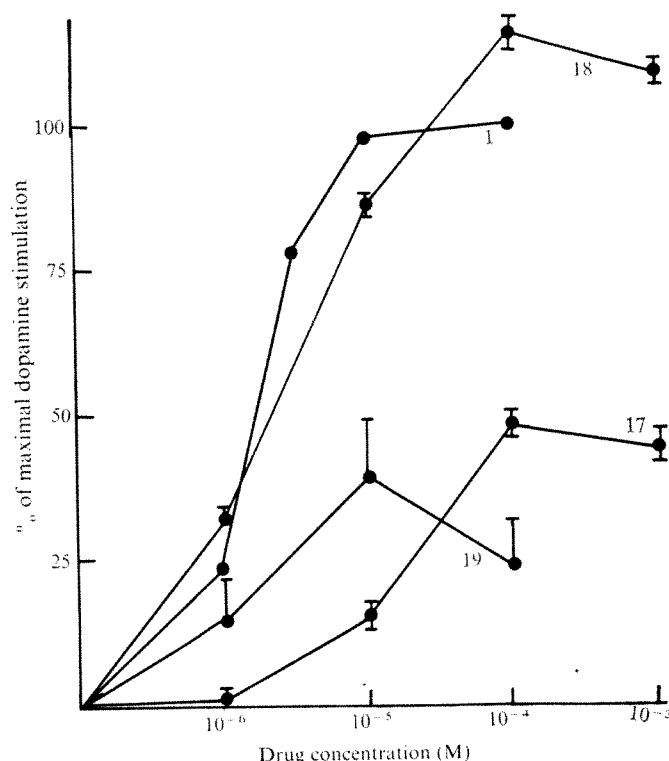


Fig. 1 Effect of cyclic analogues of dopamine on stimulation of cyclic AMP production in rat striatal homogenates. Sprague-Dawley rats weighing 180-250 g were used. The rats were decapitated and their brains rapidly removed and placed on ice. The striata were dissected as in ref. 25 and homogenised with a motor driven teflon/glass homogeniser in approximately 25 vol (w/v) of 2 mM Tris maleate buffer pH 7.4 containing 2 mM EGTA. Fifty microlitre aliquots of this homogenate were added to 250 μ l of 80 mM Tris maleate buffer, containing 2 mM MgSO₄, 0.2 mM EGTA and various drugs as indicated. The incubation tubes were kept on an ice/salt bath while ATP was added to a final concentration of 0.5 mM. The incubation tubes were then placed in a shaking water bath at 30°C for 2.5 min. At the end of this time the tubes were transferred to a boiling water bath for 2.5 min. The contents of each tube were centrifuged for 5 min in a microcentrifuge to sediment the denatured protein. 10 μ l aliquots of the supernatant were assayed for cyclic AMP content by the method of Brown *et al.*²⁶ using a linear standard curve produced with aliquots of authentic cyclic AMP between 0.2 and 8.0 pmol. The levels of cyclic AMP increased from a basal level of 31.8 \pm 0.96 pmol per assay tube (approximately 2 mg wet weight tissue) to 66.9 \pm 3.69 pmol per assay tube (mean value \pm s.e.m. $n=9$) in the presence of a maximally stimulating concentration of dopamine (100 μ M). Incubations with dopamine at 100 μ M was included in every experiment for comparison. As the stimulation produced by 100 μ M was slightly variable between experiments the effect of each agonist was compared with the effect of 100 μ M dopamine in the same experiment using the same striatal homogenate. The effect of 100 μ M dopamine was normalised to 100%. Results are means of between four and 10 separate incubations, standard errors were less than \pm 10%. Numbers on graphs refer to the structures given in Tables 1 and 2.

Table 1 Effect of phenylethylamine derivatives on cyclic AMP production in rat striatal homogenates

$ \begin{array}{c} \text{R}_1 \text{---} \text{C}_6\text{H}_3 \text{---} \text{X} \text{---} \text{N}(\text{R}_3)(\text{R}_4) \\ \\ \text{R}_2 \end{array} $								
Name	R ₁	R ₂	X	R ₃	R ₄	R ₅	Max. Stim. (%) [*]	EC50M [†]
1 Dopamine	OH	OH	—(CH ₂) ₂ —	H	H	—	100	2 × 10 ⁻⁶
2 Epinine	OH	OH	—(CH ₂) ₂ —	H	CH ₃	—	100	1.5 × 10 ⁻⁶
3 N,N-Dimethyl dopamine	OH	OH	—(CH ₂) ₂ —	CH ₃	CH ₃	—	48	2 × 10 ⁻⁶
4 N,N,N-Trimethyl dopamine	OH	OH	—(CH ₂) ₂ —	CH ₃	CH ₃	CH ₃ +	30	3 × 10 ⁻⁶
5 <i>l</i> -Noradrenaline	OH	OH	—CH—CH ₂ —	H	H	—	97	4 × 10 ⁻⁶
6 <i>dl</i> - α -Methyl dopamine	OH	OH	$ \begin{array}{c} \text{OH} \\ \\ \text{—CH}_2\text{—CH—} \\ \\ \text{CH}_3 \end{array} $	H	H	—	58	1 × 10 ⁻⁴
7 <i>m</i> -Tyramine	H	OH	—(CH ₂) ₂ —	H	H	—	—	—
8 <i>p</i> -Tyramine	OH	H	—(CH ₂) ₂ —	H	H	—	—	—
9 3,4-Dihydroxybenzylamine	OH	OH	—CH ₂ —	H	H	—	—	—
10 3,4-Dihydroxypropylamine	OH	OH	—(CH ₂) ₃ —	H	H	—	—	—
11 3,4-Dihydroxybutylamine	OH	OH	—(CH ₂) ₄ —	H	H	—	—	—
12 <i>d</i> -Noradrenaline	OH	OH	—CH—CH ₂ —	H	H	—	—	—
13 <i>m</i> -Methoxytyramine	OH	CH ₃ O	$ \begin{array}{c} \text{OH} \\ \\ \text{—CH}_2\text{—CH—} \\ \\ \text{CH}_2\text{—} \end{array} $	H	H	—	—	—
14 <i>p</i> -Methoxytyramine	CH ₃ O	OH	—(CH ₂) ₂ —	H	H	—	—	—
15 L-DOPA [‡]	OH	OH	—CH ₂ —CH—	H	H	—	—	—
16 <i>dl</i> -Amphetamine	H	H	$ \begin{array}{c} \text{COOH} \\ \\ \text{—CH}_2\text{—CH—} \\ \\ \text{CH}_3 \end{array} $	H	H	—	—	—

* Stimulation produced by 100 μ M dopamine is taken as 100%. For experimental details see Fig. 1.

† EC50 refers to concentration required to give half maximal stimulation produced between 10⁻⁶ and 10⁻³M. Agents shown as giving no stimulation of cyclic AMP production were not significantly effective ($P > 0.05$) at concentrations between 10⁻⁶ and 10⁻³M.

‡ Incubations included 0.4 mg ml⁻¹ of the dopa decarboxylase inhibitor NSD 1055 control incubations showed this had no effect on basal or dopamine-stimulated cyclic AMP production.

Compounds having 1–4 carbon atoms between the catechol nucleus and the primary amino group were examined. It was found that only dopamine, which has a 2 carbon side chain, was active. The analogues containing 1, 3 or 4 carbon atoms in the chain were all inactive. Incorporation of the side chain into a second ring system in an extended form as in 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene and apomorphine produced active agonists, whereas when the side chain was incorporated in a ring system in its nonextended form as in 6,7-dihydroxy-tetrahydroisoquinoline the resulting compound was much less active than the corresponding β -naphthylamine (Fig. 1). Substitution of a methyl group in the 1 position of the latter compound in salsolinol led to an inactive compound. Substitution of even larger groups at the 1 position also produced inactive compounds such as tetrahydropaveroline and the emetic drug emetine.

Compounds lacking the catechol group were inactive. Thus the *m* and *p* isomers of tyramine, *m* and *p*-methoxydopamine, amphetamine, apomorphine, dimethoxy-apomorphine and 2-amino-1,2,3,4-tetrahydronaphthalene did not stimulate the adenyl cyclase. Apomorphine, *dl*- α -methyl-dopamine and in particular 2-amino-6,7-dihydroxy 1,2,3,4-tetrahydronaphthalene, were all active in stimulating the enzyme system.

dl- α -Methyldopamine was less active than the parent compound. *l*-Noradrenaline was less than half as active as dopamine at a concentration of 1 × 10⁻⁵ M. The *d*-isomer of noradrenaline was inactive in concentrations up to 10⁻³ M. L-dopa was totally inactive.

As 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene was such a potent agonist, the effect of neuroleptic drugs on its adenyl cyclase stimulating ability was examined. The clinically efficacious neuroleptic chlorpromazine (1 × 10⁻⁶ M) potently inhibited the stimulation of the

enzyme system by this agonist, whereas promethazine 1 × 10⁻⁶ M, which is a phenothiazine antihistamine with little or no neuroleptic activity¹³ was much less active (Fig. 2). Similar results have already been reported for dopamine⁷. In addition a maximally stimulating concentration of dopamine (100 μ M) did not increase the stimulation produced by 100 μ M 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene. These observations suggest that the two agonists have the same locus of action.

It is of interest that in two other dopamine-sensitive preparations, namely the renal artery of the dog¹⁴ and certain neurones of the snail *Helix aspersa*¹⁵, epinine was also found to be equipotent with dopamine. Among the simple dopamine analogues there is an absolute requirement for a catechol grouping and a 2 carbon side chain attached to an amino group. The structural and conformational requirements of the amino group are apparently less stringent, as even the quaternary methyl ammonium compound still retains some activity. Activity is also not abolished when the amino group is incorporated into a second ring as in 6,7-dihydroxy-tetrahydroisoquinoline. Due to the greater potency of the dopamine analogues where the side chain is incorporated in an extended form, as in 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene and apomorphine, it seems that the preferred conformation of dopamine on interaction with the adenyl cyclase system is probably similar to the fully extended *trans* form. This has been shown to be the preferred conformation of dopamine in the solid state by X-ray crystallography¹⁶, in solution by NMR analysis and *in vacuo* by theoretical calculations¹⁷. Calculations based on published X-ray data for dopamine¹⁸ and apomorphine¹⁹ of the interatomic distance between the catechol oxygens O₁ and O₂ and the nitrogen atom of the amino group show that in dopamine N–O₁ is 6.83 Å, and N–O₂ is 7.83 Å. In the two *gauche* forms of dopamine

Table 2 Effect of β -naphthylamine, tetrahydroisoquinoline and aporphine analogues on rat striatal cyclic AMP production

Name	Basic Structure					Max* stim. %	EC50 M†
		R ₁	R ₂	R ₃	R ₄		
17 6,7-dihydroxytetrahydroisoquinoline	a	H	OH	OH	H	48	2×10^{-5}
18 2-amino-6,7-dihydroxy-(1,2,3,4)-tetrahydronaphthalene	b	OH	OH	—	—	115	4×10^{-6}
19 Apomorphine	c	OH	OH	—	—	45	2×10^{-6}
20 Salsolinol	a	CH ₃	OH	OH	H	—	—
21 1-methyl-7,8-dihydroxytetrahydroisoquinoline	a	CH ₃	H	OH	OH	—	—
22 2-amino-1,2,3,4-tetrahydronaphthalene	b	H	H	—	—	—	—
23 Aporphine	c	H	H	—	—	—	—
24 Dimethoxyapomorphine	c	CH ₃ O	CH ₃ O	—	—	—	—
25 Tetrahydropapaveroline	a		OH	OH	H	—	—
26 Emetine	a		CH ₃ O	CH ₃ O	H	—	—

* Stimulation produced by 100 μ M dopamine is taken as 100%. For experimental details see Fig. 1.

† EC50 is taken as the concentration giving half maximal stimulation obtained between 10^{-3} and 10^{-6} M.

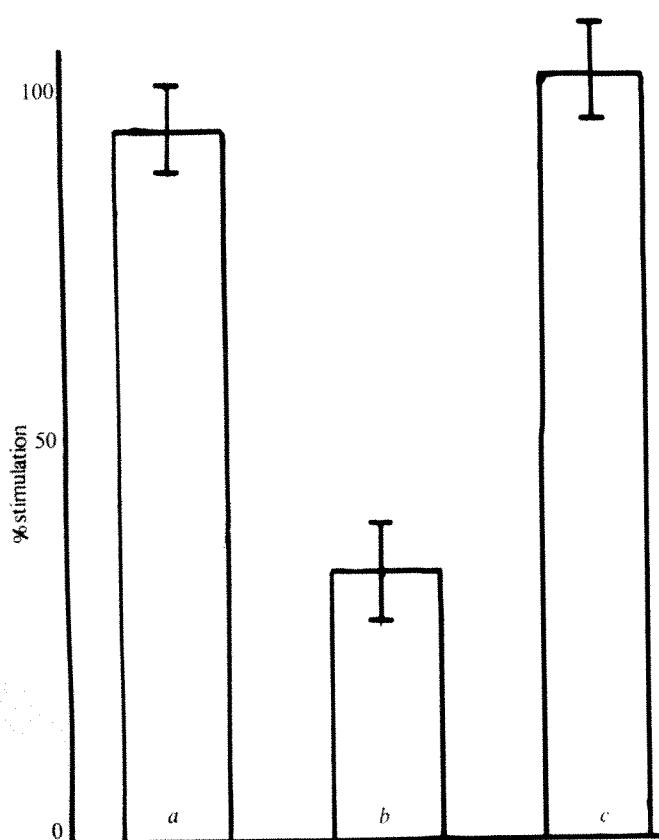


Fig. 2 Effect of *b*, 10^{-6} M chlorpromazine and *c*, 10^{-6} M promethazine on a maximally stimulating concentration of 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (10^{-4} M). *a*, 10^{-4} M 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene alone. Basal cyclic AMP levels for experiments in Fig. 2 were 32.5 pmol per assay tube (2 mg wet weight of tissue). For experimental details see Fig. 1.

(Fig. 3) these interatomic distances are significantly smaller¹⁹. This may be regarded as further evidence for the concept that the preferred active-site conformation of dopamine resembles the fully extended *trans* form. Molecular models of the rigid analogue 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene clearly show that the conformation of the amino and catechol group is very similar to that occurring in the crystal structure of dopamine, thus probably accounting for its potency as an agonist. Similar suggestions have been advanced by Rekker *et al.*¹⁹ who have argued against Kier and Truitt's²⁰ suggestion that the *gauche* conformation of dopamine, and hence the tetrahydroisoquinoline portion of apomorphine, are the im-

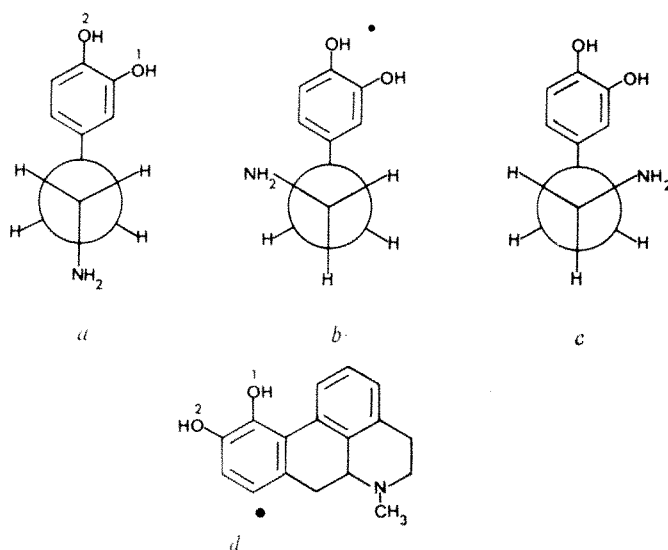


Fig. 3 *a-c*, Newman projections of dopamine conformers: *a*, *trans*; *b*, *c*, *gauche*; *d*, apomorphine.

portant conformations and moieties, respectively, for interaction with the dopamine receptor. The conclusions of Rekker *et al.* are supported by our present results. These findings also support the suggestion of Horn and Snyder²¹ that the preferred conformation of dopamine at its receptor site is similar to the fully extended *trans* form, and that this conformation could be superimposed on the X-ray structure of chlorpromazine, hence accounting possibly for the receptor blocking activity of the latter compound.

The results reported here may help in the design of future dopamine receptor stimulating agents. In particular rigid analogues of dopamine such as 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene or other similar compounds may be useful anti-Parkinsonian drugs^{22,23}. Initial studies suggest that 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene does not cross the blood brain barrier and possibly modifications of the molecule to increase its lipid solubility should be examined.

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- ¹ Klawans, H. L., jun., *The Pharmacology of Extrapyramidal Movement Disorders* (Karger, Basel, 1973).
- ² Carlsson, A., *Acta neurol. scand.*, suppl. **51**, 11–42 (1972).
- ³ Matthysse, S., *Fedn. Proc.*, **32**, 200–205 (1972).
- ⁴ Greengard, P., McAfee, D. A., and Keabian, J. W., in *Advances in cyclic nucleotide research* vol. 5, 337–357 (Raven Press, New York, 1972).
- ⁵ Brown, J. H., and Makman, M. H., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 539–543 (1972).
- ⁶ Keabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145–2149 (1972).
- ⁷ Horn, A. S., Cuellar, A. C., and Miller, R. J., *J. Neurochem.*, **22**, 265–270 (1974).
- ⁸ von Hungen, H., and Roberts, S., *Eur. J. Biochem.*, **36**, 391–401 (1973).
- ⁹ Miller, R. J., and Iversen, L. L., *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.*, **282**, 213–216 (1974).
- ¹⁰ Miller, R. J., and Iversen, L. L., *Trans. Biochem. Soc.*, **2**, 256–259 (1974).
- ¹¹ Miller, R. J., and Iversen, L. L., *J. Pharm. Pharmac.*, **26**, 142–144 (1974).
- ¹² Brown, J. H., and Makman, M. H., *J. Neurochem.*, **21**, 477–479 (1973).
- ¹³ Klein, D. F., and Davis, J. M., in *Diagnosis and Treatment of Psychiatric Disorders*, 74 (Williams and Wilkins, Baltimore, 1969).
- ¹⁴ Goldberg, L. I., Sanneville, P. F., and McNay, J. L., *J. Pharmac. exp. Ther.*, **163**, 188–197 (1968).
- ¹⁵ Woodruff, G. N., and Walker, R. J., *Int. J. Neuropharmac.*, **8**, 279–289 (1969).
- ¹⁶ Bergin, R., and Carlstrom, D., *Acta Cryst.*, **B24**, 1506–1510 (1968).
- ¹⁷ Bustard, T. M., and Egan, R. S., *Tetrahedron*, **27**, 4457–4469 (1971).
- ¹⁸ Giesecke, J., *Acta Cryst.*, **B29**, 1785–1791 (1973).
- ¹⁹ Rekker, R. F., Engel, D. J. C., and Nys, G. C., *J. Pharm. Pharmac.*, **24**, 589–591 (1972).
- ²⁰ Kier, L. B., and Truitt, E. B., *J. Pharmac. exp. Ther.*, **174**, 94–98 (1970).
- ²¹ Horn, A. S., and Snyder, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2325–2328 (1971).

- ²² Cannon, J. G., Kim, J. C., Aleem, M. A., and Long, J. P., *J. med. Chem.*, **15**, 348–350 (1972).
- ²³ Woodruff, G. N., *Comp. Gen. Pharmac.*, **2**, 43P (1971).
- ²⁴ Pinder, R. M., Buxton, D. A., and Woodruff, G. N., *J. Pharm. Pharmac.*, **24**, 903–904 (1972).
- ²⁵ Glowinski, J., and Iversen, L. L., *J. Neurochem.*, **13**, 655–669 (1966).
- ²⁶ Brown, B. L., Ekins, R. D., and Albano, J. D. M., in *Advances in cyclic nucleotide research*, vol. 2 (edit. by Greengard, P., and Robinson, G. A.), 25–40 (Raven Press, New York, 1972).

Demonstration of indolaminergic fibres in the median eminence of the duck, rat and monkey

MANY reports provide physiological evidence that monoaminergic (catecholaminergic and serotonergic) systems are involved in the control of the secretion of the hypothalamic hormones (releasing factors) at the level of the median eminence (ME) of the rat^{1–3}. Dopaminergic and noradrenergic fibres have been shown in the median eminence by histofluorescence technique^{4–7}. This technique fails to demonstrate the serotonin (5-HT) fluorophores because of their low yield and rapid fading⁸.

As serotonergic neurones are able to take up and retain selectively endogenous or exogenous 5-HT, we have used ³H-5-HT to demonstrate by radioautography⁹ serotonergic, or more generally, indolaminergic neurones in the ME of three species; the duck (Pekin), the rat (Wistar) and the macaque (*Macaca mulatta*). Adult male ducks and rats and adult female monkeys were used as experimental animals. They were all pretreated with a monoamine oxidase inhibitor (nialmide) injected intraperitoneally at the rate of 200 mg kg⁻¹ for 4 h before administration of the tracer which was given either *in vivo* by intraventricular^{6,10} injection, or *in vitro* by incubation.

In the *in vivo* experiments, the animals were killed 30 min to 3 h after tracer administration. The animals were perfused with saline, then with 3.64% glutaraldehyde solution in 0.05 M phosphate buffer. The dissected tissues were postfixed in 2% OsO₄ in the same buffer, dehydrated in ethanol and embedded in Epon. For *in vitro* experiments, the following procedure was used. After decapitation, the median eminence was incubated in 5 ml of a physiological medium containing 0.2 μCi ml⁻¹ and 0.02 μg ml⁻¹ of ³H-5-HT, that is a 10⁻⁷ M concentration of ³H-5-HT. This low concentration was maintained by a continuous renewal of the incubation liquid. The incubation lasted 45 min at 37° C (ref. 11). The tissues were then rinsed in saline, fixed in glutaraldehyde, and treated for electron microscopy as described above.

In each case, radioautographic treatment was performed as follows: thick sections were placed on glass slides, then coated with K5 (Ilford) emulsion and exposed for 15 d before development in D19B (Kodak). Thin sections were put on celloidin-coated slides, stained with uranyl acetate and lead citrate and coated with a carbon film and a monolayer of L4 (Ilford) emulsion. After exposure for 3–6 weeks, the slides were developed in Microdol X (Kodak). The treated sections were transferred to copper grids and observed in an electron microscope¹².

In the three species, locations of radioautographic reactions after intraventricular injection of the tracer gave results identical to *in vitro* incubation. In the duck, very dense accumulations of silver grains were mainly localised in the most external layers of the ME in the vicinity of hypophysial primary portal vessels (Fig. 1). In the rat, clusters of silver grains were seen throughout the ME and were particularly abundant in the most external layers (Fig. 2a). In the monkey, the radioautographic reaction affected all layers of ME (Fig. 2b). Moreover, dense



Fig. 1 Electron microscope radioautographs of two adjacent thin sections of duck ME after intraventricular injection of ^3H -5-HT. There are dense accumulations of silver grains on reactive fibres while the background is practically free. The comparison of the two pictures shows the labelling of the same fibre in the two sections. This fibre reaches the basement membrane (BM). Insets show *a*, a degenerating fibre in the same region after 5,6-DHT treatment and, *b*, an enlarged, labelled fibre with characteristic 1,000 Å dense granules.

and elongated clumps of silver grains frequently overlaid axonal bundles in the internal region.

In all three cases, electron microscopic examination revealed that labelled structures were axonal varicosities (from 0.3–1 µm) representing less than 1% of axonal sections in the ME. These labelled fibres contained densely packed inclusions: electron lucent vesicles and more scarce but very typical dense granules of larger size (1,000 Å) (Figs. 1 and 2). Some labelled terminals were seen in contact with the basement membrane close to portal vessels which are external and internal in mammals but only external in the duck. Other fibres made contact with a tanyocyte process but no synaptic differentiation could be seen at this level.

Our results give rise to three main questions concerning the nature of the radioactive molecules retained in the tissues, the identity of the labelled fibres and the function of such an innervation.

As the main pathway of catabolism of 5-HT is its oxidation by MAO (ref. 13), which was inhibited in our experiments, it seems probable that observed radioautographic reactions marked mainly the molecules of ^3H -5-HT.

The second question raises the problem of the specific labelling of indolaminergic neurones by ^3H -5-HT, as at high concentrations, 5-HT may be taken up into adrenergic neurones¹⁴. Thus, the most convincing evidence for selective uptake of ^3H -5-HT by 5-HT fibres comes from results of *in*

vitro incubations because at the concentration used (10^{-7} M), ^3H -5-HT is specifically taken up by serotonergic neurones¹⁵. Moreover, the ultrastructural features of the labelled fibres are clearly differentiated from those previously described in noradrenergic fibres^{6,7}. Noradrenergic fibres are larger and contain more scattered inclusions among which eccentric core vesicles appear to be characteristic. On the other hand, dense 1,000 Å granules seemed typical of ^3H -5-HT labelled fibres¹⁶. Finally, the pattern of labelled fibres after the administration of ^3H -5-HT was different from that obtained after administration of labelled catecholamines¹⁶. This fact was particularly clear in the duck ME where catecholaminergic fibres are concentrated in the internal zone.

A complementary pharmacological approach was attempted in this species to confirm the specificity of radioautographic results. Two intraventricular injections of 100 µg of 5,6-dihydroxytryptamine (5,6-DHT) which selectively destroys indolamine neurones^{8,17}, were administered 4 and 2 d before the application of the tracer. After this treatment, no incorporation of ^3H -5-HT was observed in the ME, and degenerating fibres appeared in the external zone (Fig. 1*a*).

All these results demonstrate the existence of indolaminergic fibres in the ME. To assume that they are truly serotonergic it would be necessary to identify them with structures containing 5-HT, the existence of which has been biochemically proved in bovine¹⁸ and duck¹⁹ ME.

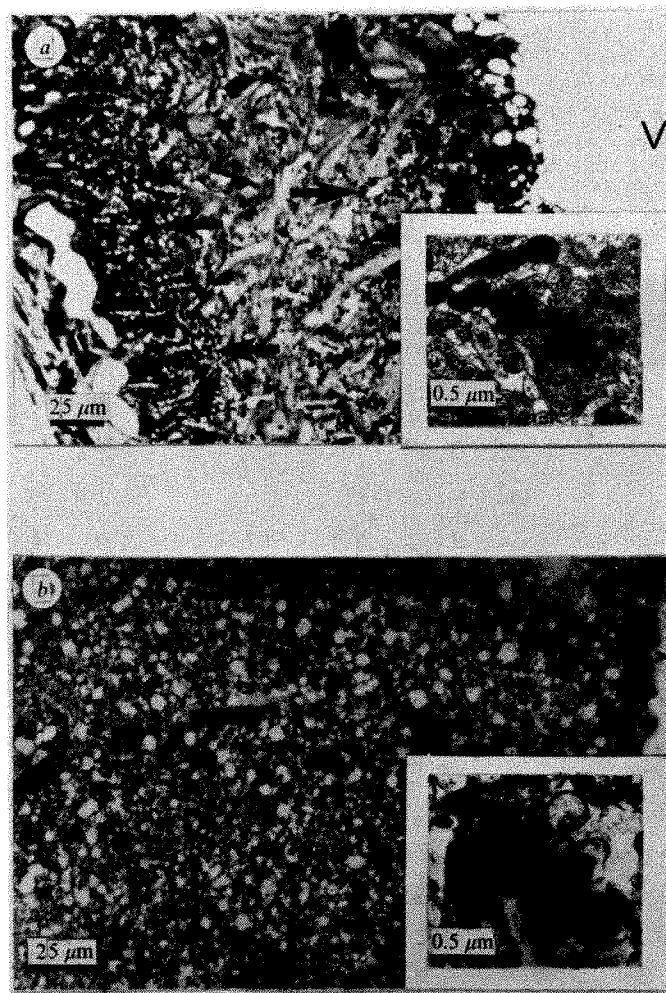


Fig. 2 Light microscope radioautographs of the ME of, *a*, the rat after intraventricular injection of ^3H -5-HT and, *b*, of the macaque after low concentration *in vitro* incubation in ^3H -5-HT. Note the localised distribution of radioautographic reaction and the low background. In the rat ME the tracer is preferentially localised near the internal and external plexuses. Insets show labelled fibres in each animal after low concentration *in vitro* incubation in ^3H -5-HT. V, Third ventricle.

In the absence of clearly defined synaptic junctions, we can only speculate on the function of the indolaminergic fibres²⁰. Their proximity to the processes of tanycytes and to external basement membrane indicates that they may be involved in the transport of hypothalamic factors by glial cells and/or their release into the portal vessels.

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- ¹ Wurtman, R. J., *Neurosci. Res. Prog. Bull.*, **9**, No. 2 (1971).
- ² Kordon, C., *Mécanismes d'actions intracellulaires des hormones*, 77–91 (1971).
- ³ Labhsetwar, A. P., *Nature*, **229**, 203–204 (1971).
- ⁴ Jonsson, G., Fuxe, K., and Hökfelt, T., *Brain Res.*, **40**, 271–281 (1972).
- ⁵ Björklund, A., Moore, R. Y., Nobin, A., and Stenevi, U., *Brain Res.*, **51**, 171–181 (1973).
- ⁶ Calas, A., *Z. Zellforsch.*, **138**, 503–522 (1973).
- ⁷ Calas, A., Hartwig, H. G., and Collin, J. P., *Z. Zellforsch.*, **147**, 491–504 (1974).
- ⁸ Baumgarten, H. G., Lachenmayer, L., and Schlossberger, H. G., *Z. Zellforsch.*, **125**, 553–569 (1972).
- ⁹ Aghajanian, G., and Bloom, F., *J. Pharmac. exp. Ther.*, **156**, 23–30 (1967).
- ¹⁰ Vincent, J. D., Arnauld, E., and Bioulac, B., *Brain Res.*, **44**, 371–384 (1972).
- ¹¹ Alonso, G., *Thèse Spéc. Physiol.*, Montpellier, 9 (1973).
- ¹² Granboulan, P., *The use of radioautography in investigating protein synthesis* (edit. by Leblond, C. P., and Warren, K. B.), **4**, 43–63 (Academic Press, New York, 1965).
- ¹³ Page, I. H., and Carlsson, A., *Handbook of Neurochemistry* (edit. by Jajtha, A.), **4**, 251–262 (Plenum Press, New York, 1970).
- ¹⁴ Fuxe, K. T., and Ungerstedt, U., *Histochemie*, **13**, 16–28 (1968).
- ¹⁵ Shaskan, E. G., and Snyder, S. H., *J. Pharmac. exp. Ther.*, **175**, 404–418 (1970).
- ¹⁶ Calas, A., *C. r. hebdom. Séanc. Acad. Sci., Paris*, **274**, 925–927 (1972).
- ¹⁷ Björklund, A., Nobin, A., and Stenevi, U., *Brain Res.*, **53**, 117–127 (1973).
- ¹⁸ Piezzi, R. S., Larin, F., and Wurtman, R. J., *Endocrinology*, **86**, 1460–1462 (1970).
- ¹⁹ Calas, A., Priam, M., and Kordon, C., *C. r. Soc. Biol.* (in the press).
- ²⁰ Baumgarten, H. G., and Lachenmayer, L., *Z. Zellforsch.*, **147**, 285–292 (1974).

Antibody to bovine choline acetyltransferase and immunofluorescent localisation of the enzyme in neurones

THE neurones of the central nervous system that contain catecholamines, or serotonin, can be visualised histochemically by the highly fluorescent products that they form in the presence of formaldehyde vapour¹. No such elegant method exists for demonstrating the cholinergic neurones. Published methods for the histochemical localisation of cholinergic terminals and choline acetyltransferase (ChAc)^{2–5}, which is considered to be exclusive to them⁶, are indirect and non-specific. Recently, ChAc has been

purified and partially characterised from bovine caudate nuclei⁷. We report here (1) the production of specific precipitating antibody against bovine ChAc in the guinea pig; (2) characterisation of the antibody by immunodiffusion, by immunoelectrophoresis, by electrophoresis of the specific immunoprecipitates and by enzyme analyses, and (3) the visualisation of the cholinergic neurones in the central nervous system by the immunofluorescent method using specific antibody against purified ChAc. The specific antibody to ChAc can now be used in conjunction with the recently improved immunohistological technique⁸ to map the cholinergic pathway in the nervous system and to establish the localisation of the enzyme within the cholinergic neurone and its processes at the cellular level. Using the horse radish peroxidase technique⁹ and specific antibody to the glial fibrillary acidic protein, this astrocyte specific protein has been localised only within fibrous astrocytes and their processes at the light and electron microscopic levels⁹.

Specific precipitating antiserum to purified ChAc⁷ was prepared in albino male guinea pigs (600–700 g) as follows. Each animal received an emulsion containing 225 µg of ChAc, 0.7 ml 0.85% saline and 0.7 ml complete Freund's adjuvant (3.5 mg *Mycobacterium tuberculosis* H37RA) by intradermal injection distributed equally among twelve sites in the back. Seven days later an emulsion containing 125 µg ChAc, 0.7 ml 0.85% saline and 0.7 ml complete Freund's adjuvant was injected into six other sites in the back. Fourteen days after the first injection, an emulsion containing 125 µg ChAc, 0.7 ml of 0.85% saline and 0.7 ml of incomplete Freund's adjuvant was injected into six other sites in the back. Thirty-five days after the first injection, the two guinea pigs were bled by heart puncture.

Immunodiffusion and immunoelectrophoresis studies with the guinea pig antisera were performed as before¹⁰. Both antisera formed specific immunodiffusion lines against the crude extract of caudate nuclei, partially purified ChAc and purified ChAc but did not react against bovine serum or haemoglobin. The preimmunisation guinea pig sera did not react with any of the ChAc-containing extracts, bovine serum or haemoglobin. When the purified enzyme was subjected to immunoelectrophoresis at pH 8.6, one precipitin line was formed which corresponded to the area of migration for the ChAc when purified ChAc or a concentrated solution of crude extract of bovine caudate nuclei were used (Fig. 1).

Precipitates of immunoglobulin-ChAc complexes were prepared for electrophoresis by incubating the ChAc-containing solution with the antiserum for 1 h at 37°C followed by 20 h at 4°C. The precipitates were pelleted by centrifugation at 2,000g for 30 min in the cold and washed twice by resuspension in saline and centrifuged as before. Disc polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS) of the crude enzyme extract, purified ChAc and immunoprecipitates were carried out as before^{10,11}.

The homogeneity of ChAc has been demonstrated by polyacrylamide gel electrophoresis at pH 9.5 and 7.3 at different gel concentrations (ref. 7). The enzyme behaved as only one sharp peak in sedimentation velocity studies (L.P.C. and F.W. unpublished results) and also only one peak was obtained on gel filtration (L.P.C. and F.W., submitted for publication). The multiple bands in the SDS-polyacrylamide gel electrophoresis are not contamination but are rather aggregated subunits of the enzyme since ChAc aggregates easily¹². Similar aggregation in SDS-polyacrylamide gel electrophoresis has been found for purified glutamic acid decarboxylase¹³, and this enzyme has been localised successfully in rat cerebellum by the immunohistochemical technique¹⁴.

Electrophoresis at pH 7 in SDS (Fig. 2) of the ChAc (gel 3a) showed a major component migrating in the range

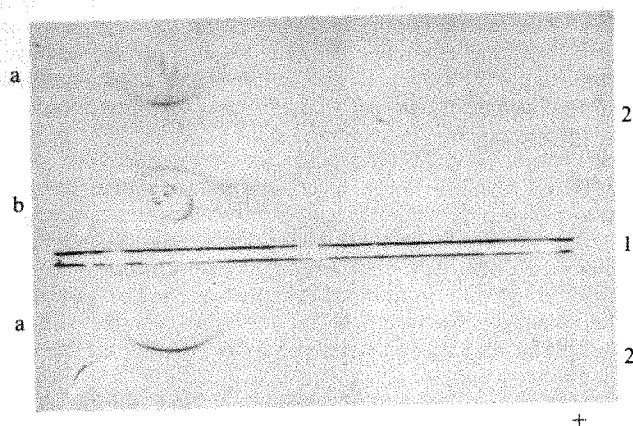


Fig. 1 Immunoelectrophoresis of purified bovine ChAc and crude extract of bovine caudate nuclei. Samples electrophoresed: *a*, ChAc; 3 mg ml⁻¹; *b*, crude extract of caudate nuclei, 17 mg ml⁻¹. Sera in the immunodiffusion troughs: 1, preimmunisation guinea pig serum; 2, serum containing specific antibody to ChAc.

of 60,000–67,000 molecular weight with additional minor components. Gels 2b and 2c are immunoprecipitates obtained by mixing the crude extract with the antiserum, and gels 3b and 3c are precipitates obtained by mixing the purified ChAc with the antiserum. In the absence of Cleland's reagent (reducing agent), the immunoprecipitates from the crude extract (gel 2b) and the purified ChAc (gel 2c) showed intact 160,000 molecular weight immunoglobulin and the ChAc bands. In the presence of Cleland's reagent, the immunoprecipitates from the crude extract (gel 3b) and the purified ChAc (gel 3c) showed the major enzyme band, the 53,000 molecular weight heavy chain, and the 25,000 molecular weight light chain of the dissociated immunoglobulin. The minor enzyme bands were obscured by the heavy chain immunoglobulin. The electrophoretic analyses of the specific immunoprecipitates demonstrate that the antiserum reacts specifically with the purified ChAc with was used to elicit the antibodies in the guinea pig.

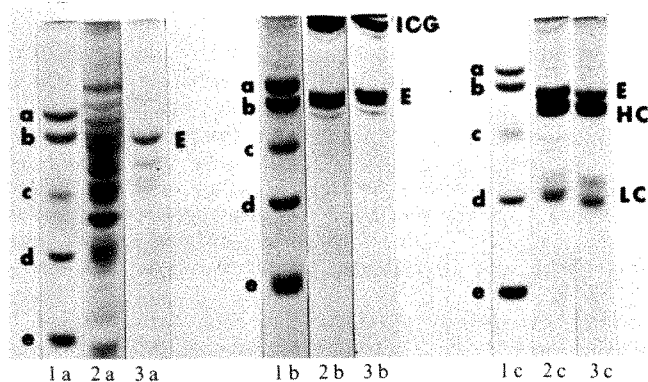


Fig. 2 Polyacrylamide gel electrophoresis in sodium dodecyl sulphate of a crude extract of caudate nuclei, purified ChAc, and immunoglobulin-ChAc complexes. Gels 1a–3a contain 7.5% acrylamide. Gels 1b–3b and 1c–3c contain 10% acrylamide. The protein samples in gels 1a–3a and 1c–3c were treated with Cleland's reagent; gels 1b–3b were not treated with Cleland's reagent. Gels 1a, 1b and 1c were mixtures of conalbumin (*a*), serum albumin (*b*), ovalbumin (*c*), chymotrypsinogen A (*d*), and cytochrome (*e*). Gel 2a was the crude extract from caudate nuclei; gel 3a was the purified ChAc; gels 2b and 2c were immunoprecipitates obtained by incubating the crude extract with antiserum, and gels 3b and 3c were immunoprecipitates obtained by incubating the purified ChAc with antiserum.

For enzymatic studies, aliquots of partially purified ChAc (50 μ l) (which is more stable than the purified ChAc)⁷ were incubated with equal amounts of each of the three following solutions: (1) 0.05 M potassium phosphate, pH 7.0, containing 0.5 mM EDTA and 7% glycerol; (2) immunised guinea pig serum; (3) preimmunised guinea pig serum. The incubations were carried out at 37°C for 1 h and then at 4°C for about 20 h. The three mixtures were centrifuged at the end of incubation. The enzymatic activity in the supernatants and their corresponding resuspended precipitates were determined by the spectrophotometric method¹⁵ in the presence of creatinine-HCl¹². No precipitates were formed in the controls (mixture 3, preimmunised serum). White precipitates could be seen in the tubes containing ChAc alone (mixture 1) or ChAc plus immunised serum (mixture 2), however, the precipitates showed no enzyme activity. The enzymatic activity recovery was 88%, 69% and 95% in the supernatants for the ChAc incubated with solution 1, 2 and 3, respectively. About 30% of the ChAc was combined with its antibody during the incubation (solution 2) and this combined ChAc was inactive. There was only 59% recovery in the supernatant when the ratio of the ChAc to antiserum was 1:2 instead of 1:1. ChAc in the presence of preimmunised serum (solution 3) was more stable to prolonged incubation than in buffer alone (solution 1). This is consistent with our finding that other proteins have a stabilising effect on ChAc⁷.

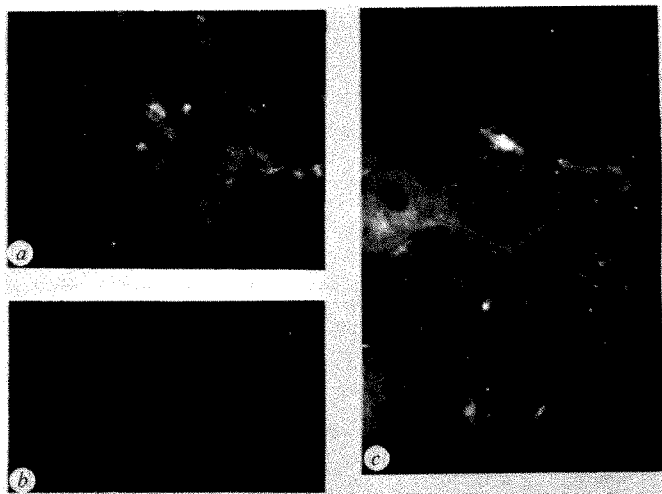


Fig. 3 Immunofluorescent localisation of choline acetyltransferase in 10 μ m frozen sections of the anterior horn of bovine spinal cord. *a*, Some of three anterior horn cells and their processes fluoresce while the adjacent white matter has only scattered spots of fluorescence. Note the absence of nuclear staining in the neurones ($\times 69$). *b*, Control anterior horn section with preimmunised guinea pig serum. *c*, Same as *a* ($\times 138$).

The anti-ChAc fluorescent antibody test was carried out by procedures previously reported¹⁰. Antiserum to guinea pig γ -globulin labelled with fluorescein was purchased from Antibodies Inc., Davis, California. Preimmunised and immunised guinea pig sera were used in 1:20 to 1:80 dilutions. Lyophilised cryostat sections (10 μ m) of the anterior horns of the lumbar enlargements of bovine spinal cord were extracted with chloroform for 15 s before incubation with antiserum. This removed the bulk of the lipid without affecting the activity of ChAc⁷. The cholinergic anterior horn cells were strongly fluorescent after incubation with guinea pig serum containing antibody to ChAc and then with the fluorescent goat antiserum (Fig. 3a). There was fluorescence also in the processes of the anterior horn cells but little in the neuropil and the adjacent white matter. Control sections using guinea pig serum before immunisation with ChAc were negative (Fig. 3b). Further studies

on the immunoenzymatic localisation of cholinergic neurones in the nervous system are in progress. While this work was in progress, antibodies to rat brain were reported¹⁶.

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- ¹ Falck, B., Hillarp, N. A., Thieme, G., and Tarp, A., *J. Histochem. Cytochem.*, **10**, 348-354 (1962).
- ² Akert, K., and Sandri, C., *Brain Res.*, **7**, 286-295 (1968).
- ³ Burt, A. M., *J. Histochem. Cytochem.*, **18**, 408-415 (1970).
- ⁴ Kasa, P., Mann, S. P., and Hebb, C., *Nature*, **226**, 812-816 (1970).
- ⁵ Kasa, P., and Morris, D., *J. Neurochem.*, **19**, 1299-1304 (1972).
- ⁶ Potter, L. T., in *Handbook of neurochemistry* (edit. by Lajtha, A.) **4**, 263-284 (Plenum, New York, 1970).
- ⁷ Chao, L. P., and Wolfgram, F., *J. Neurochem.*, **20**, 1075-1081 (1973).
- ⁸ Kawaoi, A., and Nakane, P. K., *Fedn Proc.*, **32**, 840 (1973).
- ⁹ Eng, L. F., and Kosek, J. C., *Trans. Am. Soc. Neurochem.*, **5**, 160 (1974).
- ¹⁰ Uyeda, C. T., Eng, L. F., and Bignami, A., *Brain Res.*, **37**, 81-89 (1972).
- ¹¹ Bignami, A., and Eng, L. F., *J. Neurochem.*, **20**, 165-173 (1973).
- ¹² Chao, L. P., and Wolfgram, F., *J. Neurochem.* (in the press).
- ¹³ Matsuda, T., Wu, J.-Y., and Roberts, E., *J. Neurochem.*, **21**, 167-172 (1973).
- ¹⁴ Saito, K., Barber, R., Wu, J.-Y., Matsuda, T., and Roberts, E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 269-273 (1974).
- ¹⁵ Chao, L. P., and Wolfgram, F., *Analyt. Biochem.*, **46**, 114-118 (1972).
- ¹⁶ Rossier, J., Bauman, A., and Benda, P., *FEBS Lett.*, **36**, 43-48 (1973).

Muscle membrane protein kinase in myotonic muscular dystrophy

MYOTONIC muscular dystrophy is an inherited disorder of man with manifestations in many organ systems including skeletal muscle myotonia and dystrophy, testicular atrophy, cataract formation, hypercatabolism of IgG, cranial bone malformations, and glucose intolerance with enhanced insulin levels (see ref. 1 for clinical references). Our previous experiments demonstrated a significant diminution in the phosphorylation of endogenous membrane proteins of frozen erythrocyte ghosts from myotonic patients¹. In subsequent studies the technique of electron magnetic resonance was employed to compare the erythrocyte membrane milieu of normal and myotonic patients². Following incorporation of 5-nitrooxide methyl stearate into erythrocyte membranes, the spin label was located in a less polar and somewhat more fluid region in myotonic membranes than in normal membranes. Although such experiments do not specify the molecular membrane abnormality or its possible relationship to protein phosphorylation, they do support the suggestion that myotonic muscular dystrophy is a disease

resulting from a basic membrane abnormality.

Erythrocytes were initially chosen for our study because of the ease of purifying membranes from normal and myotonic patients. But more significant manifestations of the disease are in muscle where numerous physiological investigations have demonstrated alterations of the surface membrane³⁻⁶. The potentially low yield of surface membrane from biopsy specimens together with the numerous secondary changes which may be unrelated to the primary disease process retarded our early biochemical investigations of muscle membrane abnormalities. With the demonstration of protein phosphorylation in purified fractions of rat muscle membrane, the technical capacity presented itself for evaluation of human biopsy material⁷. In the data to be presented below, endogenous protein phosphorylation of muscle membranes was found to be diminished in myotonic muscular dystrophy compared to control tissue. These results confirm involvement of membrane protein phosphorylation and its usefulness as a sensitive index of membrane abnormality in myotonic dystrophy.

Six patients with myotonic muscular dystrophy representing six separate families participated in the study. All of these individuals gave informed consent. Each experiment involved one patient and one control. The control muscle was obtained from patients undergoing orthopaedic operations involving femur or hip repair. None of the controls had any known muscle disease. Quadriceps femori muscle was used in all cases. The control biopsies were obtained within 1 h of patient biopsy; and both tissue preparations were fractionated simultaneously at 4° C according to a modification of the method of Brody⁸. The resultant membrane pellet consisted of a mixture of sarcoplasmic reticulum, sarcolemma, and other membrane particles. Following determination of protein concentration⁹, assessment of endogenous protein kinase activity was carried out by a modification of the methods previously used in our studies. This incubation mix contained freshly prepared muscle membranes and 50 mM sodium acetate buffer (pH 6.5); the time of incubation was 20 s. All other reagents were identical to previous studies¹⁰. Following solubilisation the polypeptides were electrophoresed on 7 1/2% SDS polyacrylamide gels. Gel staining and determination of radioactivity were performed as previously described¹⁰. Na⁺, K⁺, Mg²⁺-ATP-ase of the membrane fractions¹¹, adenyl cyclase¹² and phosphoprotein phosphatase¹ were assayed.

Washed muscle membrane preparations from both control and myotonic tissues were able to incorporate ³²P into membrane protein using only γ-³²P-ATP as a substrate. As noted previously in erythrocytes and in rat muscle preparations both the enzyme and the protein substrate seem to be endogenous components of the membrane. In each experiment the rate of incorporation of ³²P was lower in the myotonic than the control membrane. In six experiments the total myotonic membrane phosphorylation averaged 64% of control activity; and this diminution is similar to that observed in erythrocytes (Table 1).

When the polypeptide profile of the membrane preparations from control and myotonic tissue were compared, no reproducible differences were noted. Three major components were found to be phosphorylated (Fig. 1). Phosphorylation of component 1 (molecular weight approximately 90,000-100,000) was variable and after repeated washing of the membrane preparation in the Tris sucrose buffer used in membrane preparation, both the amount of component 1 relative to the other peaks as well as the phosphorylation were considerably decreased. These data suggest that component 1 is either an exogenous cytoplasmic protein or is phosphorylated by a cytoplasmic enzyme which is contaminating this crude particulate fraction.

Components 2 and 3 were readily phosphorylated and were relatively unaffected by additional membrane washes. Component 2 had a greater apparent molecular weight than component 3 respectively as assessed by SDS polyacrylamide gel electrophoresis (50,000 compared with 30,000). Component

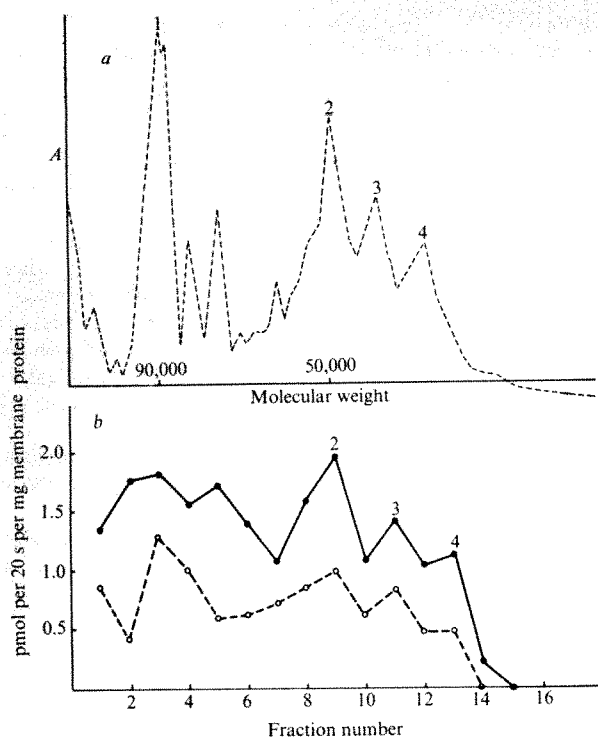


Fig. 1 *a*, Sodium dodecyl sulphate-polyacrylamide gel scan of a typical muscle membrane preparation. *b*, Radioactivity expressed as pmol per 20 s per mg membrane protein in SDS-polyacrylamide gel fractions. Experimental blanks consisting of boiled membrane or no Mg^{2+} have been subtracted. Results are from a typical experiment. ●, Control; ○, myotonic.

2 also had a slightly greater specific activity *in vitro* phosphorylation (Table 1). In all six experiments, the rate of incorporation of ^{32}P into components 2 and 3 was lower in myotonic membranes. In both components 2 and 3, the mean phosphorylation of myotonic membranes was 50% of control preparations.

Na, K (Mg) ATP-ase activities were identical in control and myotonic membrane fractions. Similarly basal, isoproterenol, and fluoride-stimulated adenyl cyclase activity demonstrated no reproducible differences (Table 2). Phosphoprotein phosphatase activity was absent in control and myotonic membranes under the conditions used in this study.

The major limitation to evaluation of enzymatic changes of membrane fractions of human muscle tissue is the limited yield of purified fractions which may be obtained from biopsy specimens. In most studies, investigators sacrifice purity for improved yield. But when doing so as in the present experiments, one should be extremely cautious about the extent to which similar membrane preparations are being examined in the control and diseased situations. The comparable polypeptide profile as assessed by polyacrylamide gel electrophoresis, the similar Na, K (Mg) ATP-ase activity, as well as hormone and

Table 2 Adenyl cyclase and ATPase in muscle membrane preparations

	Adenyl cyclase (pmol cyclic AMP per 10 min per μg protein)			ATPase (μmol P per mg protein per h)	
	Basal	Isoproterenol (0.1 mM)	NaF Mg^{2+} (10 mM)	Mg^{2+} + Na ⁺ ATPase	K^{+} + ATPase
Control	2.24	5.19	18.8	17.8	23.7
Myotonic	3.52	6.97	15.3	17.7	23.0

Data from a typical experiment. Adenyl cyclase assay was performed on fresh tissue. ATPase was measured on tissue frozen at -20° for 2 d. Each value is the mean of duplicates. In three additional experiments no differences in control or myotonic values were demonstrated, although each experiment resulted in slightly varying activities.

fluoride-stimulated adenyl cyclase all support the comparability of the membrane preparations. Nevertheless in animal experiments by Andrew *et al.* protein phosphorylation was found in a light density membrane fraction which could be separated from fractions containing the ATPase and cyclase^{7,13}. Thus our studies contrasting protein phosphorylation must be viewed with considerable caution because of the heterogeneous origins of the fractions used.

In rat muscle preparations no polypeptide larger than 30,000 was phosphorylated. In the present studies, phosphorylation of the 90,000–100,000 molecular weight component was variably affected in myotonic muscle preparations and could be altered in both normal and myotonic preparations by extensive washing. In rat muscle tissue phosphorylation of high molecular weight components is characteristic of cytoplasmic enzymes contaminating the membrane fraction. For this reason phosphorylation of this component was felt to be less significant than that of components 2 and 3.

Phosphorylation of both of these components was significantly diminished in myotonic muscle tissue. But in these muscle membranes, the altered activity cannot be definitely attributed to a diminished enzyme because comparable activity in control and myotonic membrane components 2 and 3 could be demonstrated when the pH was raised to 7.5 and assays were carried out in the presence of Tris buffer (Fig. 2). Similar

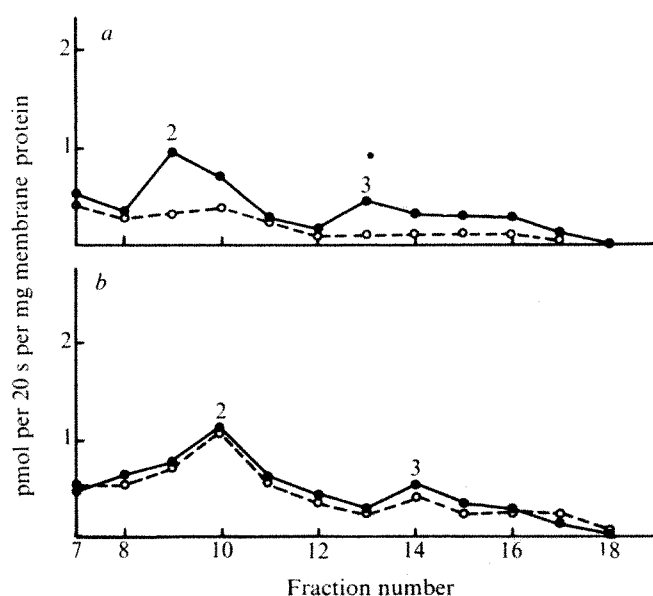


Fig. 2 *a*, Radioactivity expressed as pmol per 20 s per mg membrane protein in fractions corresponding to proteins 2 and 3 in a typical experiment using 10 mM Na acetate pH 6.5 as the buffer system. *b*, Same experiment performed using 10 mM Tris-HCl pH 7.5. ○, Control; ●, myotonic.

Table 1 Endogenous protein phosphorylation of muscle membranes

	Myotonic (pmol per mg per 20 s)	Control (pmol per mg per 20 s)	
Total membrane phosphorylation	9.95 ± 1.1	15.9 ± 1.8	$P < 0.02$
Phosphorylation of membrane component 2	0.818 ± 0.12	1.62 ± 0.21	$P < 0.01$
Phosphorylation of membrane component 3	0.545 ± 0.10	1.09 ± 0.15	$P < 0.025$

Data from all six experiments. Total membrane phosphorylation represents the sum of all protein components. All experiments were performed in duplicate with experimental blanks (boiled membranes or reactions with Mg^{2+} deleted). Values indicated are the mean \pm s.e.m. P was determined by the Student's t test.

variations of protein phosphorylation with buffer, pH and method of membrane preparation have been observed with human erythrocytes. Since the enzymatic activity of protein phosphorylation within the membrane seems so easily affected by environmental changes, it seems more reasonable to ascribe the changes noted with myotonic muscle to an altered membrane rather than to a specific genetically mediated alteration in the protein kinase enzyme. The demonstration of lowered protein phosphorylation in muscle membranes confirms the similar observations made by us on erythrocyte ghosts. Although we were initially concerned that fibrosis, fatty infiltration, dystrophy or denervation might specifically affect the outcome, none of these seem to be particularly pertinent since morphological evaluation of the biopsies employed would suggest that minimal pathology was present in the muscle segments used. Whether the altered protein phosphorylation of muscle membrane and red blood cells or the physical alteration in the red cell membrane is the more pertinent aetiological factor is not known at present. Either of these alterations might be associated with an abnormal membrane, and both would lend support to our concept of myotonic muscular dystrophy as a diffuse disorder of membranes.

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- ¹ Roses, A. D., and Appel, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1855-1859 (1973).
- ² Butterfield, D. A., Chesnut, D., Roses, A. D., and Appel, S. H., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 909-913 (1974).
- ³ Lindsley, D. B., and Curren, E. C., *Archs. Neurol. Psychiat., Lond.*, **35**, 253-269 (1936).
- ⁴ Denny-Brown, D., and Nevin, S., *Brain*, **64**, 1-18 (1941).
- ⁵ Landau, W. M., *Neurology, Minneap.*, **2**, 369-388 (1952).
- ⁶ Floyd, K., Kent, P., and Page, F., *Electroenceph. clin. Neurophysiol.*, **7**, 621-630 (1955).
- ⁷ Andrew, C. G., Roses, A. D., Almon, R. R., and Appel, S. H., *Science*, **182**, 927-929 (1973).
- ⁸ Brody, I. A., *Am. J. Physiol.*, **211**, 1277-1280 (1966).
- ⁹ Lowry, O. H., Rosebrough, N. J., Farr, A. C., and Randall, R. H., *J. biol. Chem.*, **193**, 265-275 (1951).
- ¹⁰ Roses, A. D., and Appel, S. H., *J. biol. Chem.*, **248**, 1408-1411 (1973).
- ¹¹ Wins, P., and Schoffeniels, E., *Biochim. biophys. Acta*, **120**, 341-350 (1966).
- ¹² Krishna, G., Weiss, B., and Brodie, B. B., *J. Pharmac. exp. Ther.*, **163**, 379-385 (1968).
- ¹³ Andrew, C. G., and Appel, S. H., *J. biol. Chem.*, **248**, 5156-5163 (1973).

Structural difference in sites on surface membrane of mature and immature erythrocytes

MAMMALIAN cells transformed by oncogenic viruses or chemical carcinogens undergo characteristic changes in their surface properties such as lectin-induced cell agglutination^{1,2}. Normal cells show similar changes at their mitotic phase³ or after mild protease digestion of their surface membrane^{4,5}. This suggests that cells change their surface properties under various conditions and during the cell cycle. With this concept in mind, we studied the

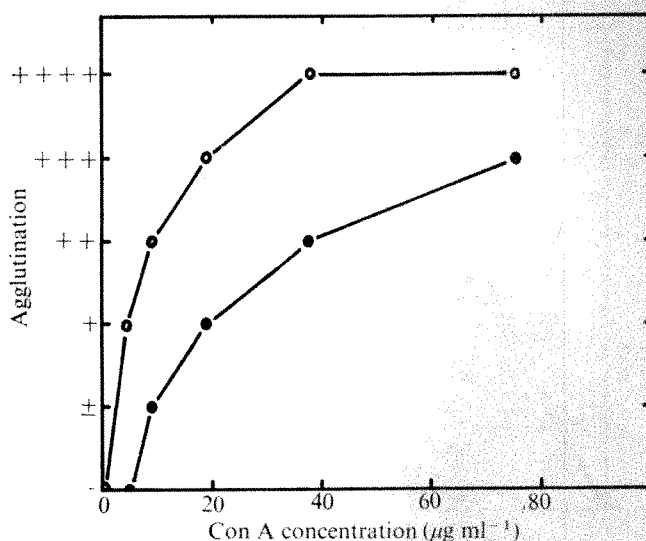


Fig. 1 Con A-induced cell agglutination of rabbit erythrocytes and reticulocytes. 10^8 cells were incubated with various concentrations of con A at 37°C for 20 min. Erythrocytes (●—●); reticulocytes (○—○).

changes on the membrane surface during the maturation of rabbit erythrocytes. We describe here the changes in cell agglutinability and in the binding sites of concanavalin A (con A) on the cell surface during erythrocyte maturation.

Reticulocytes were obtained from phenylhydrazine-injected rabbits⁶. Rabbit erythrocytes and reticulocytes were washed three times with glucose-free Hanks' balanced salt solution (HBSS). Con A and ^{125}I -labelled con A were obtained as described previously⁷. Cells were scored for agglutination on a qualitative scale from - to +++++ after 20 min incubation at varying temperature with an appropriate concentration of con A (ref. 7). The binding of ^{125}I -con A (672 c.p.m. per µg con A) was carried out at 37°C for 10 min with or without 100 mM α -methyl-D-glucoside (α -MG)⁷.

Reticulocytes agglutinated at much lower concentrations of con A at 37°C . Even at $5\mu\text{g ml}^{-1}$ con A, reticulocytes showed marked agglutination, whereas no agglutination was observed in the case of erythrocytes (Fig. 1).

The agglutination of both these cells was temperature dependent (Fig. 2). Erythrocyte agglutination was, however,

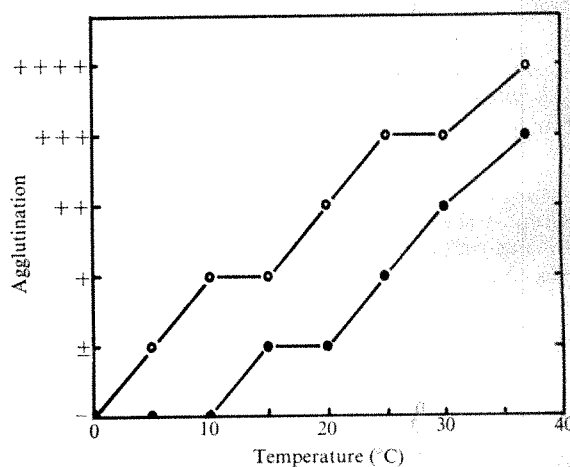


Fig. 2 Temperature-dependent agglutination of rabbit erythrocytes and reticulocytes. The concentration of con A used was $75\mu\text{g ml}^{-1}$. Other conditions as in Fig. 1. Erythrocytes (●—●); reticulocytes (○—○).

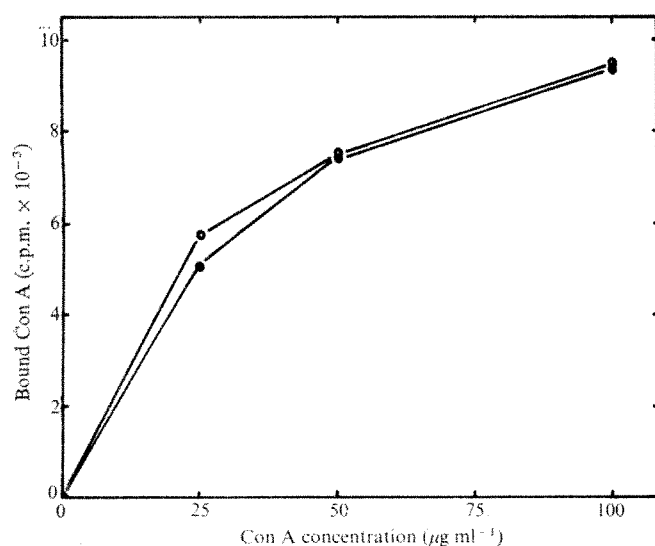


Fig. 3 Binding of ^{125}I -con A to rabbit erythrocytes and reticulocytes. 10^8 cells were incubated with varying concentration of ^{125}I -con A at 37°C for 10 min, with or without 100 mM α -methyl-D-glucoside, and were washed three times with HBSS. Erythrocytes (●—●); reticulocytes (○—○).

induced only at temperatures above 15°C whereas reticulocyte agglutination was observed even below 10°C . The difference in the agglutinability and in the temperature-dependence of these two cell types may be due to the difference in the number of surface receptors for con A.

The number of ^{125}I -con A binding per cell was, however, almost equal for each cell type (Fig. 3). This suggests that membrane changes other than the number of con A binding sites are likely to account for the difference in the agglutinability of these cells. The differences we have observed parallel the difference in the agglutinability of normal and transformed cells, and may be caused by the difference in membrane fluidity and/or topographical distribution of con A receptor sites on the cell surface. We are now doing a comparative study on surface structure and membrane components to understand the changes which occur during erythrocyte maturation.

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- ¹ Burger, M. M., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 994 (1969).
- ² Inbar, M., and Sachs, L., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 1418 (1969).
- ³ Fox, T. O., Shappard, J. R., and Burger, M. M., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 244 (1971).
- ⁴ Inbar, M., and Sachs, L., *Nature*, **223**, 710 (1969).
- ⁵ Lis, H., Sela, Ben-Ami, Sachs, L., and Sharon, N., *Biochim. Biophys. Acta*, **211**, 582 (1970).
- ⁶ Percy, A. K., Schmell, E., Earles, B. J., and Lennarz, W. J., *Biochemistry*, **12**, 2456 (1973).
- ⁷ Inoue, M., *J. Cell Sci.*, **14**, 197 (1974).

Isolation of a collagen-dependent cell attachment factor

Most untransformed mammalian cells require an appropriate surface for survival and growth *in vitro*; this phenomenon has been termed anchorage dependence¹. Glass, tissue culture plastics, fibrin clots and collagen surfaces have long been recognised as substrates able to support the attachment and growth of cells. Since the cell plasma membrane is separated from plastics substrates by a 450 Å layer of electron opaque material², the nature of the 450 Å would appear to be more important in cell attachment than the chemical composition of the plastic. It is demonstrated here that cell attachment to collagen is mediated by a high molecular weight protein present in serum.

Petri plates were coated with collagen as described in the legend to Fig. 1. When 2×10^5 SV-3T3 cells were added to collagenised Petri plates and incubated in the presence of serum for 1.5 h, the number of cells attached increased as the percentage of calf serum was increased from 0.03% to a plateau level at 3% (Fig. 2). Cell spreading was observed in the 10% and 3% points in 1.5 h. The sum of the attached and unattached cells (*a*) varied slightly with serum concentration and (*b*) was consistently slightly less than the number of cells inoculated (this may have been due to lysis of injured cells). By varying the time of the incubation, it can be seen that cell attachment started after 5 min, and continued for 6 h (at which time cell growth started in the high serum points) (Fig. 2*a*). The number of units of attachment factor, as defined in the legend to Fig. 1, varied as a linear function of time (Fig. 2*b*). The number of units of attachment factor did not vary greatly when the number of cells inoculated ranged from 1.5×10^6 to 2×10^5 (Fig. 3). It should be noted that the number of units of attachment factor varied between different commercial lots of serum.

The ionic requirements for cell attachment were determined by omitting components from Eagle's medium. Cells attached and spread in a medium consisting of NaCl, KCl (Mg^{2+} , or Ca^{2+}), glucose (at concentrations found in Eagle's medium) and 10 mM Hepes (pH 7.5) (no CO_2 or bicarbonate were used). The minimal attachment medium was 65% as effective as Eagle's medium as determined from the ratio of units of attachment activity in both media (omission of glucose or KCl results in less effective cell attachment). Cell attachment depended on either Mg^{2+} or Ca^{2+} , whereas Sr^{2+} and Ba^{2+} were inactive in the assay (Fig. 4). The pH optimum for cell attachment is in the physiological range, between pH 6.0 and 8.5 (high pH results in precipitation of Ca^{2+} and Mg^{2+} as their hydroxides).

The attachment factor was purified by batch preparation procedures as described in Table 1. The DEAE purified factor (Fig. 5) was half inactivated by treatment for 5 min at 65°C and had a pH of 4.8 (Fig. 6). Purified attachment factor (DEAE step) was stable to treatment at 37°C for 16 h with $10 \mu\text{g ml}^{-1}$ of either trypsin or pronase. The factor seemed to be of molecular weight greater than 200,000 since it was eluted at the void volume of both Sephadex G-100 and G-200 columns.

The rate of binding of attachment factor to collagen was assayed by (*a*) exposing air-dried collagenised plates to a series of dialysed cell attachment factor (second AmSO₄ step) concentrations in the presence of 0.9% NaCl (*b*) washing the plates eight times (2 min per wash) with 0.9% NaCl to remove unbound factor and (*c*) assaying cells in Eagle's medium without serum in order to determine the amount of factor that has been bound to the collagen. Figure 8 demonstrates that factor binds rapidly to collagen in the absence of divalent actions. Up to 30 min, the amount of factor bound increased linearly with time; the non-linearity in factor binding after 30 min may have been due to an increase in the number of collagen binding sites as the air-dried collagen swelled slightly during rehydration.

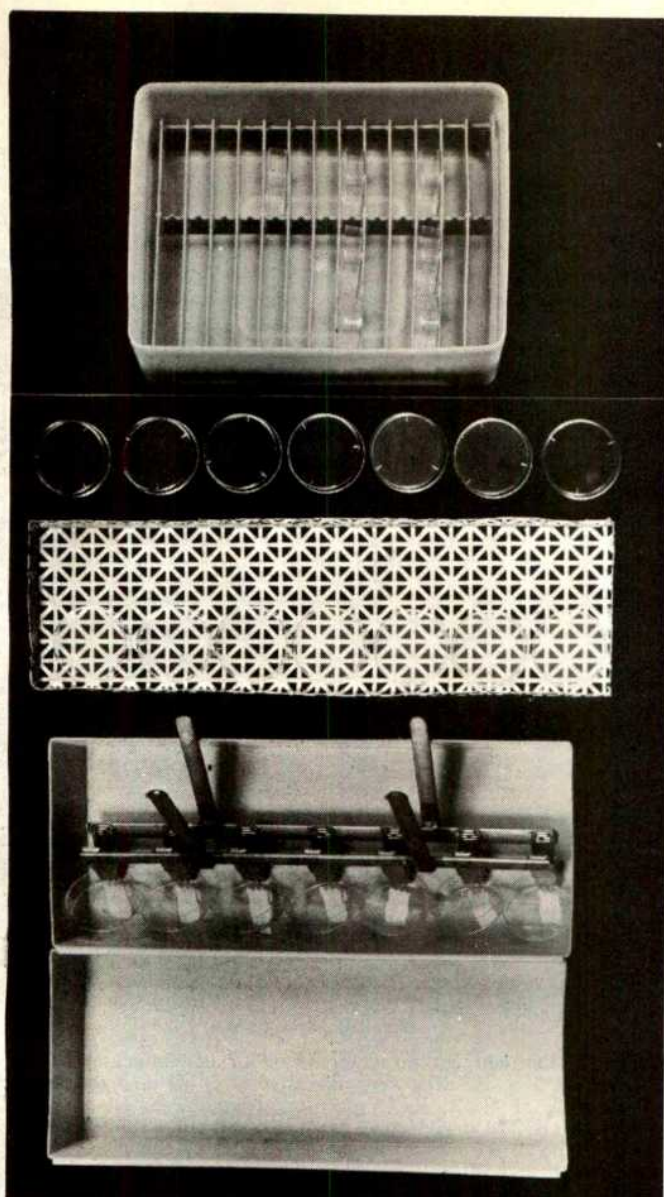


Fig. 1 Top, Petri plate washer, made from plastic box and bottom of a Rubbermaid (No. 6049) dish drainer; Middle, Petri plate tray; Bottom, Rubbermaid plastic boxes for saline and discarded solutions (used in washing Petri plates at the end of the assay); Bottom, Petri plate decanter, made from spring-loaded pants clamps and metal rod. Petri dishes were coated with collagen as follows. Collagen was prepared by acid extraction, and salt and ethanol precipitation^{4,6}. Freshly dissected rat tail tendons (8 g) were stirred overnight in 1 l of 0.2% citric acid and insoluble material was removed by filtration on a Buchner funnel. Collagen was precipitated by adding 10% (w/v) NaCl and was spun down at 10,000g for 15 min. The white precipitate was solubilised by stirring it in 0.2% acetic acid for several hours. The collagen solution was reprecipitated by adjusting the pH to 8.0 with NH_4OH , adding 20% (v/v) ethanol, and pelleting at 10,000g for 15 min. The collagen was lyophilised and stored for up to 6 months at 15° C. Bacteriological Petri plates (Falcon No. 1007) were coated with collagen by placing the bases in a large plastic tray and dispensing into them 1.5 ml of 0.25% collagen in 0.2% acetic acid plus 0.002% phenol red with a 2-ml Cornwall pipetter. After rocking the tray to ensure uniform coating, the collagen was gelled by placing a partially NH_4OH -soaked, cloth-lined lid over the tray. The plates turned a metallic red colour in 5-10 min, when the lid was removed and the plates were air-dried. To reduce control levels in the assay, the completely air-dried plates were treated with 8 M urea for 20 min in Petri plate washing trays. The bottoms were washed three times with distilled water (5 min per wash) and then air-dried. The collagen-coated plates can be stored at room temperature for at least 2 months.

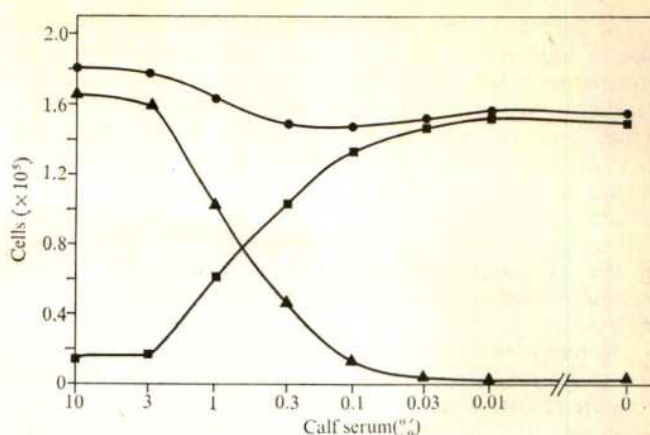


Fig. 2 Cell attachment assay. 2×10^6 cells were incubated at 37° C for 1.5 h on collagenised plates in the presence of serum. \blacktriangle , attached cells; \blacksquare , unattached cells; \bullet , attached + unattached cells. Petri plates were loaded with 5 ml of Eagle's minimal essential medium (Gibco catalogue No. F-11) containing $200 \mu\text{g ml}^{-1}$ bovine serum albumin (Sigma) with the required amount of serum or factor and preincubated at 37° C in a 5% CO_2 in-air incubator for 1 h (to allow the factor to bind to the collagen). Generally, serum concentrations in increments of one-half decade from 0.001% to 10% serum were used. During the Petri plate preincubation, semiconfluent SV-3T3 cells were (a) detached by a 15 min trypsinisation at 37° C with 0.25% Bacto-trypsin (Difco) and (b) washed twice in serum free Eagle's medium containing $200 \mu\text{g ml}^{-1}$ bovine serum albumin. After the Petri plate preincubation, 2×10^6 washed cells were added to each plate and incubated at 37° C in a CO_2 incubator for 1.5 h. Petri plates were emptied with the Petri plate decanter (Fig. 1), filled with 0.9% NaCl, decanted again and trypsinised for 10-15 min with 5 ml of 0.25% trypsin. The cells removed from the collagen were then counted with an electronic cell counter. Two samples of serum were compared with respect to cell attachment factor activity by (i) making serial half decade dilutions of the two samples and (ii) determining that percentage of serum which attaches 50% of the cells attached in the 10% serum controls. A unit of attachment factor is defined as the reciprocal of that percentage of serum that attaches half of the cells attached in the 10% serum controls (unit = $1/\%$ serum $_{1/2}$). The reciprocal of serum concentration is used to assign a higher number to a more active sample. The use of reciprocals does not change the magnitude of the ratio of activities, since $x/y = (1/y)/(1/x)$. As defined, the unit of attachment factor is proportional to the concentration of factor; the unit's dependence on time of incubation and number of cells used is given in the text.

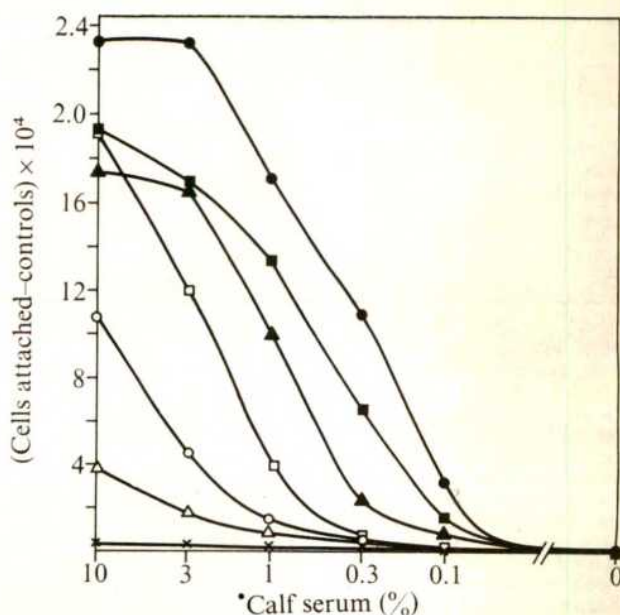


Fig. 3 Time course of cell attachment on collagen. \times , 5 min; Δ , 10 min; \circ , 15 min; \square , 30 min; \blacktriangle , 1.5 h; \blacksquare , 3 h; \bullet , 6 h.

The process of cell attachment to collagen can be divided into at least two steps. (1) A high molecular weight serum protein must bind to collagen; and (2) a divalent cation-dependent reaction is required for cells to attach to the collagen-serum factor complex. These steps can be distinguished from each other since the serum factor binds to collagen in the absence of divalent cations or cells; whereas, cells bind to factorised collagen only in the presence of Ca^{2+} or Mg^{2+} . Cell spreading on a collagen substratum may be mediated by the cell attachment factor, described here, since column fractions which mediate cell attachment also have cell spreading activity.

Collagen's role as a cell substratum may be identified as one of its major physiological functions from the following evidence. First, biochemical methods indicate that collagen represents 20% of total protein in mammalian tissues, with the exception of the nervous system³. Second, it is well known that collagen is found intracellularly in many tissues^{3,4} and is

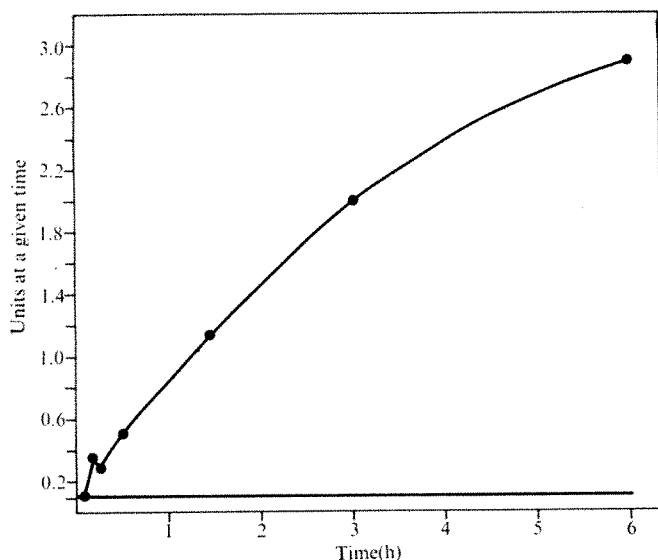


Fig. 4 Relationship between units of attachment factor and time (○).

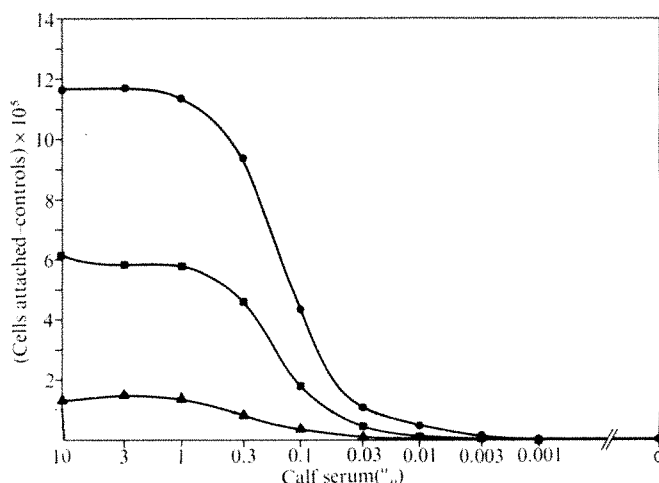


Fig. 5 2×10^5 (▲), 7.5×10^5 (■) and 1.5×10^6 (●) cells were incubated on factorised collagen for 3 h. More cells attached in low percentage serum when a high cell density was used than at high percentage serum when a low cell density was used.

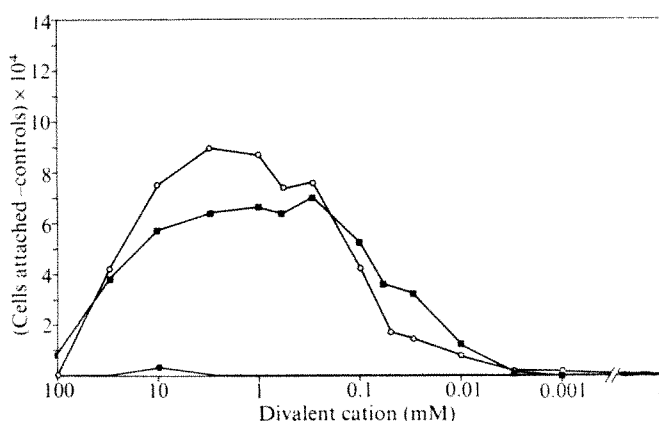


Fig. 6 Divalent cation requirement for cell attachment to collagen. 2×10^5 cells were incubated at 37°C in a medium consisting of NaCl; KCl; glucose (at Eagle's medium concentrations); 10 mM Hepes, pH 7.5; 10% calf serum equivalent of the DEAE purified factor (as assayed in complete Eagle's medium); and various divalent cations. No CO_2 was used to avoid the precipitation of carbonates. ○, Mg^{2+} ; ■, Ca^{2+} ; ●, Sr^{2+} or Ba^{2+} .

Table 1 Batch preparation of serum factor

Step	Total vol (ml)	Total protein (280nm; 1 cm path length) (fractions diluted to 1x calf serum)	Total units	Specific activity (units/280nm)	Recovery	Purification
Calf serum	1,000	71	14,300	201	—	1
1st PEG	200	20.16	14,300	711	100%	3.55
1st AmSO_4	200	8.4	12,500	1,488	87%	7.4
2nd PEG	200	5.4	10,000	1,851	70%	9.2
CaPi gel	400	2.2	1,940	882	13.6%*	4.4
2nd AmSO_4	50	1.32	3,300	2,500	23%	12.5
DEAE-11	90	0.30	2,500	8,350	17.5%	41.8

The serum attachment factor was purified from calf serum by batch preparation method. 1st PEG (0→4.5% PEG): using plastic labware to which PEG does not stick, 100 ml of a 50% (w/w) solution of polyethylene glycol-6000 (PEG) (Baker) solution was added to 1l of calf serum at 4°C. The precipitate which had formed in 30 min was spun down at 18,000g for 30 min and dissolved in 200 ml of 0.1 M imidazole + 0.15 M NaCl (pH 6.8) (standard buffer). 1st AmSO_4 (0→28.6% AmSO_4): 80 ml of saturated $(\text{NH}_4)_2\text{SO}_4$ was added slowly at 4°C to 200 ml of the 1st PEG material; after 20 min, the precipitate was collected by centrifugation at 18,000g for 15 min and dissolved in 200 ml of standard buffer. 2nd PEG (1→4.5% PEG): 4 ml of 50% PEG was added to 200 ml of the 1st AmSO_4 cut, and after 30 min the precipitate was removed by centrifugation at 18,000g for 10 min. 16 ml of 50% PEG was then added to the 1% PEG supernatant; after 30 min, the precipitate was collected by centrifugation at 18,000g for 20 min and dissolved in 200 ml of a standard buffer. CaPi gel: calcium phosphate gel (Brushite) was prepared according to Levin⁶. 200 ml of CaPi was added with stirring to 200 ml of the 2nd PEG material at room temperature. After 30 min, the gel was (i) spun down at 5,000g for 10 min; (ii) resuspended in 200 ml of 0.1 M aspartate (pH 6.8); (iii) spun down at 5,000g for 10 min, and (iv) eluted twice with 200 ml of 0.5 M aspartate + 0.1 M imidazole + 0.15 M NaCl (pH 6.8). 2nd AmSO_4 (0→30% AmSO_4): to the 400 ml of combined eluate was added 320 ml of saturated $(\text{NH}_4)_2\text{SO}_4$ at 4°C; the resulting precipitate was spun down at 18,000g for 15 min, and redissolved in standard buffer to a final volume of 50 ml. DEAE-11 chromatography: 25 ml of the 2nd AmSO_4 material was diluted to 100 ml with cold water and precipitate which had formed in 30 min was removed at 10,000g for 10 min. The soluble material was then applied to a 4.5×20 cm DEAE-11 column which had been washed with 0.5 M KH_2PO_4 (pH 6.8) and 200 ml of one-quarter strength standard buffer. The column was then washed with 60 ml of 0.25 M standard buffer. The active material was eluted with 1l of 0.25 M NaCl + 0.1 M imidazole (pH 6.8) and the active peak was pooled (Fig. 5).

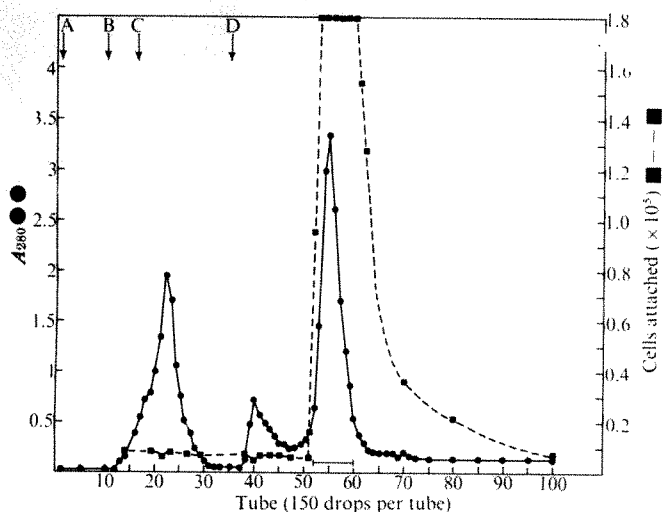


Fig. 7 DEAE-11 chromatography of 2nd AmSO_4 fraction. At each arrow, the following solutions were added to the 4.5×20 cm column: (A) 100 ml of diluted factor; (B) 60 ml of 0.25 strength standard buffer; (C) 200 ml of 0.65 strength standard buffer; (D) elution with 1l of 0.25 M NaCl + 0.1 M imidazole (pH 6.8). Tubes 52 to 60 were pooled.

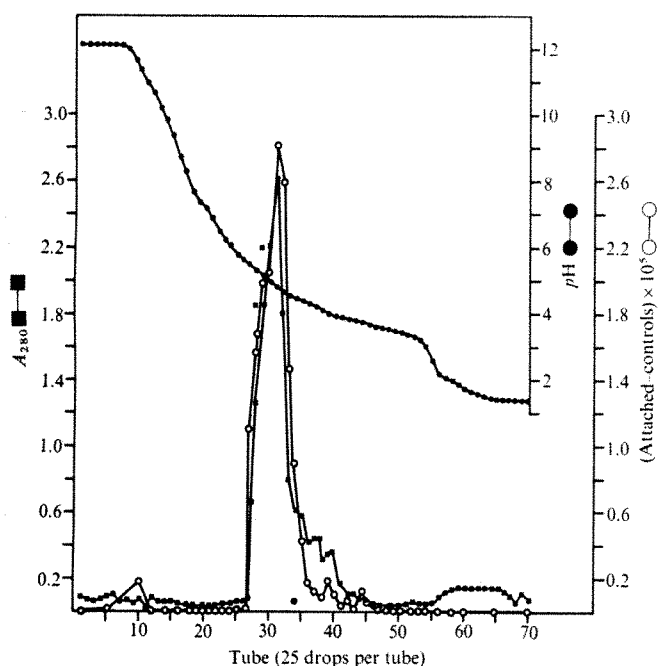


Fig. 8 Isoelectric focused in a LKB 110 ml column, in a sucrose gradient containing 1.4 ml pH 3-6 LKB ampholytes and 0.14 ml pH 3-10 LKB ampholytes.

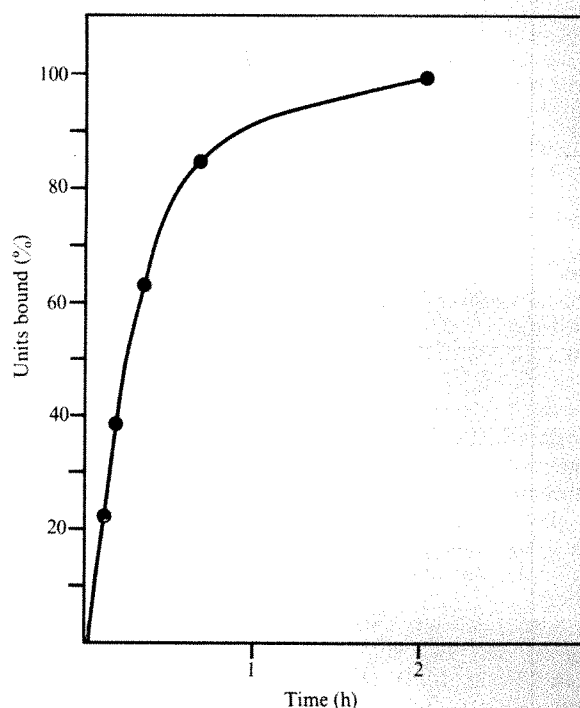


Fig. 9 Binding of purified attachment factor to collagen in the absence of divalent cations or cells. Collagenised plates were treated with a series of dialysed factor concentrations in 0.9% NaCl and treated as described in the text. The washed plates were assayed for attachment activity in Eagle's medium (with Ca^{2+} and Mg^{2+} + $200 \mu\text{g ml}^{-1}$ bovine serum albumin).

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- 1 Stoker, M. G. P., O'Neill, C., Berryman, S., and Waxman, V., *Int. J. Cancer*, **3**, 683 (1968).
- 2 Flaxman, B. A., Lutzer, M. A., and Scott, E. J. V., *J. Cell Biol.*, **36**, 406 (1968).
- 3 Harness, R. D., *Biol. Rev. Cambridge*, **36**, 399 (1961).
- 4 Bailey, A. J., *Comprehensive Biochem.*, **26B**, 297 (1962).
- 5 Goldberg, B., and Green, H., *Proc. natn. Acad. Sci., U.S.A.*, **59**, 1110 (1968).
- 6 Jackson, D. S., and Cleary, E. C., *Methods of biochemical analysis*, **15**, 25 (1967).

Effect of zinc on haemoglobin binding by red blood cell membranes

RESULTS of recent studies in our laboratory have suggested that zinc plays an important role in sickle cell anaemia. A significant proportion of sickle cell patients are zinc deficient¹. Zinc binds to haemoglobin and increases oxygen affinity^{2,3}. Sickle cells treated *in vitro* with zinc show markedly improved filterability at concentrations too low to be explained on an oxygen affinity basis⁴, and zinc may interact with the membranes to affect filterability. A recent report suggests that sickling involves the accumulation of calcium⁵ which is known to reduce red cell membrane deformability⁶. We have now found that zinc decreases the amount of haemoglobin associated with red cell membranes and inhibits the effect of calcium in causing haemoglobin retention by membranes.

Single stage red cell membranes were prepared by the technique of Hoffman⁷ with minor modifications. Agents to be incorporated into these membranes were added during

produced by most tissue culture cell lines⁵. Finally, cell attachment to collagen has been shown here to depend on physiological parameters; namely, a requirement for divalent cations as well as a specific high molecular weight protein.

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Table 1 Haemoglobin retention by ghost cells

Sample type	Hb (g% in initial packed cells)	Hb (g% in final ghost pellet)	Mean cell volume (μm^3)	Hb (g% corrected for change in MCV)	% Retention of Hb	t test
No addition (control)	25.21 \pm 1.8	1.86 \pm 0.6	73.6 \pm 1.9	1.86 \pm 0.6	7.4 \pm 2.5	$t = 6.3$ $P < 0.001$
ZnSO ₄ ghosts	24.94 \pm 0.8	0.79 \pm 0.2	22.9 \pm 2.5	0.25 \pm 0.1	1.0 \pm 0.3	
CaCl ₂ ghosts	25.81 \pm 1.0	21.79 \pm 2.4	21.5 \pm 1.6	6.39 \pm 0.9	24.6 \pm 4.1	$t = 4.5$ $P < 0.01$
CaCl ₂ + ZnSO ₄ ghosts	24.48 \pm 2.1	10.41 \pm 4.0	22.1 \pm 2.3	2.50 \pm 0.5	10.7 \pm 2.6	

All values are the mean \pm 1 s.d. of four observations. ZnCl₂ had approximately the same effects when substituted for ZnSO₄.

haemolysis. The principle of this technique is based on the knowledge that erythrocyte membranes lose their selective permeability at haemolysis and immediately thereafter, facilitating the introduction of various normally nonpenetrating compounds into the cells^{8,9}. In our experiments normal red cells to be haemolysed were divided into four aliquots and treated as follows: no addition (control), zinc sulphate (1.5 mM), calcium chloride (1 mM), and calcium chloride (1 mM) plus zinc sulphate (1.5 mM). Hypotonic exposure lasted 20 min. In all but the control aliquot enough sodium chloride was added to achieve the same ionic strength as the calcium chloride plus zinc sulphate solution. After hypotonic exposure with 10 volumes of water the red cell membranes were resealed with isotonic saline-0.01 M Tris buffer, pH 7.4, and washed until the supernatant was clear of haemoglobin. The haemoglobin in these ghost cell preparations was measured by the cyanmethaemoglobin method. The size and the mean cell volume (MCV) of the ghost cells were calculated using a Coulter counter with multichannel particle size analyser and recorder. Haemoglobin concentrations in the membrane preparations were corrected for the decreased size of the red cell membranes compared with original red cells, since shrinkage of these ghost cells results in some increase in concentration of entrapped haemoglobin.

Table 1 shows that the amount of haemoglobin retained in the membrane preparations after final washing was much less in the presence of zinc compared with control membranes. In the presence of calcium a large amount of haemoglobin was retained, as reported before^{10,11}. When zinc was present together with calcium, however, much less haemoglobin was retained compared with calcium alone, although in both cases the membranes were similar in size (both were small). Equimolar concentrations of lanthanum chloride, but not magnesium, also decreased the haemoglobin-retaining effect of calcium in these preparations (data not shown).

Calcium is known to interact with the interior of the red cell membrane, altering its configuration, decreasing passive permeability¹⁰, decreasing deformability^{6,11} and increasing haemoglobin retention^{10,11}. Zinc seems to counteract the retention of haemoglobin by red cell membrane both in the presence and absence of added calcium. One effect of calcium may be to crosslink haemoglobin and membrane sites. If so, calcium may be involved in the pathogenesis of irreversibly sickled cells by promoting such crosslinking. It is tempting to speculate that zinc improves the filterability of sickle cells, and promotes the elution of haemoglobin from red cell membranes, by blocking our hypothesised calcium-induced crosslinking of haemoglobin to membranes.

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- Schoomaker, E. B., Prasad, A., Oelshlegel, jun., F. J., Ortega, J., and Brewer, G. J., *Clin. Res.*, **21**, 834 (1973).
- Oelshlegel, jun., F. J., Brewer, G. J., Prasad, A., Knutsen, C., and Schoomaker, E. B., *Biochem. biophys. Res. Commun.*, **53**, 560 (1973).
- Oelshlegel, jun., F. J., Brewer, G. J., Knutsen, C., Schoomaker, E. B., and Prasad, A., *Clin. Res.*, **21**, 833 (1973).
- Brewer, G. J., Dash, S., and Oelshlegel, jun., F. J., *J. clin. Invest.*, Abstr. (in the press).
- Eaton, J. W., Skelton, T. D., Swofford, H. S., Kolpin, C. E., and Jacob, H. S., *Nature*, **246**, 105 (1973).
- Weed, R. I., Lacelle, P. L., in *Red cell membrane structure and function* (edit. by Jamieson, G. A., and Greenwalt, T. J.), 318-338 (Lippincott, Philadelphia and Toronto, 1969).
- Hoffman, J. F., *J. gen. Physiol.*, **45**, 837 (1962).
- Hoffman, J. F., *J. gen. Physiol.*, **42**, 9 (1958).
- Seeman, P., *J. Cell Biol.*, **32**, 55 (1967).
- Weed, R. I., Lacelle, P. L., and Merrill, E. W., *J. clin. Invest.*, **48**, 795 (1969).
- Palek, J., Curby, W. A., and Lionetti, F. J., *Am. J. Physiol.*, **220**, 19 (1971).

Nuclear segregation in *Bacillus subtilis*

JACOB, BRENNER AND CUZIN¹ suggested that prokaryote nuclear segregation was achieved by cell surface extension between surface sites to which the chromosome is permanently attached (Fig. 1a). Their model (model A) showed nuclear segregation occurring in newly divided cells, with all surface extension in one cycle occurring between the nuclei. Thus at cell division, nuclei would be arranged symmetrically in the half cell but asymmetrically during the interdivisional period. If nuclear segregation occurs during mid-cycle and cell extension is continuous throughout the cycle, the nucleus cannot reach a symmetrical position (that is at 25% of the cell length) by the end of the cycle relying solely on length extension. To overcome these difficulties, Clark² proposed that the nucleus is always located at the junction of old and new membranes with growth occurring on one side of the nucleus (model B, Fig. 1b). At nuclear segregation, the growing point divides allowing growth of the cell envelope between the nuclei as shown in Fig. 1b. Nuclei remain attached at the site of envelope extension, giving a newborn cell with an asymmetrically arranged nucleus. Donachie and Begg³ have presented evidence for terminal growth regions in slow growing cells as predicted by Clark's model. A third possibility is that the nucleus is located centrally in new born cells and moves to a position at the centre of a half cell at the time of segregation (model C, Fig. 1c). These, and other possibilities, can be explored by determining the position of nuclei in relation to cell length in exponential phase cells.

Bacillus subtilis (168/S) (an asporogenic derivative of 168 tryp⁻ thy⁻ able to grow on succinate as sole carbon

source at a generation time of 115 min) was grown in either succinate or glucose based minimal medium to minimise the cell separation time⁵ and to give substantial numbers of mononucleate cells⁴. Using Giemsa, nuclei appeared as small spherical dark blue bodies in a pale pink-to-colourless cytoplasm. The number and appearance of nuclei was highly reproducible under constant cultural conditions. Cells contain an average of 1.3 and 1.8 nuclei per unit on succinate and glucose media respectively. Each bacterial particle has been regarded as a unit even while containing a septum. The time interval between nuclear segregation and cell separation, calculated from the age distribution theorem⁵ is 48.5 and 45 min for succinate and glucose (generation times 115 and 57 min). Large numbers of cells were photographed and, from the negatives projected on to a screen, positions and diameters of nuclei and lengths of cells were determined. Less than 5% of any population could not be scored because of poor staining and a slightly larger proportion was discarded that were not in focus. All measurements of the position of nuclei were taken from the distal edge of the nucleus to the centre of the cell. These data are shown in Fig. 2.

The critical difference between model C and the other two is the abrupt transition of the nucleus from the centre of the cell to the centre of the half cell. If nuclei move apart gradually at early stages of segregation the nuclear attachment sites should be sufficiently close that nuclei would overlap and be scored as one larger than average nucleus. When plotted against cell length, the distance between cell centre and the distal edge of the nucleus should vary continuously as the two nuclei become visible. Figure 2 indicates that this is not so, and in fact the distal edges of nuclei of 85% of binucleate cells are at least 2.5 radii from the cell centre. Nuclei vary only slightly in size between mononucleate and binucleate cells. The best line connecting binucleate and mononucleate cells has a slope of about 1.5 in both media indicating that the nucleus moves along the cell apparently three times faster than the rate of length extension. The frequency distribution of distances from the cell centre to centre of nuclei are shown for two size classes of binucleate cells in Fig. 3. The peak for all cells is between 20 and 24% from the centre, but there is a skew towards the centre in smaller cells. The data are shown relative to the average nuclear radius of mononucleate cells and there is only minimal overlap between mono and binucleate cells. The peak of the distribution of distance from the cell centre is slightly less than 25%, possibly because

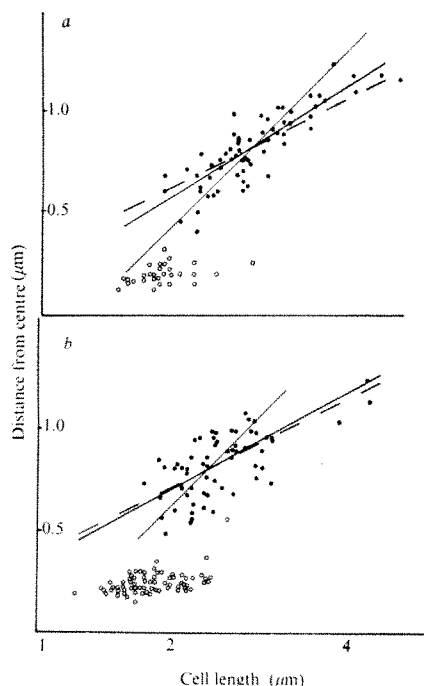


Fig. 2 Position of nuclei relative to cell length. *a*, Glucose grown cells (96 units in sample); *b*, Succinate grown cells (247 units in sample). To stain the nuclei, bacteria were heat fixed onto glass slides, treated with 1 N hydrochloric acid at 60°C for 7 min, washed in phosphate buffer (pH 7.4), and stained with Giemsa (diluted 1:10 with distilled water) for 5 min. ●, Binucleate cells; ○, mononucleate cells; —, graph of 25% of cell length; —, graph of 50% of cell length; —, line of best fit (obtained by method of least squares) for distal edge of nucleus. Regression coefficients for the glucose grown cells (*a*) for distal edge and nuclear centre respectively were 0.31 (significantly different to 0.25 at 1% probability using Student's *t* test) and 0.27 (not significantly different at 5% probability) respectively. The correlation coefficients were 0.87 and 0.76 respectively. Regression coefficients for the succinate grown cells (*b*) for distal edge and nuclear centre respectively were 0.25 and 0.23 and were not significantly different to 0.25 at 5% probability. The correlation coefficients were 0.67 and 0.65 respectively.

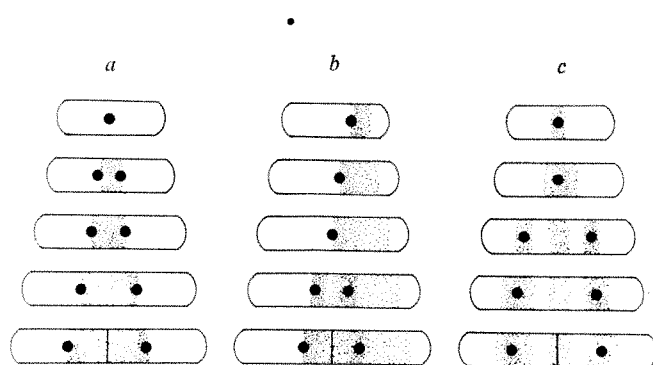


Fig. 1 Models of nuclear segregation in bacteria. *a*, The model of Jacob, Brenner and Cuzins¹ (model A). All surface growth occurs between the nuclei. *b*, The model of Clark² (model B) in which nuclei are rigidly attached to a particular site and growth occurs only to one side of the nucleus. *c*, An alternative model (model C) in which nuclei move to a site 25% of the cell length from the poles. Surface growth occurs to both sides of the nucleus. Most intense stippling indicates newest cell envelope.

cell length has been systematically slightly overestimated. As less than 5% of mononucleate cells show any deviation from the centre of the cell, the nuclei are probably located at 25% from the cell centre immediately before cell division.

Model B proposes that nuclei should be arranged asymmetrically in most cells of an exponential population. Less than 5% of mononucleate cells show any asymmetry on either medium, and the data in Fig. 3 indicate that nuclei of most binucleate cells are arranged symmetrically in each half cell.

Model C differs from A and B in the slope of the line relating nuclear position with cell length. Models A and B predict a slope of 0.5 whereas model C predicts a slope of 0.25. Although for both sets of data the scatter is considerable, the data favour model C rather than A or B (Fig. 1). Using autoradiography, Mendelson⁶ has shown that nuclei in binucleate cells of *Bacillus subtilis* 168 are predominantly 25% from either pole but mononucleate cells were not included in his analysis.

The rapid transition (less than 6 min) indicated by these data may be illusory, as in growing cells the nucleus may be arranged more diffusely than stained preparations suggest. If so, the location of the fixed nucleus probably represents the focus of the chromosomes' strongest interactions with the cell surface. The nucleus, however, may form a loose link with a new site earlier in the cell cycle. In *Escherichia coli*, segregation occurs close to the time of

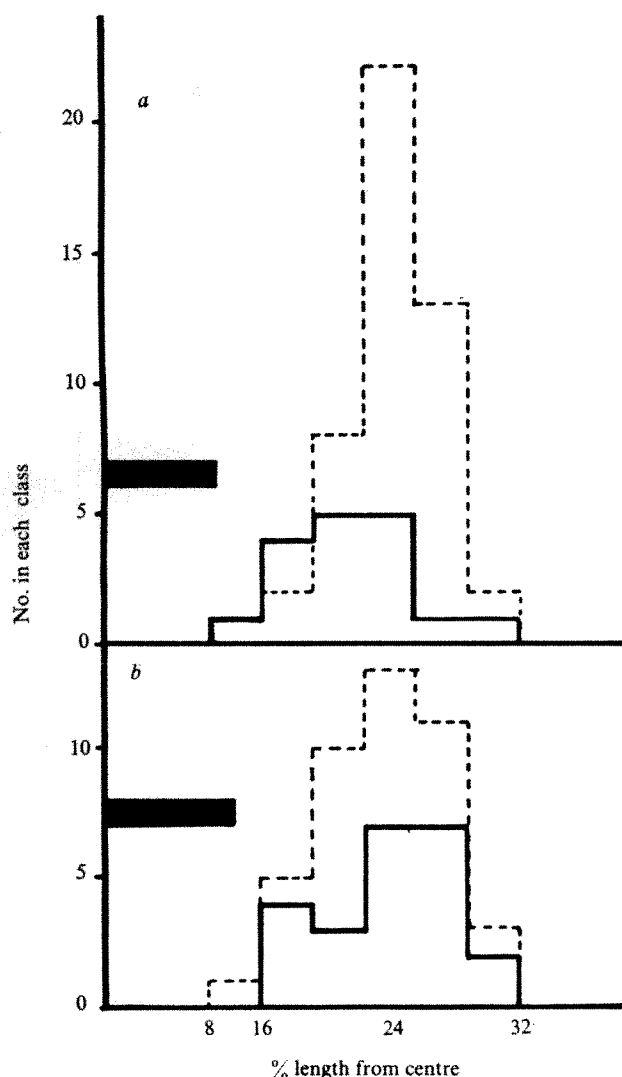


Fig. 3 Frequency distribution of distances from cell centre to centre of nuclei. *a*, Data from Fig. 2*a*. —, Data from binucleate cells less than 2.8 μm long. ---, Cells of greater length. *b*, Data from Fig. 2*b*. —, Binucleate cells less than 2.6 μm long. ---, Cells of greater length.

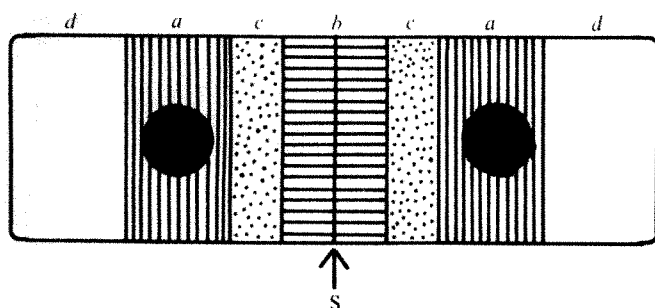


Fig. 4 Postulated origin of cell surface for a cell at division. Length of zone calculated assuming linear rate of surface extension from sites occupied by nuclei and that segregation occurs at mid-cycle (S, septum). *a*, Synthesised in interval between segregation and cell division; *b*, synthesised before segregation in current cycle; *c*, synthesised in interval between segregation and cell division in previous cycle; *d*, synthesised before segregation in previous cycle.

chromosome termination⁷ and this may represent the signal for pre-existing weak interactions with new sites to become predominant.

If the site of nuclear attachment is a major site of cell envelope extension and the rate of length extension is linear at each growth zone, the pattern of surface growth shown in Fig. 4 follows from the pattern of nuclear segregation in model C. The diagram shows the postulated distribution of cell surface synthesised during four periods for a cell which is about to divide. Lengthwise growth between segregations (Fig. 4*b* and *c*) must equal half the length of a cell at segregation. Therefore, if nuclei move 25% of the cell length, they would move to the junction of old and new surface formed one generation previously. As this was the site occupied by the nucleus at the time of the previous segregation, 'the pre-existing weak interactions', discussed here, may have been established at this time.

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- ¹ Jacob, F., Brenner, S., and Cuzin, F., *Cold Spring Harb. Symp. quant. Biol.*, **28**, 329 (1963).
- ² Clark, D. J., *Cold Spring Harb. Symp. quant. Biol.*, **33**, 823 (1969).
- ³ Donachie, W. D., and Begg, K. J., *Nature*, **227**, 1220 (1970).
- ⁴ Sargent, M. G., *J. Bact.*, **116**, 736 (1973).
- ⁵ Paulton, R. J. L., *J. Bact.*, **104**, 762 (1970).
- ⁶ Mendelson, N. H., *Cold Spring Harb. Symp. quant. Biol.*, **33**, 313 (1969).
- ⁷ Cooper, S., and Helmstetter, C. E., *J. molec. Biol.*, **31**, 519 (1968).

Chronic response of dogs to parathyroid hormone infusion

WHEN parathyroid hormone is infused chronically at a near-physiological rate, it causes hypercalcaemia by a mechanism fundamentally different from that which follows acute injections. Hormones, like drugs, have characteristic rates of metabolism and excretion and their distribution in the body is affected by binding to plasma proteins and membranes. Thus similar dependence of the pattern of response on entry rate is to be expected in the case of other hormones with short half lives, acting on multiple receptors of varying sensitivity.

As discussed in a recent review¹, parathyroid hormone (PTH) is an 84-residue single-chain polypeptide, which is probably secreted continuously at a rate regulated by the calcium concentration of the plasma. Its principal function seems to be to raise the calcium concentration in the plasma and extracellular fluid. Most of its multiple actions contribute to this effect and the rate of secretion of the parathyroid glands is inversely related to the circulating calcium concentration, providing close negative feedback control of the plasma calcium level. PTH accelerates bone breakdown and increases renal calcium retention and intestinal calcium absorption, this being the order of relative contribution of these actions to the hypercalcaemia elicited by injecting a large dose. Consideration of their individual dose-response curves, however, suggested a very different relative importance in normal physiology and led us to make a direct test of the effects of infusing minute doses continuously for periods of several weeks.

A miniature syringe-type pump (the Mill Hill infuser) was developed for us by the Engineering Division of the National Institute for Medical Research to enable continuous infusion during investigations and experimental therapy in freely mobile patients and large animals². Large dogs, whose calcium metabolism is similar to that of man, were equipped with indwelling cannulae in the external jugular vein³ and trained to wear the infusers on a light leather harness. Two cannulae were used, a technique permitting withdrawal of large well-mixed blood samples without interruption of the hormone infusion.

The results of infusions lasting up to 3 weeks are illustrated in Fig. 1. A dose rate of 25 ng kg⁻¹ h⁻¹ (0.05 U kg⁻¹ h⁻¹) continued for 3 d left the plasma calcium level of two dogs unchanged, but 100 ng kg⁻¹ h⁻¹ caused marked hypercalcaemia in each of four animals, reaching a plateau within 24 h and continuing throughout the infusions.

These results permit conclusions that could not be drawn from experiments by subcutaneous injection, in which the rate of absorption and the extent of local destruction of the hormone are both uncontrolled. For example, they make possible an estimate of the plasma level of intact PTH required to produce marked hypercalcaemia. From published estimates of the half time of disappearance of intact PTH (approximately 0.1 h (refs 8–10)), it can be calculated that an infusion of 0.1 µg kg⁻¹ h⁻¹ could only support an equilibrium circulating level of about 30 pg ml⁻¹ of intact hormone. From the 'black box' relationship: content at equilibrium = (entry rate × $t_{1/2}$)/0.693. Content is converted to concentration by assuming distribution throughout body water (50% of body weight). This estimate of the hypercalcaemic level is strikingly lower than present radioimmunoassay estimates of the PTH level in normal health (for example 1 ng ml⁻¹ (ref. 11)). The discrepancy underlines the fact that immunoassays can estimate biological activity only very indirectly. For example, biological activity of PTH is associated with N-terminal fragments, whereas current immunoassays for this hormone measure principally C-terminal fragments of long half life^{1,12,13}.

The results in Fig. 1 also give an indication of the normal rate of parathyroid secretion. The hypercalcaemia shows that the circulating level of bioactive PTH must have been greatly increased by infusion of 100 ng kg⁻¹ h⁻¹, although on immunoassay evidence the output of the animal's own parathyroids was presumably reduced virtually to zero at the calcium concentration reached^{1,14}. Copp and his colleagues¹⁵, using crude parathyroid extract, estimated that the dose required to maintain plasma calcium in acutely parathyroidectomised dogs kept under anaesthesia was 0.1 U kg⁻¹ h⁻¹. This estimate of the endogenous secretion rate is close to that which the present evidence suggests, in spite of the many differences in method.

At first sight the evidence obtained on the mechanisms contributing to hypercalcaemia at this low level is surprising. For example, signs of osteolysis were looked for in bone biopsy samples from the iliac crest before and after each infusion and alcohol-soluble hydroxyproline was measured in the plasma (an index of collagen breakdown¹⁶ which correlates closely with bone destruction^{17,18}). The biopsies, examined by Darby⁴, showed no increase in osteoclast numbers or in the areas involved in resorption and the hydroxyproline levels did not increase during hypercalcaemia in any of the four animals (Table 1).

Little support, however, can be found for the assumption that the acceleration of bone breakdown is a normal physiological response to parathyroid hormone. It occurs in patients with an actively secreting parathyroid adenoma and is known to be a direct effect of exposing bone to high concentrations of PTH (ref. 1). But the doses required to elicit a half-maximal hypercalcaemic response involving calcium mobilisation from bone are of the order of

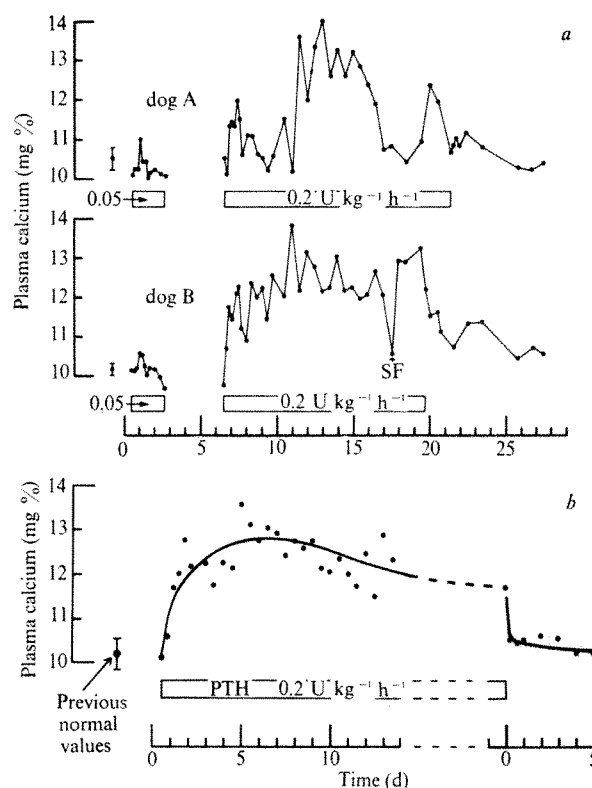


Fig. 1 *a*, Plasma calcium levels of two dogs during and after infusions of highly purified bovine parathyroid hormone at 25 and 100 ng kg⁻¹ h⁻¹ (0.05 and 0.2 MRC units kg⁻¹ h⁻¹). Horizontal bars indicate the duration of the infusions, which were separated by an interval of 3 d. The mean and s.d. of the plasma calcium level during 5 d preceding the first infusion is shown on the left. The letters SF denote a temporary syringe failure. Preliminary descriptions of these experiments were presented at the Endocrinology 1973 meeting⁴ and the Tenth European Symposium on Calcified Tissues⁵. Infusions were carried out at 2 ml per 24 h, in a vehicle consisting of 10% dog serum in 1% (w/v) sodium acetate trihydrate. This was heated at 56° C for 1 h (pH ≈ 7) to destroy proteolytic enzymes, adjusted to pH 4 with concentrated HCl, sterilised by membrane filtration (Millipore membranes, mean pore size 0.45 µm) and stored at -20° C. It is a nonantigenic variant of the vehicle used in our laboratory for bioassay of parathyroid hormone⁶. PTH dissolved in this vehicle at only a few µg ml⁻¹ was shown by the intravenous chick assay to be protected from surface absorption and to be stable for at least one week at room temperature. Calcium and phosphate analyses were carried out by atomic absorption spectrophotometry⁷ and had a coefficient of variation of less than 1%. *b*, Mean values of the plasma calcium level at corresponding times during infusions at the hypercalcaemic rate (100 ng kg⁻¹ h⁻¹) to four dogs. As two infusions lasted for 2 weeks and two for 3 weeks, the time scale is interrupted and restarts at the end of the infusions. The solid line is drawn to give an overall impression of the average effect, emphasising the slow onset of hypercalcaemia and the rapid fall in calcium level which followed termination of the infusion in all dogs. This time course is consistent with the known slowness of induction of enhanced intestinal absorption and rapid recovery from the renal action¹. PTH, parathyroid hormone.

20 U kg⁻¹ in dogs³ and 50 U kg⁻¹ in chicks⁶—both sensitive species. Experiments including potentiation of the PTH response with a small intravenous calcium injection suggest that the slow osteolytic process is initiated by (and long outlasts) a very rapid cellular response involving calcium influx¹ as well as activation of adenyl cyclase¹⁹, but these responses required doses of hormone corresponding to initial plasma levels thousands of times greater than the hypercalcaemic level calculated above for the present infusion study.

The hypercalcaemia due to infusion of 100 ng PTH kg⁻¹ h⁻¹ therefore presumably involves more sensitive responses and we looked for effects on calcium absorp-

tion and excretion. The calcium absorption coefficient (α =calcium absorbed/calcium ingested) was measured by giving ^{47}Ca by mouth and ^{45}Ca intravenously²⁰ and following the isotope ratio in plasma until it became constant. Analysis of urine from these large dogs was achieved only by housing two of them in metabolic cages for 24 h and comparing the concentrations of calcium and creatinine.

A striking effect on calcium absorption was observed during each infusion, α rising to between two and four times control values⁴. This action of PTH is well established¹, but its relative sensitivity was previously unknown. Its mechanism may involve enhanced production of 1,25 dihydroxycholecalciferol, a biologically active metabolite of vitamin D (refs 21 and 22) and induction of the vitamin D-dependent calcium-binding protein described by Wasserman and Taylor²³.

The urinary calcium: creatinine ratio fell during PTH infusion in both dogs studied, in spite of the marked hypercalcaemia and consequent increase in filtered load. This calcium-retaining action of the hormone is also well established¹ and was recently studied in dogs by micropuncture during infusion of $2 \text{ U kg}^{-1} \text{ h}^{-1}$, a dose which greatly enhanced distal tubular reabsorption²⁴. Interestingly, the phosphaturic action of PTH has a proximal tubular mechanism²⁵, so presumably there are two anatomically separate sets of PTH receptors in the kidney alone. Although phosphaturia is known to be evoked by lower doses than bone breakdown²⁶, neither urine nor plasma phosphate concentrations changed during the present infusions.

Table 1 Plasma hydroxyproline ($\mu\text{g ml}^{-1}$)

Dog	Control	During hypercalcaemia
A	7.2	7.2
B	4.2	3.0
C	3.5	3.2
D	4.5	4.0

Heparinised plasma for analysis (1.5 ml) was obtained by pooling aliquots from 3–5 successive days during control or infusion periods, each sample having been separated within 30 min and stored at -20°C . Non-protein bound (alcohol-soluble) hydroxyproline was measured by the method of Le Roy, *et al.*¹⁶.

These findings suggest the need to reassess current views of the biological role and clinical significance of parathyroid hormone. The contrast between the effects of administration in a physiological manner and the well known response to single or intermittent injections is so great as to virtually reverse the currently accepted picture of parathyroid hormone as an agent of bone destruction. The evidence indicates rather that its physiological role may be to promote calcium retention and bone formation and focuses attention on earlier studies in which PTH given chronically to rats in low dose was shown to have an anabolic effect on bone^{27–29}.

This conclusion has clinical implications of two kinds. Pathologically, the qualitative difference between high-dose and low-doses responses to parathyroid hormone implies that the syndrome of hyperparathyroidism may need to be subdivided. The demineralising condition currently regarded as typical may result only when there is gross over-secretion by the gland³⁰, though it will be difficult to prove this until radioimmunoassay findings can be translated into circulating levels of bioactive PTH. Manifestations of milder forms of the disease, including the frequent formation of renal calculi, are probably much influenced by the dietary levels of calcium and vitamin D (ref. 31), but it is particularly interesting that there is evidence for general or localised increase in bone density in some cases of

primary³² and secondary³³ hyperparathyroidism. Therapeutically, the present study strengthens the case for clinical trial of parathyroid hormone in osteoporosis, alone or in combination with calcitonin³⁴. As it seems that the renal and intestinal effects of parathyroid hormone can be obtained at a dose level which does not significantly increase bone breakdown, low doses may be capable of inducing a positive calcium balance and enhancing bone formation.

Finally, the importance of close attention to physiological dose levels and entry rates in future studies of hormone action can be briefly illustrated from other recent findings. The very name 'vasopressin', commonly used as a synonym for antidiuretic hormone, provides an example of a misleading physiological conclusion reached on the basis of inappropriate dosage. The word suggests a vasoconstrictor and pressor agent, yet pressor effects are first seen after doses of $10\text{--}40 \mu\text{g}$ ($5\text{--}20 \text{ U}$) in man³⁵, which must produce blood levels of several ng ml^{-1} . The normal blood level of this hormone is known now to be about 4 pg ml^{-1} ($2 \mu\text{U ml}^{-1}$), rising to 40 pg ml^{-1} during water deprivation³⁶. At this concentration, the hormone seems to act exclusively on the kidney. The need to imitate physiological entry rates was also illustrated by work of Knobil and his colleagues^{37,38}, showing that the 'pre-ovulatory' surge of luteinising hormone can be triggered by a small dose of oestrogen in ovariectomised monkeys. There was no dose which would produce the response unless a physiological basal oestrogen level had been established during the previous week by subcutaneous injections of oestrogen in oil or implanting crystals in a slow-release capsule.

Insulin is another hormone with a wide variety of dose-related effects and it may be that the incidence of pathological changes in chronically maintained diabetics could be greatly reduced by imitating physiological blood levels more closely than the depot-insulins permit.

Parathyroid hormone was prepared from fresh-frozen beef glands in the large scale laboratory of the National Institute for Medical Research. We thank Dr J. L. H. O'Riordan, Middlesex Hospital, for assistance in the final stages of its purification and the Armour Pharmaceutical Company, Eastbourne, who provided facilities for sterile ampouling.

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- Parsons, J. A., and Potts, J. T., jun., *Clin. Endocr. Metab.*, **1**, No. 1, 33 (1972).
- Sharpe, J., Lewin, J., and Parsons, J. A., *Lancet* (in the press).
- Parsons, J. A., Neer, R. M., and Potts, J. T., jun., *Endocrinology*, **89**, 735 (1971).
- Parsons, J. A., Darby, A. J., and Reit, B., in *Endocrinology 1973* (edit. by Taylor, S.) (Heinemann Medical Books, London, in the press).
- Reit, B., Rafferty, B., and Parsons, J. A., in *tenth Eur. Symp. Calcified Tissues* (edit. by Kuhlencordt, F.) (Springer, Berlin, in the press).
- Parsons, J. A., Reit, B., and Robinson, C. J., *Endocrinology*, **92**, 454 (1973).
- Parsons, J. A., Dawson, B., Callahan, E., and Potts, J. T., jun., *Biochem. J.*, **119**, 791 (1970).
- Potts, J. T., jun., Keutmann, H. T., Niall, H. D., Deftos, L. J., Brewer, H. B., and Aurbach, G. D., in *Parathyroid hormone and Thyrocalcitonin (Calcitonin)* (edit. by Talmage, R. V., and Belanger, L. F.), 44. (Excerpta Medica, Amsterdam, 1968).
- Silverman, R., and Yalow, R. S., *J. clin. Invest.*, **52**, 1958 (1973).
- Segre, G. V., Niall, H. D., Habener, J. F., and Potts, J. T., jun., *Am. J. Med.*, **56**, 774 (1974).
- Potts, J. T., jun., Murray, T. M., Peacock, M., Niall, H. D., Tregear, G. W., Keutmann, H. T., Powell D., and Deftos,

- L. J., *Am. J. Med.*, **50**, 639 (1971).
- ¹² Habener, J. F., Segre, G. V., Powell, D., and Potts, J. T., jun., *Nature new Biol.*, **238**, 152 (1972).
 - ¹³ Segre, G. V., Habener, J. F., Powell, D., Tregear, G. W., and Potts, J. T., jun., *J. clin. Invest.*, **51**, 3163 (1972).
 - ¹⁴ Care, A. D., Sherwood, L. M., Potts, J. T., jun., and Aurbach, G. D., *Nature*, **209**, 55 (1966).
 - ¹⁵ Copp, D. H., Moghadam, H., Mensen, E. D., and McPherson, G. D., in *The parathyroids* (edit. by Greep, R. O., and Talmage, R. V.), 203 (Thomas, Springfield, Illinois, 1961).
 - ¹⁶ LeRoy, E. C., Kaplan, A., Udenfriend, S., and Sjoerdsma, A., *J. biol. Chem.*, **239**, 3350 (1964).
 - ¹⁷ Bates, W. K., McGowen, J., and Talmage, R. V., *Endocrinology*, **71**, 189 (1962).
 - ¹⁸ Harris, E. D., and Sjoerdsma, A., *J. clin. Endocr.*, **26**, 358 (1966).
 - ¹⁹ Chase, L. R., Fedak, S. A., and Aurbach, G. D., *Endocrinology*, **84**, 761 (1969).
 - ²⁰ De Grazia, J. A., Ivanovitch, P., Fellows, H., and Rich, C., *J. Lab. clin. Med.*, **66**, 822 (1965).
 - ²¹ Omdahl, J. L., and Deluca, H. F., *Physiol. Rev.*, **53**, 327 (1973).
 - ²² Avioli, L. V., and Haddad, J. G., *Metabolism*, **22**, 507 (1973).
 - ²³ Wasserman, R. H., and Taylor, A. N., in *Mineral metabolism* (edit. by Comar, C. L., and Bronner, F.), **111**, 321 (Academic Press, New York, 1969).
 - ²⁴ Agus, Z. S., Gardner, L. B., Beck, L. H., and Goldberg, M., *Am. J. Physiol.*, **224**, 1143 (1973).
 - ²⁵ Agus, Z. A., Puscheck, J. B., Senesky, D., and Goldberg, M., *J. clin. invest.*, **50**, 617 (1971).
 - ²⁶ Treacher, R. J., *J. Endocr.*, **35**, 229 (1965).
 - ²⁷ Selye, H., *Endocrinology*, **16**, 547 (1932).
 - ²⁸ Kalu, D. N., Pennock, J., Doyle, F. H., and Foster, G. V., *Lancet*, **i**, 1363 (1970).
 - ²⁹ Walker, D. G., *Endocrinology*, **89**, 1389 (1971).
 - ³⁰ O'Riordan, J. L. H., Watson, L., and Woodhead, J. S., *Clin. Endocr.*, **1**, 149 (1972).
 - ³¹ Stanbury, S. W., Mawer, E. B., Lumb, G. A., Hill, L. F., Holman, C. A., Taylor, C. M., and Torkington, P., in *Clinical Aspects of Metabolic Bone Disease* (edit. by Duncan, J., and Frame, B.) 562 (Excerpta Medica, Amsterdam, 1973).
 - ³² Connor, T. B., Stoner, R. E., Freijanes, J., Martin, L. G., and Jowsey, J., *Trans. Am. clin. climatol. Asso.* (in the press).
 - ³³ Doyle, F. H., *Br. med. Bull.*, **28**, 220 (1972).
 - ³⁴ Robinson, C. J., Rafferty, B., and Parsons, J. A., *Clin. Sci.*, **42**, 235 (1972).
 - ³⁵ Brazeau, P., in *The Pharmacological Basis of Therapeutics* (edit. by Goodman, L. S., and Gilman, A.) 4th ed. 880 (Macmillan, New York, 1970).
 - ³⁶ Lauson, H. D., *Am. J. Med.*, **42**, 713 (1967).
 - ³⁷ Yamaji, T., Dierschke, D. J., Hotchkiss, J., Bhattacharya, A. H., Surve, A. H., and Knobil, E., *Endocrinology*, **89**, 1034 (1971).
 - ³⁸ Karsch, F. J., Dierschke, D. J., Weick, R. F., Yamaji, T., Hotchkiss, J., and Knobil, E., *Endocrinology*, **92**, 799 (1973).

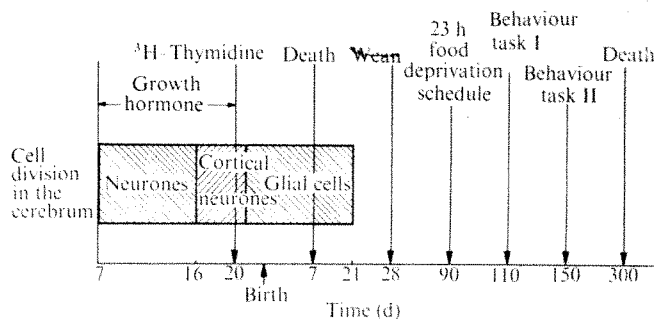


Fig. 1 Time course for the experimental procedure.

received the same volume of vehicle alone. Injections were given from day 7 of gestation, at the time of formation of the neural tube, until day 20 when each pregnant rat was injected intraperitoneally with 500 μ Ci of tritiated thymidine (3 H-TdR). Neurones in the cerebral cortex were labelled specifically by administration of 3 H-TdR at the time of their origin on day 20 of gestation². The rate of

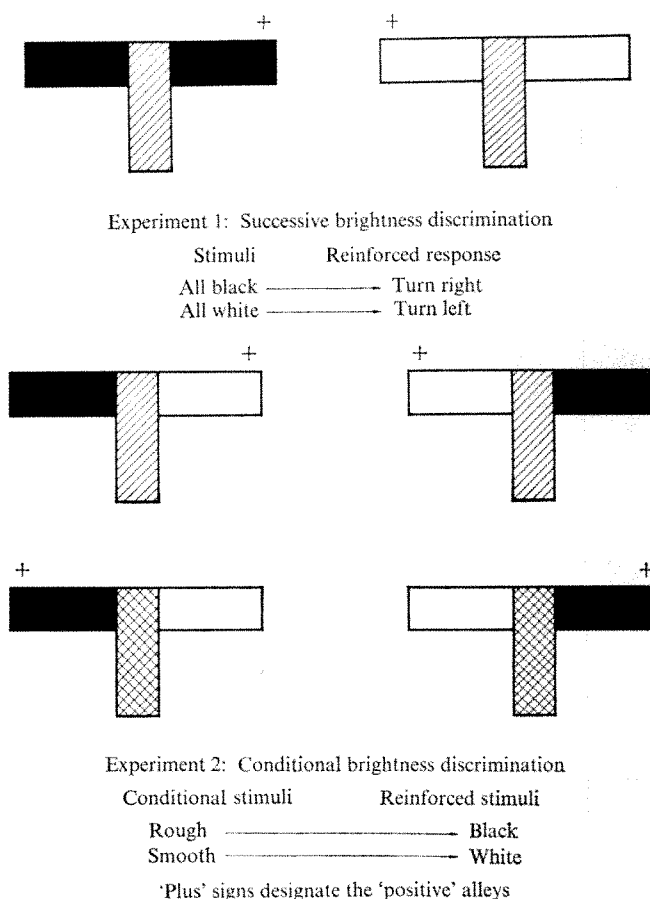


Fig. 2 Procedures used in the conditional discrimination tasks. Experiment 1: successive brightness or spatial conditional discrimination where the animal is trained to turn right for reinforcement when both arms are black, and left when both are white. Experiment 2: brightness conditional discrimination where the animal is trained to turn to the black arm for reinforcement when the floor of the alley is rough, and to the white arm when the floor of the alley is smooth. +, Denotes the positively reinforced discriminanda and the cross-hatching of the alley arm represents the rough tactile stimulus.

Prenatal action of growth hormone on brain and behaviour

IMPLICIT in all physiological theories of learning is the concept that learning ability depends on neuronal interaction, a function of both the number of neurones and the extent of their interconnections. Although learning ability varies with axo-dendritic development¹, a similar relationship to neuronal number has not been demonstrated, possibly because neuronal proliferation in most species, including rat² and man³, has ceased *in utero*. As alterations in neuronal number must therefore occur before birth we hypothesised that any prenatal growth factor would increase the number of cortical neurones and enhance subsequent learning ability. We have investigated this question, using young rats which had received pituitary growth hormone as the prenatal growth stimulus.

The experimental procedure is summarised in Fig. 1. Pregnant Wistar rats were treated at random in one of five ways: experimental animals received daily subcutaneous injections of either 3 mg, 1 mg, 100 μ g or 10 μ g of purified porcine growth hormone (0.6 U mg⁻¹) and controls

Table 1 Effect of various doses of growth hormone on brain structure and learning ability

Group	Brain weight* (mg)	³ H-TdR uptake* into brain DNA (d.p.m.)	Packing* density (d.p.m. mg ⁻¹ of brain tissue)	Number of trials† to obtain criterion performance on the first behavioural task‡	Number of trials† to obtain criterion performance on the second behavioural task‡
Control	387 ± 81	14,497 ± 4,763	43 ± 15	36 ± 12	88 ± 25
3 mg PGH	566 ± 50	30,703 ± 5,703	54 ± 11	24 ± 7	59 ± 6
1 mg PGH	562 ± 60	23,480 ± 6,458	43 ± 11	21 ± 3	30 ± 27
100 µg PGH	640 ± 55	25,380 ± 7,755	41 ± 12	18 ± 5	30 ± 19
10 µg PGH	451 ± 63	23,069 ± 1,771	53 ± 7	25 ± 7	51 ± 10
F	43.4	8.2	2.3	11.1	14.8
d.f.	4,70	4,43	4,43	4,63	4,62
P	<0.001	<0.001	>0.05	<0.001	<0.001

Results are expressed as mean ± s.d. PGH, porcine growth hormone.

* Data analysed by a one-way fixed effects analysis of variance for unequal group sizes.

† Data analysed by a two-way fixed effects analysis of variance of unweighted means for unequal group sizes.

‡ A learning criterion of 10 consecutive correct responses was used.

proliferation within this discrete population was determined subsequently by the incorporation of ³H-TdR into brain DNA, and the validity of the procedure was assessed by fine-resolution autoradiographic localisation. On the seventh day after birth the brains of half the offspring in each litter were examined. DNA⁴ was extracted from each brain and its radioactivity was determined. Autoradiograms were prepared from one animal in each litter.

The remaining offspring were examined for learning ability at maturity. On day 90 they were placed on a 23 h food deprivation schedule and tested for performance on a series of conditional discrimination tasks of increasing order of difficulty (Fig. 2). Animals were killed on day 300, blood collected and plasma concentrations of thyroxine⁵, testosterone⁶, insulin⁷ and corticosterone⁸ in response to stress measured to determine any hormonal changes which may have influenced behaviour.

Table 1 shows that growth hormone produced a significant increase in brain weight and cellular content. The number of cortical neurones increased as measured by the incorporation of ³H-TdR and subsequent localisation by autoradiography. In all autoradiograms a band of heavily labelled cells was found only in the supragranular layers of the cortex, indicating that ³H-TdR uptake specifically reflects the proliferation of neurones within this region. When examined for functional capacity, offspring of mothers treated with growth hormone during pregnancy showed enhanced ability, obtaining criterion performance in significantly fewer trials than controls. This difference became particularly apparent on the second two-cue learning task, where after 100 free trials, 63% of control animals had failed to reach criterion performance. In comparison, only one animal treated with growth hormone was unsuccessful by this time. Enhanced performance could not be attributed to activation changes as determined by both latency of responding and hormonal response to stress. No change was found in adult body weight or plasma hormone concentrations ($P > 0.05$). Indeed at maturity, brain structure and functional capacity seemed to be the sole consequences of growth hormone treatment.

These results show that the administration of growth hormone during the time of neuronal proliferation produces a permanent increase in the number of cortical neurones and that there is a subsequent enhancement of learning ability. Although this period has been largely neglected as a vulnerable period of brain development, these findings demonstrate that it is critical not only for growth but for subsequent intellectual capacity.

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1. Eayrs, J. T., *Hormones in development* (edit. by Hamburg, M., and Barrington, E. J. W.) (Appleton-Century Crofts, New York, 1971).
2. Berry, M., and Rogers, A. W., *J. Anat.*, **99**, 691 (1965).
3. Dobbing, J., and Sands, J., *Nature*, **226**, 639 (1970).
4. Munro, H. N., and Fleck, A., *Methods of biochemical analysis*, XIV, 113 (1966).
5. Thorson, S. C., Mincey, E. K., McIntosh, H. W., and Morrison, R. T., *Br. med. J.*, **2**, 67 (1972).
6. Horton, R., Kato, T., and Sherins, R., *Steroids*, **10**, 245 (1967).
7. Hales, C. N., and Randle, P. J., *Biochem. J.*, **88**, 137 (1963).
8. Murphy, B. E. P., *J. clin. endocr. Metab.*, **27**, 973 (1967).

Depolarisation of *Onchidium* neurone by glycine

INTRACELLULAR recording of cholinergic synapses in the *Onchidium* oesophageal ganglia revealed one neurone, V-3, in the visceral ganglion in which neither membrane potential nor conductance changes after perfusion with 0.5 mM acetylcholine. Significant depolarisation with increased membrane conductance was caused by 1.3 mM glycine. This response to glycine is opposite to that of the mammalian spinal motoneurone¹⁻³. Neurones examined in the ganglia, except V-3, were not affected by glycine. Here we report the ionic mechanism of glycine depolarisation, and compare the effect of strychnine on glycine depolarisation with its antagonistic action of mammalian glycine inhibition⁴.

The excised oesophageal ganglia were continuously perfused with artificial seawater (NaCl 457.6 mM, KCl 9.6 mM, CaCl₂ 10.4 mM, MgCl₂ 48.5 mM, pH, 7.8 by Tris-HCl, at 23° C). Two microelectrodes were inserted into the V-3 neurone, one for recording the membrane potential, the other for applying current to polarise the membrane. The resting membrane potential was -56.3 ± 4.9 mV (mean ± s.d.) (30 cells). Some spontaneous excitatory postsynaptic potentials, but no spontaneous firings were observed. The cells were depolarised about 40 mV with the membrane conductance increased to about twice normal and they fired vigorously when 13 mM glycine was added to the perfusing solution. Ouabain (3×10^{-4} M) had no effect on the level of resting potential or glycine depolarisation. Thus, the depolarisation is probably not caused by the inactivation of an electrogenic Na pump⁵ or the activation of the outward Cl pump⁶. The glycine depolarisation did not change significantly when the chloride ions in the bathing solution were replaced by

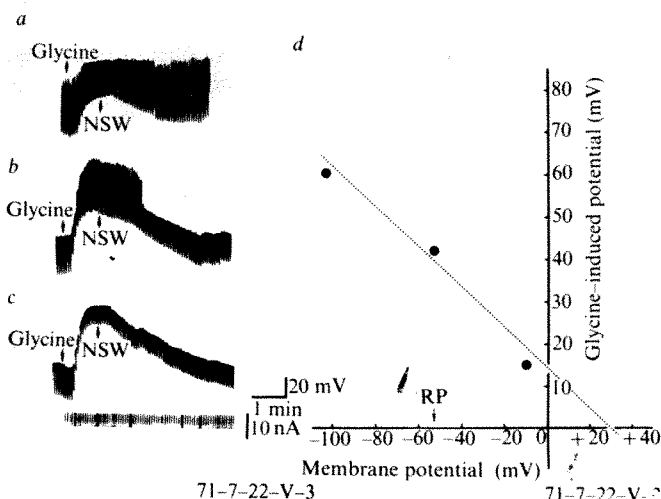


Fig. 1 Effect of displacement of membrane potential on amplitude of glycine depolarisation (glycine, 13 mM). *b*, Glycine depolarisation at original resting potential (-53 mV); *a*, at depolarised membrane potential of 43 mV; *c*, at hyperpolarised membrane potential of 50 mV; *d*, Amplitude of glycine depolarisation plotted against three different levels of membrane potential. The membrane potential at the point where the dotted line crosses the abscissa was taken as the equilibrium potential for glycine depolarisation. RP, original resting membrane potential (-53 mV). NSW, normal seawater.

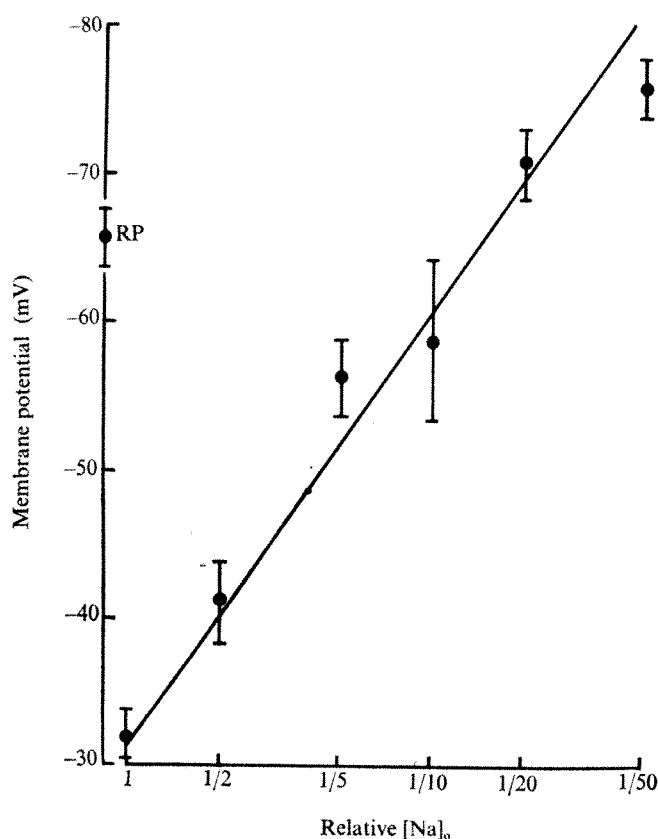


Fig. 2 Relationship between amplitude of glycine (13 mM) depolarisation and $[Na]_o$ in seawater. Amplitude of glycine depolarisation (mean \pm s.d., three V-3 neurones) plotted against $[Na]_o$. Average of original resting membrane potential is shown as RP. The resting potential was stepwise hyperpolarised a total of approximately 17 mV by stepwise decrease from 1 to 1/50 in $[Na]_o$. Thus each glycine depolarisation was the shift from the new resting potential at each new $[Na]_o$ concentration.

acetate. Increase of $[K]_o$ to twice normal did not change glycine depolarisation.

To determine the equilibrium potential for glycine depolarisation, the membrane potential was changed by current application. Figure 1 shows an equilibrium potential of +30 mV obtained by extrapolation of three experimental values. This high potential indicates that the ions involved in the generation of glycine depolarisation are probably Na, or Ca ions, or both.

The next series of experiments therefore consisted in changing $[Na]_o$ to modify this concentration gradient across the cell membrane (NaCl replaced by Tris-Cl). Stepwise decrease in $[Na]_o$ from 457.6 to 9.1 mM caused a corresponding stepwise decrease in the level of depolarisation by 13 mM glycine (Fig. 2). Note that the normal resting potential was hyperpolarised in steps corresponding to the stepwise decrease in $[Na]_o$ from 1 to 1/50. The total amount of hyperpolarisation was approximately 17 mV. The reported depolarisation values are those measured from each respective new 'resting potential'. The change in depolarisation for 10 times change in $[Na]_o$ was about 30 mV. Tetrodotoxin (3×10^{-5} M) had no effect on glycine depolarisation. This indicates that the glycine effect is probably taking place at the subsynaptic membrane⁷⁻⁸. For a five-fold increase of $[Ca]_o$, the glycine depolarisation remained at the same level. Hence, it may be concluded that glycine depolarisation is due exclusively to an increased permeability of the membrane to Na. Doubling the concentration of Mg (97 mM) in seawater had no effect on the glycine depolarisation. The lack of effect of Mg as well as Ca indicates that the glycine action is subsynaptic rather than presynaptic. Glycine was also effective when applied locally to the soma and neck of the V-3 neurone by passive diffusion from a glass microelectrode (tip diameter, about 3 μ m). The neck portion of this neurone was much more sensitive to glycine than was the soma. Synapses of the *Onchidium* nervous system are always axo-axonic and are located in the neuropile at various distances from the soma (T. Yamamoto, unpublished observation).

Interestingly, the effect of glycine on the V-3 neurone was augmented, not blocked, by 2.5×10^{-4} M strychnine. Figure 3a shows depolarisations at various concentrations of glycine from 0.1 to 130 mM; Fig. 3b shows the augmenting effect of strychnine on each glycine depolarisation. The membrane conductance at the plateau phase of depolarisation by glycine plus strychnine (Fig. 3b, right) was certainly no larger than that from glycine alone (Fig. 3a, right). It may even have been smaller. Strychnine alone in this concentration had no detectable effect on the resting

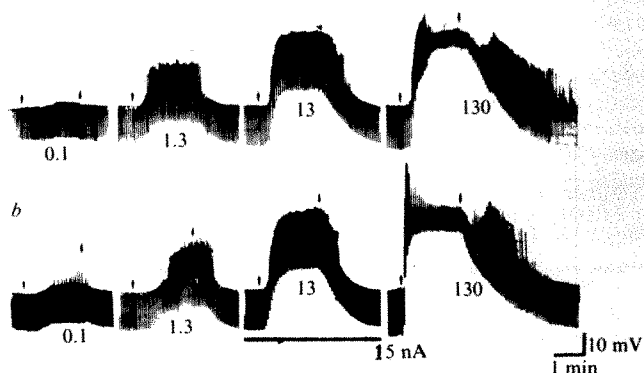


Fig. 3 Augmenting effect of strychnine on glycine depolarisation at various concentrations of glycine. *a*, Glycine alone; *b*, glycine (0.1 to 130 mM) plus 2.5×10^{-4} M strychnine. Figures indicate glycine concentration (mM). Strychnine alone had no effect on the resting membrane potential or conductance.

membrane property, in contrast to its effect on the giant axon of the lobster⁹. The equilibrium potential of glycine depolarisation was 22.7 ± 2.7 mV (four cells); that of glycine plus strychnine was 41.4 ± 3.2 mV. The increase in membrane conductance was $58.3 \pm 16.2\%$ in the former and $44.7 \pm 5.1\%$ in the latter. The differences in equilibrium potential and membrane conductance change for glycine plus strychnine compared with glycine alone indicate that the strychnine augmentation is caused by a decrease in K permeability.

Mention of the effect of GABA on the V-3 neurone may be worthwhile. α -Amino butyric acid (GABA, 10 mM) caused a few mV depolarisations of the V-3 neurone. At the same time it caused a decrease in membrane conductance to about 80% of normal. Preliminary experiments indicate that this depolarisation results in a decrease of the resting K permeability of the subsynaptic membrane. This effect is completely different from that on the vertebrate pre and postsynaptic membranes. Both membranes are depolarised by GABA due to increased Na and Cl permeability respectively¹⁰⁻¹².

In summary, glycine is excitatory in the V-3 neurone. Glycine depolarisation is due solely to increase in Na permeability at the subsynaptic membrane. Strychnine augmentation is caused by K permeability decrease during the glycine depolarisation. The effects of glycine and GABA on the membrane properties of *Onchidium* are very different from their effects on vertebrate neurones.

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- ¹ Davidoff, R. A., Shank, R. P., Graham, L. T., jun., Aprison, M. H., and Werman, R., *Nature*, **214**, 680 (1967).
- ² Curtis, D. R., Hösl, L., and Johnston, G. A. R., *Nature*, **215**, 1502 (1967).
- ³ Werman, R., Davidoff, R. A., and Aprison, M. H., *J. Neurophysiol.*, **31**, 81 (1968).
- ⁴ Larson, M. D., *Brain Res.*, **15**, 185 (1969).
- ⁵ Nishi, S., and Koketsu, K., *J. Neurophysiol.*, **31**, 717 (1968).
- ⁶ Oomura, Y., Ooyama, H., and Sawada, M., *Twenty-third Int. Congr. Physiol. Sci.*, Abstr. 389 (1965).
- ⁷ Furukawa, T., Sasaoka, T., and Hosoya, Y., *Jap. J. Physiol.*, **9**, 143 (1959).
- ⁸ Katz, B., and Miledi, R., *Proc. R. Soc.*, **B167**, 8 (1967).
- ⁹ Freeman, A. R., *J. Neurobiol.*, **4**, 567 (1973).
- ¹⁰ Bowery, N. G., and Brown, D. A., *Br. J. Pharmacol.*, **45**, 160 (1972).
- ¹¹ Adams, P. R., and Brown, D. A., *Br. J. Pharmacol.*, **47**, 639 (1972).
- ¹² Barker, J. L., and Nicoll, R. A., *J. Physiol. Lond.*, **228**, 259 (1973).

Leptospiral motility

THE motility of spirochaetes, especially the *Leptospira*, has interested many workers but has remained unexplained despite the application of modern techniques. Inadequate observations by Noguchi¹ have been used by modern workers (Ritchie and Ellinghausen²; Nauman³) to construct inaccurate models of leptospiral movement, the main inaccuracy of which is that leptospirae rotate upon their axes in free liquid. Noguchi¹, however, did observe that "when

one end is straight and the other semicircularly hooked, the organism usually progresses in the direction of the straight portion and seems to be propelled from the rear by the rotating hook". Although not correct in important details, the observation of translational movement in the direction of a straightened portion is a fundamental principle. Previous observations and explanations were at variance with the physical principles of fluid mechanics. This paper presents information which we believe to be in accord with such principles.

Observations were made using a Reichert Zetopan microscope set for dark ground operation. Videorecordings were made to aid the categorising of the movements, and direct observation was used to analyse the finer details. The organism used was *Leptospira icterohaemorrhagiae* strain 3H cultured in Korthof medium. Particulate matter was supplied to the organism either as particles of agar added to the culture on the microscope slide, or by allowing an active culture to invade semi-solid nutrient agar.

Movement in free liquid. With non-translational movement (Fig. 1a) the body remains relatively stationary with

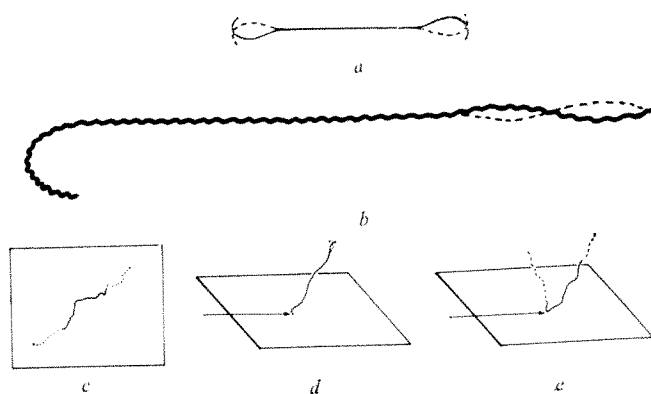


Fig. 1 Motility of *Leptospira icterohaemorrhagiae*. a, Non-translational movement in free liquid; b, translational movement in free liquid; c, crawling on a smooth surface; d, anchoring on a smooth surface; e, staple movement on a smooth surface. Arrows indicate point of fixation.

either end waving in a circular motion in opposite directions. The rapid movement gives the appearance of spinning and the hooked ends blur into the familiar 'button hole' figure. Illumination causes the organisms to move more slowly and under such conditions contra-rotation of the hooked ends may be observed. Though care must be exercised in extrapolating from the behaviour of such organisms to the normal state, the observation that organisms decelerate smoothly would suggest that an abrupt change is unlikely. The forces produced by this type of movement are seen to be equal in all directions as there is no net change in the position of the organism.

When movement is Translational (Fig. 1b), the end towards which the translation is effected performs helical waves travelling for a short distance of the length of the cell towards the trailing end. The trailing end forms a broad hook which waves in an irregular circle in the opposite direction to the propulsive helical wave to provide stability and prevent rotation of the whole body. The helical wave may also be observed when a portion of the body is fixed to or embedded in a particle and unable to rotate. An organism has been observed moving with a piece of colloidal graphite fixed to the tip of the trailing end. The particle was so massive with respect to the organism that there was no tendency for the body to rotate, emphasising the function of the hooked tail.

Movement on a smooth substrate. *Leptospira* may crawl (Fig. 1c) either like a snake or, alternatively, by lifting clear and then reapplying parts of the body. A variant on this is circular crawling in which no sideways waves of propulsion are seen. The body in this case follows a circular path, the circumference of which is normally less than the length of the body.

The organism may be anchored (Fig. 1d). One end may remain fixed to the substrate despite high rates of flow in the medium. Frequently one end of the organism is seen to be fixed to itself further along, in place of the substrate.

A third possibility is *staple movement* (Fig. 1e) so called because it can be imagined that the organism is passing through a staple or hoop fixed to the substrate. This movement often follows anchoring and is most probably due to the same process. By virtue of the small radius of the protoplasmic cylinder the surface energy would be relatively high, providing the physical adhesion necessary for this type of behaviour. When the organism moves through the 'staple' the spiral of the protoplasmic cylinder behaves as if it were a screw, threading through a small hole. This keeps the cell in intimate contact with the substrate in accordance with the surface energy concept. There is at present no direct evidence to indicate that surface energy forces are responsible for the phenomena observed. Equally, there is no evidence to indicate the presence of adhesive organelles or the secretion of adhesive substances.

Movements in semi-solid medium. Translation is effected by snaking through the medium with the body conforming to the texture of the medium. The body is very flexible. It is not obvious how movement is actually carried out but the body once again screws through the medium.

The most important point to arise from these observations is the notion that when moving in free liquid *Leptospira* do not rotate about the axis as has previously been supposed. The hook at the trailing end of an organism performing translational movement remains relatively still thereby providing an 'inert head' fundamental to the effectiveness of the helical waves of the leading end of the cell as demanded by Taylor's calculations on propulsion by helical waves¹. This basic requirement is not included in previous observations.

A certain amount of roll about the long axis is inevitable as a consequence of the helical nature of the propulsive waves², but this can be seen to be corrected for by flicking movements of the trailing hook. Any remaining rotation could not be confused with the rapid spinning described previously¹⁻³.

The rapid spinning seen in nontranslatory movement is now seen to be an illusion. There is no process available to produce rapid spinning in the absence of flagellae, cilia or jet devices, none of which have been observed. It is, however, a physical reality that the ends can wave in circles of opposite direction, each providing the necessary reaction to the other.

Any attempts to explain these movements at the sub-cellular level must be speculative at the moment. It is possible that the axial filaments might be responsible, provided that they extend along the axis of the body as a whole and are not themselves spiralled structures. This condition seems to be fulfilled³, a fact which eases the problem somewhat, since a system whereby a spiralled filament produces secondary spiral waves defies imagination. The axial filaments are known to extend from each end of the cell for a short distance towards the middle, and it is the terminal portion of the cell that performs the locomotory function.

The importance of these observations to the study of leptospirae as pathogens lies in the insight gained as to how the pathogen solves some of the problems posed by both the external environment and the internal environ-

ment of the body of the host.

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¹ Noguchi, H., *J. exp. Med.*, **27**, 575-593 (1918).

² Ritchie, A. E., and Ellinghausen, H. C., *J. Bact.*, **89**, 223-233 (1965).

³ Nauman, R. K., Holt, S. K., and Cox, C. D., *J. Bact.*, **98**, 264-279 (1969).

⁴ Taylor, G. I., *Proc. R. Soc.*, **A211**, 225-239 (1959).

⁵ Chwang, A. T., and Wu, T. Y., *Proc. R. Soc.*, **B178**, 327-346 (1971).

Bioassay of a *Drosophila* pheromone influencing sexual selection

PHEROMONES function by transmitting information from a sending to a receiving organism by chemical means. In a classic paper, Bossert and Wilson classified the effects of such information transfer into releasing and priming¹. Releaser pheromones evoke an immediate overt behavioural effect, whereas primer pheromones activate the chemoreceptor in such a way as to alter the physiology of the receiving organism^{1,2}. In general, studies involving the isolation identification of pheromones have concentrated on releasers such as sex attractants, alarm pheromones, trail pheromones and aggregation pheromones³. The advantage of using releaser pheromones in such studies is that the behavioural effects can be observed even in a single individual of the appropriate age, sex and species. This makes the development of a relatively simple and rapid bioassay a straightforward process. So far the important exception to the use of releaser pheromones has been the work of Butler *et al.* showing that 9-ketodecenoic acid inhibits ovarian development in worker bees⁴. This substance, however, was first identified as the sex attractant of the queen bee and later shown to have a primer effect. We describe here a bioassay for a substance with an effect which does not fit easily into either the releaser or primer category.

We have been attempting to isolate and identify the pheromones responsible for the rare male behaviour found in *Drosophila* of several species and in other organisms⁵⁻⁷. Rare male behaviour is characterised by females preferring the strain of males in least abundance presented to them as potential mates. This rare male advantage may have evolutionary significance, since it provides a mechanism for preserving a heterogeneous gene pool.

In particular we are concerned with two intensively studied strains of *Drosophila pseudoobscura*, homozygous Chiricahua (CH) and homozygous Arrowhead (AR) — differing in third chromosome inversions⁸. Previous work has shown that when the ratio of males (CH:AR) is sufficiently different from 1.0, males of the strain in least abundance are favoured as mates by females of both strains (Fig. 1)^{6,7}. Furthermore, a compound or compounds of low polarity extractable from CH males into light petroleum ether (and to a much lesser extent into acetone) has been

shown to be capable of producing an advantage for AR males when the AR:CH ratio is 1.0 (ref. 7).

Attempting to isolate and identify this active material, we have been led to quantify the assay, and in doing so have been able to show that the behaviour can be controlled substantially by olfactory cues alone. In the mating trials, any extract was spotted on to the filter paper bottom of an Elens-Wattiaux observation chamber⁸ and the solvent allowed to evaporate. Twelve aged females from each of the two strains were then introduced unanaesthetised and allowed to acclimate to the chamber for 1 min. Eighteen AR males and six CH males were then introduced in a similar manner and all matings were scored by direct observation with a hand lens. As Fig. 1 shows, this CH:AR ratio of 0.33 normally produced nonrandom mating frequencies favouring the rare CH males; this held true both in clean chambers and in the presence of inactive compounds, solvent or low concentrations of active material. In the presence of petroleum ether extracts of CH males, however (prepared by tissue grinding flies in acetone, centrifuging, decanting and extracting the pellet with petroleum ether, 30°–60° C), this rare male advantage could be removed. As Fig. 2 shows, this removal exhibited a threshold above which random mating (as measured by χ^2) is observed. The relatively high value of this threshold (ten to fifteen flies extracted per fly present) may indicate that the extraction was incomplete or resulted in degradation or loss of the material, that the actual pheromone release was directional (and hence more sparing of material), and/or that other cues (such as auditory, visual or tactile sensations^{9–11}) supplement the olfactory signals. The persistence of random matings even to high multiples of the threshold value lends strength to the supposition that other cues are also important.

This bioassay, and hence the behaviour which it measures, differs significantly from procedures generally used. Unlike the usual assays of releaser pheromones, our bioassay cannot be conducted with individual organisms. The pheromone appears to bias the female's response in a predictable manner, but in a way which cannot be measured for individual females. Furthermore, unlike the situation with primer pheromones, the effect is observable on a very short time scale. In a different context from the reports

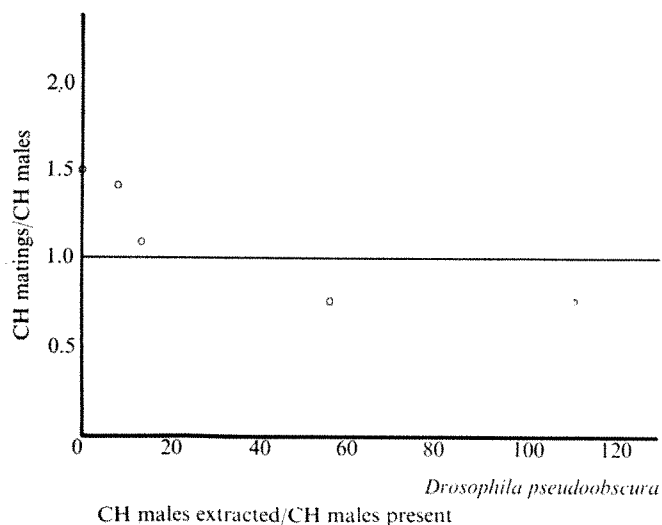


Fig. 1 As the AR:CH ratio approached 50:50, the mating advantage of the CH males disappeared. Ratios are based on a total of 96 observed matings. To guarantee the vitality of the stocks, only chambers in which all females mated are included.

mentioned above, E. O. Wilson has spoken of the "intractable" recognition substances which are "deserving targets for the best efforts of chemists in the future"¹². The females of the *D. pseudoobscura* strains studied are clearly using a chemical cue to determine the presence of males of the CH strain, and altering their behaviour accordingly. Rather than releasing an overt behavioural effect or altering the female's physiology in a long term manner, the

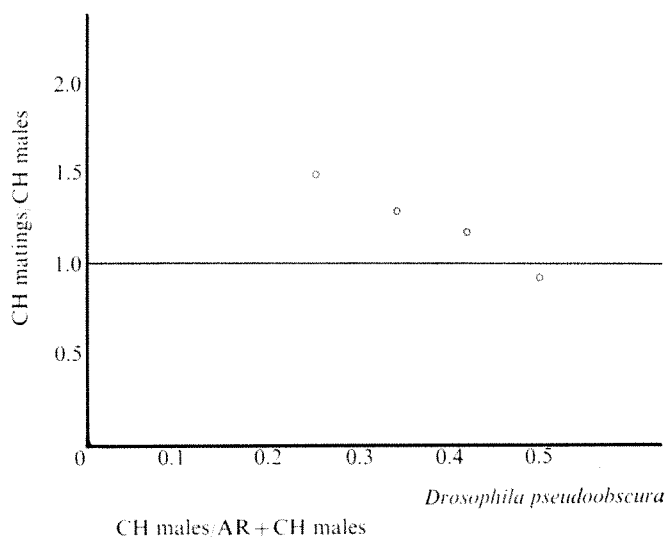


Fig. 2 As further petroleum ether extract of CH males were added to the chambers, the mating advantage of CH males disappeared. Ratios are based on 48 observed matings in chambers where all females mated. The assay can also be run using a 50:50 AR:CH ratio; a full advantage for AR (as determined by the χ^2 for randomness) can be achieved. This assay is used because it appears to provide a better internal control for the vitality of the critical CH flies. There is no significant difference in the statistical stringency of the two assays.

olfactory cue seems to act as information, pure and simple. Although other information (auditory, visual, tactile) is also utilised, the olfactory cue can strongly bias whatever mechanism operates in the females to determine their choice of mates. This work was supported by a US Public Health Service National Institutes of Health research grant.

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- ¹ Bossert, W. H., and Wilson, E. O., *J. theor. Biol.*, **5**, 443 (1963).
- ² Wilson, E. O., in *Chemical ecology* (edit. by Sondheimer, E., and Simone, J. B.), 133 (Academic Press, New York, 1970).
- ³ Regnier, F. E., and Law, J. H., *A. Rev. Biochem.*, **40**, 533 (1971).
- ⁴ Butler, C. G., *Symp. R. Ent. Soc. London*, **2**, 66 (1964).
- ⁵ Petit, C., and Ehrman, L., *Evolut. Biol.*, **3**, 177 (1969).
- ⁶ Ehrman, L., *Am. Naturalist*, **101**, 415 (1967).
- ⁷ Ehrman, L., *Behav. Genet.*, **2**, 69 (1972).
- ⁸ Elens, A., and Wattiaux, J., *Drosophila Info. Service*, **39**, 118 (1964).
- ⁹ Conolly, K., Burnet, B., and Sewell, D., *Evolution*, **23**, 548 (1969).
- ¹⁰ Ewing, A., and Manning, A., *A. Rev. Ent.*, **12**, 471 (1967).
- ¹¹ Grossfield, J., *Univ. Texas Publ.*, No. **6818**, 147 (1968).
- ¹² Wilson, E. O., *Science*, **172**, 406 (1971).

Dietary preference and diseases of age

THE frequency of a number of tumour types and several age-related diseases are readily modified by dietary means¹⁻⁸. Traditionally, in defining the influence of a dietary factor experimentally, diets of fixed composition are offered either *ad libitum* or in limited amounts for arbitrarily assigned periods. In *ad libitum* conditions, however, except for the amount of food consumed, the animals have no choice but to eat the diet provided; in restricted feeding conditions, even this option is denied them.

The element of dietary preference was introduced in the investigations reported here. This new approach to long term studies was suggested by the well-established fact that the young rat, unless it has been biased by previous experience or training, has the ability to select for itself essential dietary substances in amounts necessary to sustain life, to grow and to reproduce⁹⁻¹². That this behaviour represents a response to specific metabolic 'needs' is supported by the observations that a variety of conditions which alter metabolism generally results in compensatory changes in appetite for specific food substances¹³⁻²².

The self-selection (S-S) method of feeding seems to have several advantages over a 'fixed' dietary regimen: the diet selected is of physiological significance to the individual, at least during the growth period of life; it more closely approximates the natural condition; and the arbitrary decisions made by the investigator as to the quality of the diet without considering the needs of the individual are avoided. In addition, by permitting the animals to display their preferences, it may be possible to assess on an individual basis the long term consequences of dietary habits at different stages of life.

In this study we provided each rat fed on the S-S basis, three complete isocaloric diets⁸ in separate containers instead of the individual components. They differed only in their casein and sucrose content. To determine the influence of each of the diets separately, other rats were fed *ad libitum* in the conventional manner.

The regimen of the individually housed male Charles River COBS rats, which genetically are relatively heterogeneous, was begun when they were 21 d of age and was maintained for the remainder of the animals' lives (the age at death of the longest lived rat was 1,075 d). The amount of each of the diets consumed by each S-S rat was determined daily. The dietary history during the gestation and suckling periods has been described^{8,23}. Upon the death of each rat, a thorough necropsy was performed and samples of all normal and diseased tissues were taken for histopathological examination. The tissue sections were coded so as to preclude identification with the age and dietary history of the animal.

Far more 'spontaneous' tumours and more cases of kidney, heart, and prostate gland disease occurred among the S-S rats than among the rats fed the same diets singly (Table 1). Indeed, there were a number of tumours for which the incidence among the S-S rats was as great or greater than among all three conventionally fed groups combined. These included benign tumours without respect to type, adenomas and carcinomas without respect to site, leukaemia, tumours of the anterior pituitary, adrenal, exocrine pancreas, and skin. This also held for advanced cases of glomerulonephrosis, myocardial fibrosis, and for prostatitis. Adjustments for time of occurrence, age-specific morbidity rates, population size and length of life, confirmed the differences in risk (Table 1). In addition to the significantly higher incidences, more S-S rats had multiple affections at time of death than did those on the fixed diets; 66% of the S-S rats had at least three of these diseases as compared with 9, 26, and 28% for rats fed low, intermediate, and high protein diets (D₁₀, D₂₂ and D₅₁), respectively.

The higher incidences of the S-S rats were not attributable to longer life spans. Although the S-S rats had a higher life expectancy than rats on the fixed diets, this was limited to age periods before 400 d. Once the various diseases manifested themselves, the life expectancy of the S-S rats was lower than

those of the other groups. The change to higher age-specific mortality rates was commensurate with the consistently higher age-specific morbidity rates.

With the conventional feeding method, the frequencies of the non-neoplastic diseases (Table 1), and of several tumour types⁸, were dependent upon the proportion of protein in the diet.

Table 1 Frequencies of age-related diseases of rats as influenced by mode of feeding

	Dietary regimen*			Self-selection† (D ₁₀ , D ₂₂ , D ₅₁) Cases per 100 rats‡
	Single-fixed D ₁₀	D ₂₂	D ₅₁	
Tumour bearing rats	26	29	28	62'' (177)††
Tumours, all types	29	38	39	91'' (295)
Benign	20	28	30	7'' (232)
Malignant	9	10	9	14 (107)
Glomerulonephrosis				
All grades	38	56	73§	90** (145)
Moderate and severe	4	18	39§	56** (176)
Myocardial fibrosis				
All grades	11	42	48§	67** (167)
Moderate and severe	<1	4	18§	38** (258)
Prostatitis	5	10	12¶	62'' (639)

*Diets identified according to the proportion of the casein component.

†Average casein content of the composite diet selected throughout the greater part of life, between 27-28%.

‡Number of rats: 125 in the S-S group, 250 in each group provided one diet only.

§P, < 10⁻³. ¶P, < 0.01; disease frequency for D₅₁ significantly different from D₁₀. Absence of superscript indicates difference between groups, N. S. Fisher's exact probability test applied to empirical data. χ² test of association among the 3 groups significant for all grades and for moderate and severe cases of glomerulonephrosis and myocardial fibrosis (P, < 10⁻³; < 10⁻⁶; < 10⁻⁶; < 10⁻⁴, respectively).

**P, < 10⁻⁶; **P, < 0.003; S-S frequency significantly higher than that of the fixed dietary group with the highest frequency.

††Relative morbidity ratio (x 100), all ages. Values of 100 assigned to the fixed dietary group with the highest frequency. Values in the S-S group > 100 indicate extent of increase in risk relative to that of the fixed dietary group. Morbidity ratio = c_x / \bar{c}_x , where c_x = number of cases observed in the S-S group and \bar{c}_x = expected number of cases: $\bar{c}_x = (\bar{c}_{x1} \times l_{x1}) + (\bar{c}_{x2} \times l_{x2}) + \bar{c}_{x3} \times l_{x3}$, . . . where $\bar{c}_{x1,2,3, \dots}$ = 'standard' age-specific morbidity rates, consecutive 100 d periods and $l_{x1,2,3, \dots}$ = exposure (that is, number of living S-S rats entering an age period) consecutive 100 d periods. For each disease class, age-specific morbidity rates of the fixed dietary group with the highest frequency used as standard rates.

The increase in the prevalence of these diseases for the S-S rats cannot be explained on this basis as the protein/carbohydrate ratio of the diet selected by any of the S-S rats had to fall within the upper and lower limits of the three fixed diets.

The S-S rats grew more rapidly and attained higher body weights than rats fed the diets separately. We had shown^{23,24} that among conventionally fed rats those which grew more rapidly and attained higher body weights during maturity had a higher risk of tumours than those with lower rates of growth and lower maximum body weights. Body weight differences, however, do not explain the marked increase in disease proneness of the S-S rats. For this analysis, each of the S-S rats was weight-paired with individuals from the D₂₂ group. There was,

consistently, a greater number of tumour bearing rats in the S-S group than among conventionally fed rats. For example, on the basis of the body weight at 98 d old, 60% of the S-S rats but only 23% of rats of corresponding body weights from the D₂₂ group subsequently developed tumours. When maximum body weights were matched, 58 and 33% of the rats maintained on the S-S and D₂₂ regimens, respectively, developed tumours.

No two S-S rats exhibited the same dietary preferences. After an initial rapid increase in the amount of food consumed, the daily intake by each rat remained relatively uniform from 11 to as much as 49 weeks. By 1 y of age, 90% of the rats showed a

Table 2 Dietary preference and disease frequency

	Dietary preference *†			Food intake (g)§		
	Casein content of diet (%)‡			Inter-		
	Low	mediate	High	Low	mediate	High
	Disease frequency (%)					
Tumour-bearing rats	67	70	54	60	63	68
Benign tumours	75	90	73	75	85	78
Malignant tumours	20	15	10	13	13	20
Epithelial tumours¶	78	90**	59	70	78	78
all Adenomas	58	58	46	48	55	59
Endocrine tumours¶	55	58	42	48	53	54
all Anterior pituitary	40	33	29	28	35	39
Pancreatic	13	13	10	18	10	7
Glomerulonephrosis	88	98	95	90	95	95
Myocardial fibrosis	60	73	76	65	70	73
Prostatitis	60	58	76	68	60	66

*†Rats classed according to dietary choice during 200–300 d of age.

†Number of rats in each subclass was 40 or 41 (4 rats died before 100 d).

‡Class limits: low = 12.0–24.8%; intermediate = 25.2–30.3%; high = 30.4–43.0%.

§Class limits: low = 16.1–20.2 g; intermediate = 20.3–21.9 g; high = 22.0–26.8 g.

¶Among rats maintained on fixed diets, the highest frequency was: all epithelial tumours, 27%; adenomas, 20%; all endocrine tumours, 20%; anterior pituitary tumours, 10%; pancreatic tumours, 6%. Additional values given in Table 1.

"All grades of severity.

**Frequency of epithelial tumours of the intermediate dietary subclass significantly higher than that of the high-protein subclass ($P < 0.02$). Absence of superscript indicates no significant difference between subclasses. (Fisher's exact probability test applied to empirical data). χ^2 test of association among the three subclasses also not significant.

marked and progressive increase in food intake. Before death, only a few animals failed to decrease their intake of food. The level of intake during each of these phases differed widely from rat to rat.

Within the first 5 weeks of feeding, most of the rats established the level of protein and carbohydrate that each would continue to select for a prolonged period. Despite variation in relative preference for the three diets or in the amount of food consumed, the protein: carbohydrate ratio of the resulting composite diet was maintained by some rats for as long as 700 d.

The associations between disease susceptibility and dietary preferences were evaluated by separate rankings of the S-S rats according to the protein content of the composite diet selected and the amount of food consumed. The tumour data are limited to several tumour types, as the numerous tumour classifications that should be made cannot be adequately treated in a short communication.

The data shown in Table 2 for the S-S rats are, in general, representative of the relationships seen at earlier and successive 100 d age periods until 600 d of age was attained. Beyond this time, the progressive decrease in number of animals precluded

further analyses; that is, of the number of rats started in the experiment, 45 and 75% had died by 600 and 700 d, respectively.

The prevalence of the four diseases among the S-S rats, classed according to the protein content of the diet, was almost without exception greater at any level of protein than those of rats maintained on any of the fixed regimens. In addition, whereas the incidences of the renal, myocardial, and prostate gland diseases for the rats on the fixed diets (Table 1) are strongly dependent on the proportion of the protein component of the diet, among the S-S rats the protein dependencies were weak or nonexistent (Table 2). Irrespective of the protein content of the diet the frequency of several types of tumours tended to increase slightly among animals whose food intake was higher. The mean length of life, however, tended to decrease 690 ± 124 , 622 ± 123 , and 580 ± 104 d, respectively). Even so, the differences in the incidences for the neoplastic and non-neoplastic diseases among the subgroups were not significant. In contrast, with fixed regimens the trends for most tumours were consistently striking and statistically significant²⁴.

Unless the members of an experimental population are highly inbred or subjected to extraordinary environmental experiences, not all of the individuals will develop any of the common age-related diseases. In conventional *ad libitum* feeding practices, changes in the prevalence and severity of the observed idiopathic diseases could be taken to represent a dose-response relationship. In S-S conditions, the diet chosen differs from animal to animal and yet equivalent or nearly equivalent effects are exerted by any one diet on disease susceptibility. The dose-response interpretation cannot be applied to the free choice situation. Rather, the impressive increase in the risk of developing tumours or of other diseases of age must be viewed as the direct consequence of the specific selections made by the individual in satisfying its unique metabolic 'needs'.

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- McCay, C. M., Crowell, M. F., and Maynard, L. A., *J. Nutr.*, **10**, 63–79 (1935).
- Tannenbaum, A., in *Approaches to Tumor Chemotherapy*, (edit. by Moulton, F. M.), 96–127 (American Association Advances Science, 1947).
- Silberberg, M. and Silberberg, R., *Physiol. Rev.*, **35**, 347–362 (1955).
- Ross, M. H., *Fedn Proc.*, **18**, 1190–1207 (1959).
- Berg, B. N., and Simms, H. S., *J. Nutr.*, **71**, 255–263 (1960).
- White, F. R., *Cancer Res.*, **21**, 281–290 (1961).
- Bras, G., and Ross, M. H., *Toxicol. appl. Pharmacol.*, **6**, 247–262 (1964).
- Ross, M. H., and Bras, G., *J. Nutr.*, **103**, 944–963 (1973).
- Richter, C. P., Holt, L. E., jun., and Barelare, B., jun., *Am. J. Physiol.*, **122**, 734–744 (1938).
- Mayer, J., Dickie, M. M., Bates, M. W., and Vitale, J. J., *Science*, **113**, 745–746 (1951).
- Valenstein, E. S., Kakolewski, J. W., and Cox, V. C., *Science*, **156**, 942–943 (1967).

- ¹² Thiel, A., Schemmel, R., Mickelsen, O., Johnson, J. T., and Gill, J. L., *Nutr. Rep. Int.*, **5**, 101–110 (1972).
- ¹³ Richter, C. P., *Am. J. Physiol.*, **115**, 155–161 (1936).
- ¹⁴ Richter, C. P., and Eckert, J. F., *Am. J. Med. Sci.*, **198**, 9–16 (1939).
- ¹⁵ Richter, C. P., and Schmidt, E. C. H., jun., *Endocrinology*, **28**, 179–192 (1941).
- ¹⁶ Lat, J., in *Handbook of Physiology*: Section 6, **1**, (edit. by Code, C. F. and Heidel, W.), 367–386 (American Physiological Society, Washington, 1967).
- ¹⁷ Soulairac, A., in *Handbook of Physiology*: Section 6: **1**, (edit. by Code, C. F. and Heidel, W.), 387–398 (American Physiological Society, Washington, 1967).
- ¹⁸ Griffiths, W. J., jun., *Ann. N. Y. Acad. Sci.*, **67**, 1–10 (1956).
- ¹⁹ Richter, C. P., and Barellare, B., jun., *Endocrinology*, **23**, 15–24 (1938).
- ²⁰ Richter, C. P., *Am. J. Physiol.*, **135**, 781–787 (1942).
- ²¹ Donohoffer, S., *Triangle*, **4**, 233–239 (1960).
- ²² Jacobs, H. L., *J. comp. Physiol. Psychol.*, **51**, 304–310 (1958).
- ²³ Ross, M. H., Bras, G., and Ragbeer, M. S., *J. Nutr.*, **100**, 177–189 (1970).
- ²⁴ Ross, M. H., and Bras, G., *J. Nutr.*, **87**, 245–260 (1965); *J. natn. Cancer Inst.*, **47**, 1095–1113 (1971).

Evidence for the inhibition hypothesis in expanded angle illusion

THE illusory expansion of acute angles has long been suspected to be an important contributory factor in a number of well known illusions, such as the Zollner, the Wundt-Hering and the Poggendorf^{1,2}. Recently Blakemore, Carpenter and Georgeson³ proposed that angle expansion is a side effect of an inhibitory process that improves the orientation resolution of the visual system. Neural line and edge detectors considered in isolation, are assumed to respond to a wide range of orientations, but when incorporated into a functional system inhibitory interactions occur between them which sharpen the specification of a line or edge⁴. When two spatially contiguous lines of neighbouring orientations are exposed simultaneously, however, the activity peaks in the population of orientation detectors are shifted away from each other because of the inhibitory interactions. Consequently, the orientations of the lines comprising the angle are perceived wrongly. This process of central lateral inhibition is thought to be similar to that which operates in peripheral sensory structures⁵. It seems natural to ask how far this similarity extends.

Hartline and associates⁶ have described the following major features of inhibition in the compound eye of *Limulus*⁶. First, the distance effect—as the test and inducing stimuli are moved further apart the effectiveness of the inhibition decreases. In the orientation domain experimental evidence indicates that following an initial rapid build up, the illusion diminishes as the angular separation increases^{3,7}. Second, the area effect—the larger the number of neighbouring facets illuminated, the greater the inhibition of the test ommatidium. Extrapolated to the visual cortex, one might expect the induced change in orientation of a test line will be proportional to the number of parallel lines of neighbouring orientation exposed in its vicinity. Experimental data provided by Ogasawara⁸ and Wallace and Crampin⁹ show the larger the number of parallel lines that cross a contour, the greater its angular distortion. If the inducing lines are not parallel then inhibitory interactions will occur between them thus ‘disinhibiting’ their potential combined action on the test line. Evidence that disinhibition does occur in the case of interactions between orientations has been provided by Carpenter and Blakemore⁷. Third, the intensity effect—for a given distance between inducing and test omma-

tidia, the more intense the illumination falling on the inducing facet the more intense the inhibition of the test ommatidium. Where two facets are unequally illuminated, the inhibitory interaction increases the differences in their outputs. Extrapolated to the case of interactions between orientations, these effects suggest the following predictions. If two lines of neighbouring orientations are unequally illuminated, then the angular distortion of the two lines will be unequal because proportionally more inhibition, and therefore more angular distortion, of the dimmer line by the brighter will occur than *vice versa*. Where the luminance of both lines is equal, little change would be expected in the magnitude of the mutual distortion as the absolute luminance is varied over a fairly wide range, as the inhibition should remain proportionately similar. Because verification of these predictions would constitute valuable support for the theory it was decided to test them specifically.

The subject, his chin supported by a rest, looked at the display through a 4 mm artificial pupil with his right eye. The display consisted of a pair of lines, each subtending $2.0^\circ \times 0.17^\circ$, forming an acute angle, whose luminance could be varied independently, or in unison, with neutral density filters. The illusion size was measured in the same way as that employed by Blakemore *et al.*³. A small comparison line, subtending $1.2^\circ \times 0.1^\circ$, was positioned 2.0° from the base line (*B*) of the angle (Fig. 1) by means of a half-silvered mirror. By moving a lever, which rotated a Dove prism, the orientation of the comparison line was adjusted to appear parallel to *B*. The maximum luminance of the angle lines was 8.5 cd m^{-2} and the comparison line was constant at 2.0 cd m^{-2} . Before each estimate of the orientation of *B*, the comparison line was offset $30\text{--}40^\circ$. The subject placed his head on the rest and the room lighting was turned off. The subject adjusted the comparison line, usually in 5–6 s, then the room lighting was turned on and the reading

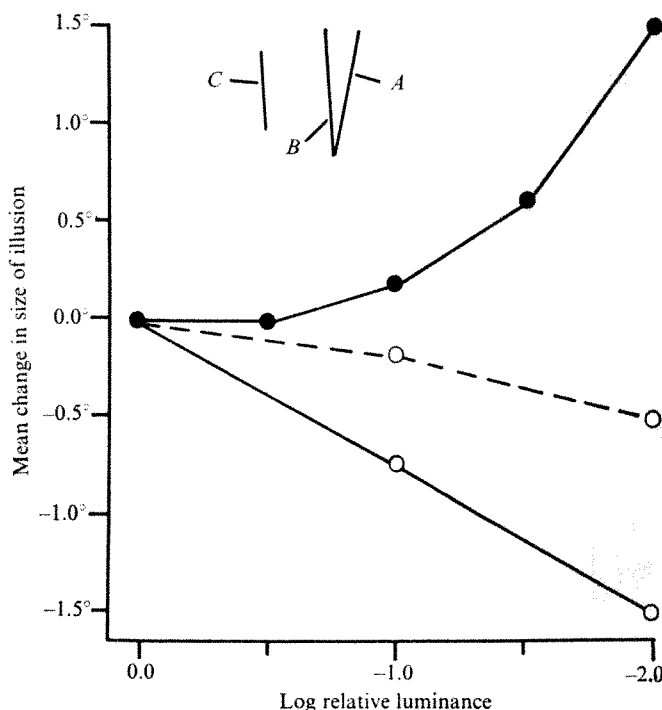


Fig. 1 The inset shows the arrangement of lines used in the present study. *A*, angle line; *B*, base line; *C*, comparison line. Angle of *A* and *B* = 15° . ●—●, *A* constant, luminance of *B* decreased. ○—○, Luminance of *A* and *B* decreased concomitantly. ○—○, *B* constant, luminance of *A* decreased. In all cases illusion magnitude was calculated as orientation of *B* in absence of *A* minus orientation of *B* in presence of *A*.

noted. Each of five subjects made seven estimates of the orientation of *B* under each luminance condition for the three experiments (15 subjects in all). The order in which the estimates were made was changed for each subject.

The results presented in Fig. 1 are for the case where the orientation of *B* with respect to the vertical was 5° anticlockwise, and *A* was positioned 10° clockwise. The upper curve in Fig. 1 shows the progressive increase in the induced tilt of line *B* as it is made dimmer than *A*, indicative of a greater inhibitory effect of *A* on *B*. Analysis of variance on the data showed that this change was significant ($P < 0.01$). The lower curve in Fig. 1 plots the significant reduction ($P < 0.01$) in the induced tilt effect as the luminance of *B* is held constant and that of *A* reduced, indicative of a reduced effect of *A* on *B* as *A* becomes dimmer. The middle curve shows the nonsignificant change in illusion size ($F = 0.735$, d.f. = 2, 8), as the brightness of both *A* and *B* was varied concomitantly over two log units. These results show that the size of the induced tilt effect varies systematically as a function of the relative, but not the absolute luminance of the test and inducing lines. The effect is not confined to this particular orientation of *A* and *B*, nor is it a function of the particular angle used here (15°). The effect is also obtainable when a subjective vertical criterion is used to estimate the orientation of *B*, so the results cannot be due to any interaction with the comparison line.

It is known that the relative luminance of spatially adjacent areas affects their perceived brightness¹⁰. These results show that the relative luminance of orientationally adjacent contours affects their perceived orientation and offer evidence in favour of the hypothesis that angle expansion is the result of an inhibitory process. While the results are similar to a relative contrast phenomenon recently demonstrated for the tilt after effect (TAE)¹¹, the explanation of the present results in terms of adaptation must be rejected for other reasons. The angle expansion illusion is known to be spatial frequency specific¹² while the TAE is not^{11,13}. The TAE also shows an 'indirect effect' when the adapting stimulus is in the region of 90° from the test stimulus¹³, which is not true of the illusion.

The similarities in the operation of inhibition processes in simple visual systems and the interactions between lines indicate that lateral inhibition may be a more general phenomenon than is commonly realised and suggests that certain illusions which involve neighbouring orientations may be explained without the theoretical involvement of constancy mechanisms¹⁴.

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- ¹ Helmholtz, H. von, *Treatise on Physiological Optics*, 111 (Dover, New York, 1962).
- ² Robinson, J. O., *The Psychology of visual illusion* (Hutchinson, London, 1972).
- ³ Blakemore, C., Carpenter, R. H. S., and Georgeson, M. A., *Nature*, **228**, 37 (1970).
- ⁴ Andrews, D. P., *Nature*, **205**, 1218 (1965).
- ⁵ Hartline, H. E., Wagner, H. G., and Ratcliff, F., *J. gen. Physiol.*, **39**, 651 (1956).
- ⁶ Ganz, L., *Psychol. Rev.*, **73**, 128 (1966).
- ⁷ Carpenter, R. H. S., and Blakemore, C., *Expl Brain Res.*, **18**, 287 (1973).
- ⁸ Oyama, T., *Psychologia*, **3**, 7 (1960).
- ⁹ Wallace, G. K., and Crampin, A. J., *Vision Res.*, **167** (1969).
- ¹⁰ Diamond, A. L., *J. exp. Psychol.*, **45**, 304 (1953).
- ¹¹ Parker, D. M., Q., *J. exp. Psychol.*, **24**, 1 (1972).
- ¹² Georgeson, M. A., *Nature*, **245**, 43 (1973).
- ¹³ Campbell, F. W., and Maffei, L., *Vision Res.*, **11**, 833 (1971).
- ¹⁴ Day, R. H., *Science*, **175**, 1335 (1972).

Form-specific colour after effects in scotopic illumination

THE retina of the human eye contains rod and cone receptors, and in very dim light the rods alone function (scotopic illumination) so that objects appear colourless. At higher light levels, cones also function (photopic illumination) and colours are seen.

There is evidence that rods interact only with rods and that the various colour mechanisms, the Stiles π mechanisms, act independently¹⁻⁵. These events probably occur early in the retina, for the various channels interact even within the retina⁶, and the later stages of the visual system show a strong opponent colour organisation. Other evidence suggests that rod and cone signals interact; in these studies colour sensations are elicited through rods. For example, if one first views a coloured flash that affects cones, a complementary coloured afterimage is seen upon viewing a dim flash that affects only rods⁷. The rods seem to trigger this after image⁸, for its hue remains constant as the wavelength of the dim test flash is varied, and the light level sufficient to trigger the after image coincides with the dark adaptation curve of the rods. Simultaneous contrast colours of blue and green are seen in a bipartite field when one half is illuminated with dim light, that stimulates only rods and the other half is illuminated with long wavelength light that stimulates cones⁹⁻¹⁰. Similarly, many colours are seen in a Land two-colour projection produced with two illuminants that stimulate differently long wavelength cones and rods¹¹. These studies suggest that rod and cone signals converge at some level of the visual system.

I have found that form-specific colour after effects—the McCollough effect—can be seen on test patterns observed in dim light that stimulates the rods but not the cones. This shows that rod signals feed into colour mechanisms. McCollough¹² discovered that after adapting for several minutes to a vertical grating in orange light alternating with a horizontal grating in blue light, black and white test gratings with retinal orientation similar to the adapting patterns were tinged with colours complementary to the adapting colours. For example, the vertical test grating appeared blue and the horizontal orange.

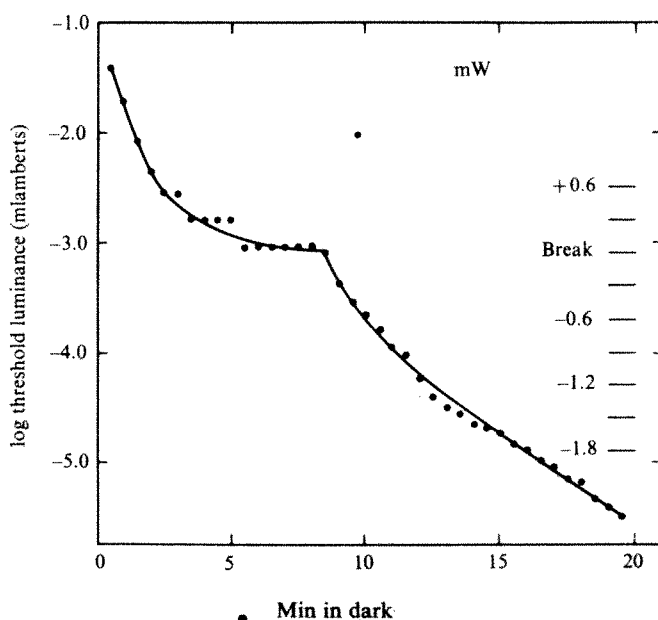


Fig. 1 Dark adaptation curve for one observer. The bars on the right indicate various luminance values for white bars of test patterns.

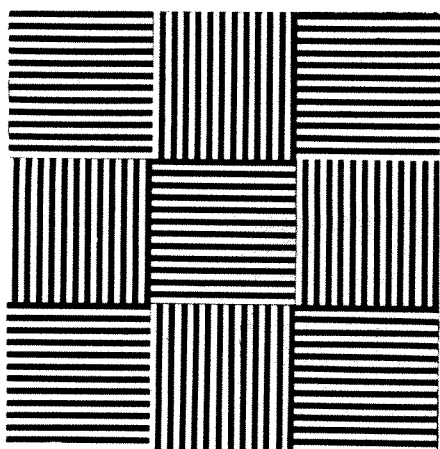


Fig. 2 Test pattern. Gratings had spatial frequency of 0.75 cycles per degree.

Of the three observers I used, two were highly experienced with the after effect and one was uninformed about the experiment. Dark adaptation curves were first measured on the two experienced observers. After strong light adaptation, the observer fixated a tiny red light in the centre of a 10° diameter disk of white paper on a black background and adjusted a neutral wedge, which controlled the illumination level, so that the disk remained just below threshold. These materials had the same reflectance as the test patterns used later. The light came from a regulated projector with accessory lenses and was filtered through a narrow-band yellow-green filter (Wratten 74, dom λ 538nm). The curve for one observer is shown in Fig. 1. The vertical axis represents the luminance of the white disk. The curve for the other observer was very similar. The level of the rod-cone break agrees with the value measured by Chapanis¹³ for a green spot 3° in diameter, 7° from the fovea, which was flashed on for 0.2 s. It was also noticed, subsequently, that the appearance of the test patterns (Figs. 2 and 3) changed markedly near the break: the bars of the gratings appeared sharp just above the break and diffuse just below the break. Rods alone presumably function below the break, for the break is generally assumed to indicate cone threshold.

Colour after effects were next built up with high-contrast square-wave gratings of 0.75 cycles per degree, rear-projected on a diffusing screen. Gratings subtended an area about 30° square. A tiny light was placed in the centre of the screen. Gratings were projected in alternate vertical and horizontal orientations and interchanged every second. On each presentation the gratings were projected in the same position or shifted 180° in phase by mirrors. The shift was determined randomly. The observer stared at the light to help adapt the eyes evenly. The observer adapted for 20 min to the alternating vertical grating in green light (Wratten 40 filter) and the horizontal grating in complementary magenta light (Wratten 31 filter). Mean luminance was about 70 m lamberts. The observer next dark adapted for 30 min and viewed the test pattern (Fig. 2) illuminated with the light used to measure the dark adaptation curves. The luminance of the white areas of the test gratings was set at values indicated by the bars in Fig. 1, starting at the lowest level and moving upward. The spatial frequency of the test pattern was the same as the adapting patterns, 0.75 cycles per degree. The pattern was about 48° square.

The vertical and horizontal gratings of the test pattern appeared faintly pinkish and greenish, respectively, to all observers at the lowest light level, -1.8 log units below the rod-cone break. The colour became more saturated

as the light was increased. The naive observer later matched from memory the strongest scotopic after effects to Munsell colour chips viewed in Macbeth daylight=6750 K. The colours chosen were 10 RP 7/8 and 10 GY 7/8. All observers claimed that the scotopic colours were quite saturated.

The McCollough effect is somewhat specific to the spatial frequency of the adapting pattern¹⁴. The present experiment demonstrates that the colour effect stays tied to the adapting frequency even for test patterns in scotopic light. The previous experiment was essentially repeated using two vertical adapting gratings of 0.5 and 2.0 cycles per degree. The observer again viewed the fixation light in the centre of the adapting field. The patterns were about 30° square and centred on the fixation light. The 0.5 cycles per degree pattern alone was projected in the same position or shifted 180° in phase, as before; the 2.0 cycles per degree pattern was always projected in the same position. Observers adapted for 40–60 min to one grating in green light, alternating with the second grating in magenta light. For some observers, the two gratings were shown for unequal amounts of time to build up good pink and green after-effects. (Low frequency grating were used, because below the rod-cone break gratings above 5 cycles per degree cannot be resolved¹⁵.) After colour adaptation, the test pattern (Fig. 3) was viewed in Macbeth daylight and then, after dark adaptation, in scotopic light at levels from -1.0 to -0.3 log units below the rod-cone break. The pattern was about 15° wide and 30° high. The test gratings appeared appropriately coloured. For example, adaptation to 0.5 and 2.0 cycles per degree gratings in magenta and green light, respectively, produced a green after effect on the 0.5 cycles per degree test grating and a pink after effect on the 2.0 cycles per degree grating. All observers claimed that the colours were more saturated at scotopic than photopic levels.

That the after effect stays tied to the adapting spatial frequency suggests that the receptive field of the mechanism underlying these effects does not lose its antagonistic surround at scotopic levels. Loss of the surround would cause pattern of low spatial frequency to elicit both the pink and green after effects, and no colour would be seen since the two colours are complementary and would thus cancel. Adaptation to luminance gratings at low mesopic levels has been shown to raise the threshold only for grat-

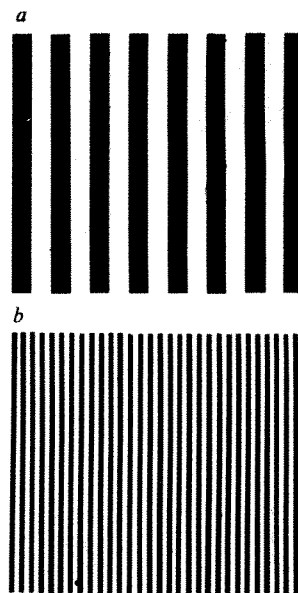


Fig. 3 Test pattern. The spatial frequencies of the test gratings were 0.5 cycles per degree (a) and 2 cycles per degree (b).

ings of similar spatial frequencies¹⁶. My results suggest that adaptation is frequency-selective at even lower scotopic levels. The existence of lateral inhibition at scotopic levels is further suggested by studies on simultaneous brightness contrast¹⁷, Mach bands¹⁸, and ganglion cells in Rhesus monkey¹⁹.

My results show that rod signals influence colour mechanisms. Rod and cone signals converge onto single ganglion cells in the monkey²⁰, and many ganglion cells have a receptive field that receives an excitatory signal from either red, green or blue cones in the centre and an inhibitory signal from a different cone type in the periphery²¹. Similar mechanisms are found in the lateral geniculate nucleus (LGN), and many of these units undergo a Purkinje shift when dark adapted, thus showing a rod input²². Ganglion and LGN cells have circular receptive fields; however, many units in the striate cortex are sensitive to line orientation and colour²³. The cortical units have receptive fields whose excitatory and inhibitory areas receive inputs from the same cone type²⁴, and thus they are highly sensitive to the spatial properties of a red or green pattern—unlike the precortical cells. Adaptation to a coloured grating might thus depress sensitivity in cortical cells tuned to the adapting colour, orientation and spatial frequency. A test pattern of the same orientation and spatial frequency would then produce less response in these units, and this imbalance may signal the complementary colour. Rod signals may also converge onto these mechanisms, and thus the colour effect may be seen at scotopic levels.

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- ¹ Stiles, W. S., *Documenta. ophth.*, **3**, 138 (1949).
- ² Stiles, W. S., *Proc. natn. Acad. Sci. U.S.A.*, **45**, 100 (1959).
- ³ Alpern, M., *J. Physiol. Lond.*, **176**, 462 (1965).
- ⁴ Alpern, M., and Rushton, W. A. H., *J. Physiol. Lond.*, **176**, 473 (1965).
- ⁵ McKee, S., and Westheimer, G., *J. Physiol. Lond.*, **206**, 117 (1970).
- ⁶ Gouras, P., *J. Physiol. Lond.*, **199**, 533 (1968).
- ⁷ Stabell, U., and Stabell, B., *Scand. J. Psychol.*, **6**, 195 (1965).
- ⁸ Stabell, B., and Stabell, U., *Scand. J. Psychol.*, **9**, 129 (1968).
- ⁹ Willmer, E. N., *P. Physiol. Lond.*, **111**, 17P (1950).
- ¹⁰ Stabell, U., and Stabell, B., *Scand. J. Psychol.*, **9**, 133 (1968).
- ¹¹ McCann, J. J., *Science*, **176**, 1255 (1972).
- ¹² McCollough, C., *Science*, **149**, 1115 (1965).
- ¹³ Chapanis, A., *J. gen. Physiol.*, **30**, 423 (1947).
- ¹⁴ Stromeier, C. F., III, *Vision Res.*, **12**, 717 (1972).
- ¹⁵ Shlaer, S., *J. gen. Physiol.*, **21**, 165 (1937).
- ¹⁶ Graham, N., *Vision Res.*, **12**, 53 (1972).
- ¹⁷ Hollins, M., *Vision Res.*, **11**, 1459 (1971).
- ¹⁸ Fiorentini, A., and Maffei, L., *Vision Res.*, **13**, 73 (1973).
- ¹⁹ Gouras, P., *J. Physiol. Lond.*, **192**, 747 (1967).
- ²⁰ Gouras, P., and Link, K., *J. Physiol. Lond.*, **184**, 499 (1966).
- ²¹ Gouras, P., *J. Physiol. Lond.*, **199**, 533 (1968).
- ²² Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, **29**, 1115 (1966).
- ²³ Dow, B. M., and Gouras, P., *J. Neurophysiol.*, **36**, 79 (1973).
- ²⁴ Gouras, P., *P. Physiol. Lond.*, **238**, 583 (1974).

Infantile obesity and later weight control in the baboon

RECENTLY widely quoted studies imply that overfeeding in infancy is associated with increased formation of adipose cells and predisposes to obesity later in life. This has been documented for newborn rats in whom the high adipose cell count persisted after overfeeding was discontinued¹. In rats which had been fed sparsely as newborns for up to 21 d, the fat cell count did not increase when they were fed more generously later. The establishment of regulatory controls over food intake has also been related to early experiences.

Rhesus monkeys raised in isolation and then given access to unlimited food were found to overeat to the same degree as monkeys with hypothalamic lesions². Clinical observations suggest that the inability of some obese people to control their food intake is related to deficits in early learning experiences, with inability to differentiate between nutritional need and other signs of discomfort³. Experimental observations have confirmed that obese and normal subjects differ in their eating responses to internal and external cues⁴.

We report here evidence which casts doubt on the prevailing opinion. Using a group of baboons (*Papio* sp.) raised in the laboratory, some of whom had been fat as infants, we have been able to evaluate the relative importance for later weight regulation of excessive fat formation in infancy and of defects in the regulatory mechanisms. No adipose cell count was done during the period of infantile obesity.

The baboons were delivered by Caesarean section within a 6-week period, with birth weights ranging from 0.65 to 1.1 kg. They were fed a mixture simulating the composition of natural baboon milk, which was given by bottle at scheduled intervals in measured amounts to insure normal growth and weights⁵. This liquid diet was composed of a commercial human infant formula, SMA/S-26 (Wyeth), water and corn oil in the ratio of 90:80:1. At the end of the first month some infants were put on a self-feeding device, permitting *ad libitum* feeding; these animals gained excessive weight and became visibly fat⁶. After 7 to 8 months all animals were weaned gradually on to solid food, a commercial monkey biscuit which was approximately

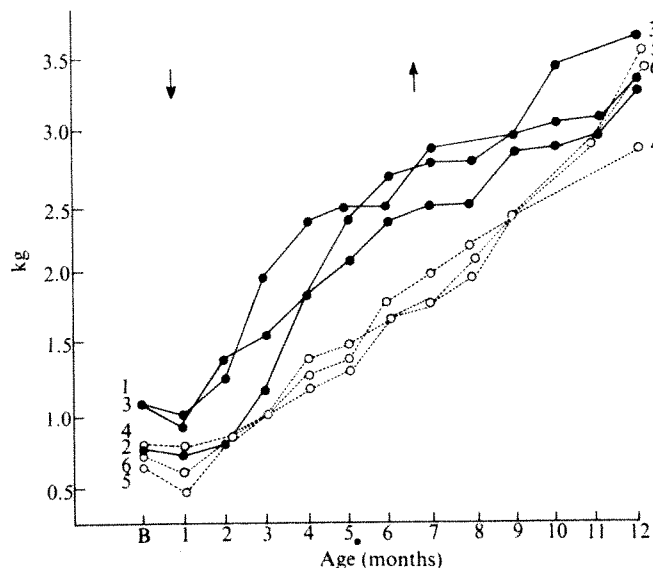


Fig. 1 Weight of baboons during infancy. The heavy lines represent three animals on a self-feeding device; the onset is indicated by the first arrow (↓). The dotted lines show the weights of three animals on measured feeding. The second arrow (↑) indicates onset of gradual weaning of all animals to solid food.

25% protein, 5% fat, 4.5% fibre and 3% mineral. The weight gain of the fat infant baboons slowed down. All animals were weighed regularly every month.

From birth until 1 month of age these animals were housed in stainless steel boxes and were then transferred to conventional stainless steel primate cages. They were kept singly but could hear and see each other in rooms which housed several baboons. They were cared for by a minimum number (two or three) of animal care personnel which remained the same during the whole period.

For our experiment we used approximately 2.5-yr-old animals (pre-puberty): three had formerly been obese, and three, which were of equal age and had not been fat as infants, served as controls. Figure 1 shows the patterns of weight gain in these

Table 1 Weekly changes in weight on *ad libitum* feeding

No.	June 3	July 6	13	20	27	August 3	10	17	24	September 7	14	21	28	October 5	12	19	26	Nov. 2	Dec. 14
<i>Formerly fat baboons (self-feeding in infancy)</i>																			
1	7.5	7.5	+7	+1	+1	0	+3	0	+1	+2	-1	+4	+1	9.2	-7	-1	0	-1	+2
														(+22%)					
2	6.7	6.6	+4	+4	-2	+1	+1	-1	-1	+4	+2	-1	+3	-1	7.9	-6	0	-1	+1
														(+20%)					
3	7.7	7.7	+6	0	+1	0	+2	0	+1	+3	+2	+1	+2	0	9.5	-6	-1	0	+1
														(+23%)					
<i>Experimental controls (measured feeding in infancy)</i>																			
4	6.9	7.0	+6	0	0	+2	0	0	+1	+1	+2	0	+3	0	8.5	-6	-1	-2	+1
														(+21%)					
5	7.6	7.6	+7	+1	-1	+2	+3	-3	+2	+4	-3	+1	+1	0	9.2	-5	-4	+1	-1
														(+21%)					
6	6.5	7.1	+3	-2	0	+4	0	-2	+2	0	0	+1	+2	0	7.9	-3	0	+1	+2
														(+12%)					

Figures are kg. *Ad libitum* feeding began on July 6 and measured feeding recommenced on October 5.

six animals during infancy. Animals on the self-feeding device had a slightly higher birth weight, 0.9 kg as average, compared with an average of 0.73 kg for the others. On the self-feeding device weight increased at an excessive rate, and reached 110% of birth weight at the end of the fourth month and 190% at the end of 6 months. The corresponding increases for the animals on measured feeding were 75% and 130% respectively. At 12 months, the weights of all animals were in the same range.

Our experiment consisted of offering an inexhaustable food supply by keeping the food containers filled with monkey biscuits at all times. Two to three times as much food was consumed by the experimental animals than by those on measured laboratory feeding. Because of the wasteful feeding habits of baboons, it was not possible to measure the exact amounts eaten. The rapid gain in weight reflects the greatly increased food intake. Animals were weighed weekly (Table 1). Total body weights during and following the experimental period are shown in Fig. 2. The overall weight gains during the 3 months of the experiment were very similar for five animals—20 to 23% of the starting weight. One animal (No. 6) who had gained weight rapidly during the preceding month,

gained only 12%. Three other baboons of similar ages who continued on the measured laboratory feeding, showed only 4% weight gain during this period.

When excess feeding was discontinued and the animals were again given measured amounts, there was rapid loss of weight during the first week and gradual loss of weight and then stabilisation during the next few months; patterns were similar for all animals.

We found that with access to an unlimited food supply, the formerly fat and nonfat animals reacted in the same way. They all ate excessively and gained weight at a similar rate. Although the immediate control over food intake seemed deficient, as had been described for monkeys raised in isolation², there seem to be limiting regulatory mechanisms for what can be assimilated, without recognisable difference in the formerly fat and nonfat animals.

The lack of difference in the rates of gain and loss between the formerly fat and nonfat animals contrasts with expectation according to reports for experimental rats on excess feeding during infancy. In baboons the later rate of weight gain, and partial retention of the excess weight, is for all practical purposes identical in formerly fat and nonfat animals. Perhaps over-feeding was not continued long enough to cause changes that would influence eating patterns and facilitate fat storage later on. This is in agreement with clinical observations. Though it is true that infantile obesity is frequently followed by life-long obesity, this occurs only when the conditions that make for over eating and obesity persist⁷.

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- Hirsch, J., and Han, P. W., *J. Lipid Res.*, **10**, 77-82 (1969).
- Miller, R. E., Mirsky, I. A., Caul, W. E., and Sakata, T., *Science*, **165**, 1027-1028 (1969).
- Bruch, H., *Psychiatric Quart.*, **35**, 458-481 (1961).
- Schachter, S., *Science*, **161**, 751-756 (1968).
- Voss, W. R., in *Medical primatology 1970—proceedings of the second conference on experimental medicine and surgery in primates*, New York, 1969, 903-911 (Karger, Basel, 1971).
- Voss, W. R., Buss, D. H., and Carroll, L. W., *Lab. Anim. Sci.*, **21**, 901-903 (1971).
- Bruch, H., in *Eating disorders: obesity, anorexia nervosa and the person within* (Basic Books, New York, 1973; Routledge and Kegan Paul, London, 1974).

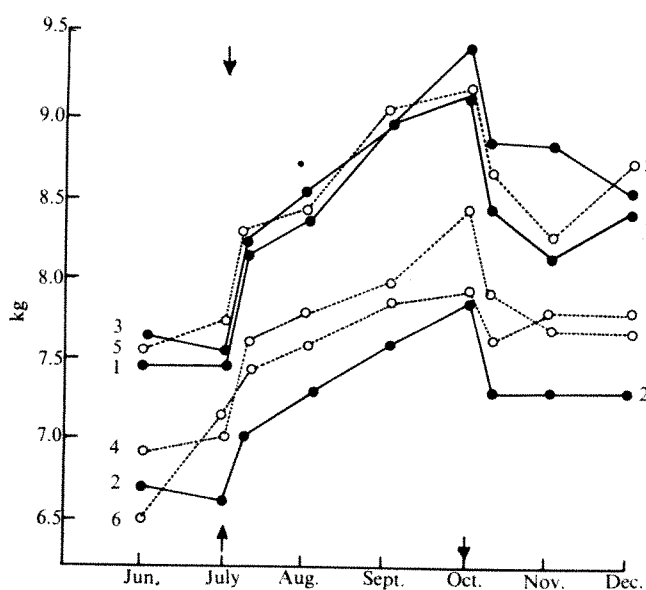


Fig. 2 Weight changes of baboons during *ad libitum* feeding. The heavy lines represent three formerly fat baboons, the dotted lines three animals who had not been fat. The first arrows (↑) indicate onset of unlimited food supply, the second arrow (↓) return to measured feeding for all animals.

Evidence from Lincolnshire of the age and intensity of the mid-Devensian temperate episode

THE study of fossil beetles from several sites has indicated a period of temperate climate in the middle of the Devensian (Weichselian)¹. Upton Warren (Worcestershire), dated at about 42,000 yr BP, yielded an assemblage composed, in part, of thermophilous beetles². At Four Ashes (Staffordshire) deposits of the same age which contain warm species overlie Devensian silts of arctic aspect³. A further warm fauna of this age has been investigated from Isleworth, Middlesex (personal communication from G. R. Coope). New faunal and ¹⁴C evidence from this critical period of mid-glacial climatic change is currently being investigated from Tattershall Castle Pit, Lincolnshire (map ref. TF570210).

At this pit organic horizons of several ages lie in sands and gravels above a till rich in Jurassic and Cretaceous erratics. The lowest organic deposit, a shell marl containing a terrestrial snail fauna characteristic of open conditions, may be of Upper Wolstonian age (personal communication from J. G. Evans). This is overlain by compact woody peat assigned on pollen evidence to subzone Ip IIb of the Ipswichian interglacial (personal communication from L. Phillips). An extensive beetle fauna extracted from this peat includes non-British beetles such as *Brachytemnus submuricatus* Schön. and *Valgus hemipterus* L. previously recorded from interglacial deposits^{4,5} and *Isorhipis melasoides* Lap. and *Pycnomerus terebrans* Ol., species noted in Postglacial deposits^{6,7} but now extinct in Britain⁸. Wood-boring genera such as Cerambycidae and Scolytidae are well represented and species of Anobiidae include *Xestobium rufovillosum* (Deg.), *Anobium fulvicorne* Sturm and *A. punctatum* (Deg.). Examples of other tree-dependent species, *Melasis buprestoides* (L.), *Dryophthorus corticalis* Pk., the oak leaf-miner *Rhynchaenus quercus* (L.) and the predators *Colydium elongatum* (F.) and *Dasytes plumbeus* (Müll.), demonstrate the presence of mature, mainly deciduous forest. Other environmental factors—rich humic soils, areas of marshy vegetation, influent streams and in particular, warmer summer temperatures than in South England today—are indicated by this interglacial fauna.

Above this peat are two mid-Devensian organic silt horizons. The lower of these, which occurs within frost-disturbed gravels or directly on the peat, has ¹⁴C ages of 42,100 ⁺¹⁴⁰⁰/₋₁₁₀₀ yr B.P. (Birm 309) and 44,300 ⁺¹⁶⁰⁰/₋₁₃₀₀ yr BP (Birm 408), and it has yielded a cold, impoverished fauna. Characteristic foreign species include *Diachila arctica* Gyll., *D. polita* Fald. and *Dyschirius septentrionum* Munst. (Carabidae), *Helophorus obscurus* Popp. and *Ochthebius kaninensis* Popp.⁹ (Hydrophilidae), *Olophrum boreale* Pk., *Boreaphilus henningianus* Sahlb. and *B. nordenskiöldi* Pk. (Staphylinidae). These are now largely confined to areas of high latitude or extreme continentality. Several other species are now restricted to Northern Britain. This fauna indicates tundra conditions, with tracts of sterile soil between low plants and ephemeral ponds.

Stratigraphically above and thus demonstrably younger than this 'arctic' silt is a more continuous horizon which has yielded a remarkably thermophilous fauna. Its ¹⁴C ages of 43,000 ⁺¹⁴⁰⁰/₋₁₁₀₀ yr B.P. (Birm 341) and 42,000 ± 1,000 yr BP (Birm 409), apparently overlapping those of the 'arctic' silt, underline the closeness in age of the two horizons, despite their marked faunal contrast. Noticeable among the several hundred species which make up the warm assemblage are certain non-British beetles such as *Hydrochus flavipennis* Kust. (R. B. Angus, unpublished work) which is today found in Southern Europe and *Aphodius bonvouloiri* Har., now found only in Spain. *Pseudocleonus cinnereus* Schrank and *Chrysolina limbata*

L. are Central and Southern European species, and *Helophorus discrepans* Rey lives in East Europe and at high altitudes in Spain. A high proportion of the species today found in Britain, including *Nebria livida* (L.), *Porcinolus murinus* (F.), *Crypticus quisquilius* (L.) and *Otiorrhynchus ligustici* (L.) occur only in the southern half of the country. Clearly this assemblage requires average July temperatures at least as high as those of South England. Despite the occurrence of certain East European species, there is no evidence for marked continentality and it is unlikely that the total fauna would survive particularly low winter temperatures.

The duration of this warm, mid-glacial episode was short. A cold, continental fauna from deposits of 39,300 ⁺¹³⁵⁰/₋₁₁₆₀ BP (Birm 333) at Queensford, Oxfordshire (G. R. Coope personal communication) indicates that a considerable degree of cooling had occurred by this time. At Tattershall the reduction in the numbers of thermophiles in successively younger samples from this horizon may be the result of falling temperatures. The structure and ecological requirements of this interstadial fauna provides further evidence of the shortness of this climatic oscillation; this may be illustrated by comparison with the earlier interglacial fauna. Both assemblages indicate warm conditions at the time they were deposited, but the mature, deciduous forest habitat of many of the interglacial beetles obviously developed during a long period of climatic stability. Such vegetation maturity is not demanded by the interstadial fauna which is largely composed of species that feed on low plants and grasses, as well as abundant Scarabaeidae associated with the large mammal population which grazed the area. This insect fauna, able to respond rapidly to climatic change by colonising a newly favourable area⁴, lacks any species whose habitats require a long period of development. In particular, tree-dependent species are absent although temperatures were high enough to permit forest growth. This fact, while dispelling any idea that this fauna is interglacial, supports the stratigraphical and radiocarbon evidence of its mid-glacial age.

I thank Dr R. B. Angus, Dr G. R. Coope and Mr P. J. Osborne for help with this project, and Professor F. W. Shotton for reading this manuscript. This research is being undertaken during the tenure of a Natural Environment Research Council grant.

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¹ Coope, G. R., Morgan, Anne, and Osborne, P. J., *Palaeogeogr., Palaeoclimat. Palaeoecol.*, **10**, 87–101 (1971).

² Coope, G. R., Shotton, F. W., and Strachan, L., *Phil. Trans. R. Soc.*, **B244**, 379–421 (1961).

³ Morgan, A., *Boreas*, **2**, 173–212 (1973).

⁴ Coope, G. R., *A. Rev. Ent.*, **15**, 97–120 (1970).

⁵ Shotton, F. W., and Osborne, P. J., *Phil. Trans. R. Soc.*, **B248**, 353–378 (1965).

⁶ Osborne, P. J., *Phil. Trans. R. Soc.*, **B263**, 327–368 (1972).

⁷ Buckland, P. C., and Kenward, H. K., *Nature*, **241**, 405–406 (1973).

⁸ Osborne, P. J., *Int. Congr. Ent. Lond.*, 1964, 456–457 (1965).

⁹ Balfour-Browne, J., *Entomol. Mon. Mag.*, **89**, 23–24 (1948).

book reviews

Second generation environmentalists

Environmental Issues: Population, Pollution and Economics. By Lawrence G. Hines. Pp. ix+339. (Norton: New York, December 1973.) \$9.75 cloth; \$3.25 paper.

Society and the Assessment of Technology: Premises, Concepts, Methodology, Experiments, Areas of Application. By F. Hetman. Pp. 420 (OECD: Paris; HMSO: London, 1973.) 38 francs; £3.36; \$9.50.

NEITHER of these books is an original contribution to knowledge, but they are nevertheless both very useful. They reflect the extent to which environmental concerns and technology assessment have become part of normal policy making and analysis. The typical early literature of this genre had an evangelistic and strong emotional tone, which was often conveyed in the title as well as the text. One thinks, for example, of *Our Plundered Planet*, *Silent Spring* and the *Population Bomb*.

Both of these books belong emphatically to the second generation. Not that they are lacking in concern; Hines in particular makes quite explicit the strength of his attachment to the preservation of natural amenity and wilderness. But both are very much alive to the complexity of the issues and the trade-offs which are inevitably involved. This means that they are rather duller books than the best of the pioneering single-minded first generation advocacy; but as against this they are much more realistic and much more acceptable to administrators.

The scope of Hines's book is wider than that of Hetman's in one sense: its first section is devoted to the world population and resource problem brought into the limelight by the MIT "World Models". His sceptical critique of the MIT work, although much less thorough than that of the Sussex Science Policy Research Unit, comes to similar conclusions. The second part deals with problems of cost/benefit analysis and the third part with policies to combat pollution. Hetman's book does not discuss the "Limits to Growth" problem at all, nor does it discuss specific policies in relation to pollution. It is devoted entirely to a critical review of the various techniques of 'technology assessment' of which 'cost-benefit' is treated as one example.

Although there are important similarities,

the books differ greatly in style and presentation. Hines's book is far better written, and does not suffer from poor translation into the curiously effete and de-humanised English jargon of the international bureaucracies. The Hines book is far better produced and edited, and the diagrams, tables and references are of a standard to which we have become accustomed in the best American college textbooks. The OECD publication by contrast is rather poorly produced and many of the tables are clumsy. Some of them (for example, Tables 15 and 16) are simply long quotations from other sources.

This is a misfortune as Hetman's book has some outstanding merits. It manages to synthesise a great deal of information about the burgeoning technology assessment movement. It is very balanced in its own assessment of the various techniques and sensible in its awareness of their strengths and limitations. It avoids the temptation of staking everything on one particular technique—a fault which bedevils so much of this literature. Finally it recognises the importance of the political process in relation to any technique or combination of them. In its all-round grasp of a very complex problem the book compares very favourably with much of the American technology assessment literature. It is therefore a thousand pities that the book is so badly edited and produced. It is too long, often repetitive and sometimes unreadable. The two greatest faults are first: the inclusion of large chunks of material (especially in chapter 3) which should have been relegated to an appendix or the (otherwise excellent) reference list. Second, the failure to bring the book to life by thorough discussion of some real-life cases such as the third London airport or supersonic transport. Many examples are cited, but the form of citation is long enough to be boring but not thorough enough to give the full flavour and excitement of a real political problem. Perhaps these faults may be partly attributed to the inevitable limitations of publication through an international organisation, but the OECD has sometimes been outstanding in its toleration of lively and critical material (even when it was embarrassing to member governments) and in the quality of its publications.

By contrast, one of the best chapters in the Hines book is on the "Middle Snake River Hydro-electric Project". This makes the essential points far better than any theoretical analysis, since it shows so clearly just how cost-benefit analysis was manipulated in practice to serve particular interest groups, and the real practical difficulties involved in the attempt to put a market valuation on some of the intangibles. Hines is a competent professional economist but he has taken the trouble to learn a good deal about water and air pollution, and he therefore makes a modest success of his ambitious goal to transcend the normal disciplinary boundaries of economics. One must hope that this approach will become a new fashion in economics, either through interdisciplinary teams or by the individual efforts of men like Hines. He explains in a lucid, albeit very elementary manner, the failure of the market mechanism in decisions about the environment, and the inevitability of public responsibility and policy. In this respect, of course, his book is a welcome return to an old fashion: the original classical discipline was political economy.

C. FREEMAN

Tetrahymena

Biology of Tetrahymena. Edited by Alfred M. Elliott. Pp. x+508 (Dowden, Hutchinson and Ross: Stroudsburg, Pennsylvania; Wiley: Chichester, December 1973.) £19.25.

OF all the protozoa *Tetrahymena* is probably the most studied genus. *T. pyriformis* (formerly called *Glaucoma pyriformis*), which occurs in ponds and streams the world over, was first successfully cultivated axenically, that is, in the absence of the other organisms which comprise its normal diet, in 1923 by André Lwoff. By 1951 Kidder and Dewey had succeeded in growing it in a chemically defined, though very complex, medium. Later developments have been the discovery by Elliott and his colleagues of mating types of *Tetrahymena*, following similar work in *Paramecium*, thus opening up the possibilities of doing genetic work, and these have been exploited by Nanney, Allen and others. On technical grounds, however, *Tetrahymena* has turned out to be rather less suited to

from abacus,
through...bubble chamber...
carposporangium...
flying tuck...
pentaerythritol tetranitrate...
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genetic experiments than *Paramecium*, though for biochemical work *Tetrahymena* is far superior. Finally *Tetrahymena* has recently been used for developmental studies, especially of the elaborate surface patterns of basal bodies, cilia, oral apparatus and so on. Thus the organism is now available for many different kinds of work, and a general review of the current state of knowledge about it would be very valuable.

This volume consists of some thirteen chapters written by about as many specialists and ranges over the taxonomy, cytology, life cycle, biochemistry, genetics and cortical development of the organism, with a final chapter on *Tetrahymena* as a nutritional and pharmacological tool. The styles of the different chapters are very diverse, from the relatively prosaic descriptive accounts of the taxonomy, life cycle, and some of the biochemistry, to far flung speculations in the genetics chapter of Allen and Gibson. Little attempt seems to have been made to interrelate the material in different chapters, and there is a certain amount of overlapping. The book is really a series of separate articles, tied together only by the fact that the same organism is mentioned in all of them. Viewed in this way, it is a useful source of information about the state of knowledge of various special topics in 1972

or slightly earlier, and for anyone planning to use the organism experimentally, it is essential reading.

The chapter by Frankel and Williams on "Cortical Development" is very comprehensive and clear, and the book ends with a bibliography prepared by Dr Elliott, after consultation with John Corliss, of no fewer than 1,700 citations stated to comprise all major and most minor publications on *Tetrahymena* up till 1972. This is one of the most valuable features of the book.

It seems to me that research on *Tetrahymena* may be approached from two opposing viewpoints. The first involves using the organism as a model cell whose behaviour resembles closely enough that of higher animals to enable it to be used for trials of pharmacological and dietary substances intended for use on higher animals or man. This is discussed in the final chapter by Hutner and others. It seems that one should be cautious in accepting the results of such trials, especially of drugs suspected of having carcinogenic, toxic, or other harmful effects on human cells, though the use of *Tetrahymena* as an agent for assaying preparations of particular amino acids or vitamins which are an essential requirement in its diet is more reliable.

The second approach to research on *Tetrahymena* is to study it for itself, as a remarkably interesting biological system, with many technical advantages for experimental work, helping us to understand all sorts of biological processes, whether biochemical, genetic or developmental. Here it does not matter so much that *Tetrahymena* differs markedly from cells of higher animals, for example in its presence of two kinds of nuclei, its extraordinarily elaborate surface structures, and finally its 'protozoan' nature—compressing all life processes into one and the same compartment.

Biologists expert in other matters, or those with more general interests, would be disappointed to find in this volume so little effort at assessing the overall importance of the work, and no attempt at all to write a single coherent account.

G. H. BEALE

Resistant plants

Breeding Plants for Disease Resistance: Concepts and Applications. Edited by R. R. Nelson. Pp. xii+401. (Pennsylvania State University: University Park and London, November 1973.) £6.85.

PLANT diseases are major factors limiting agricultural production throughout the world. Resistant varieties, which are less damaged by disease than others, are widely used to control diseases. This book attempts to summarise the

present state of our knowledge concerning the production of resistant varieties, with particular reference to some of the most economically important crop species.

The book is divided into two main parts. In part 1 the editor gives his views (many of which he admits are controversial) on the concepts, principles and terminology of breeding for disease resistance. Part 2 consists of sixteen chapters in each of which work on breeding for resistance in a particular crop species is summarised. Each chapter has been written by specialist pathologists, geneticists and plant breeders, mainly from the United States, with research experience in that crop. The format of these chapters varies from crop to crop but generally involves a short introductory section on the origin, economic importance and breeding system of the crop concerned and a description of the main sources of disease resistance. This is followed by accounts, which are often extremely brief, of breeding for resistance to some of the main virus and fungus diseases of the crop. An example of this brevity is afforded by the chapter on disease resistance in peas in which there are subsections on eighteen diseases in less than seventeen pages. Conversely, the chapter on soybeans deals in much greater detail with only four diseases in fourteen pages.

This lack of uniformity of treatment in different chapters can be disconcerting but is perhaps to be expected in such a compilation by so many authors, each with a different style and approach. It would have been useful if the editor had written a third part in which he attempted to draw general conclusions from the experiences described in the crop chapters, particularly in relation to the theoretical considerations outlined in part 1. As it is, the reader is left to draw his own conclusions from work on sixteen different crop species.

I find it surprising that a book of this nature includes no line drawings or photographs. Illustrations of disease symptoms and of examples of differences between resistant and susceptible varieties after exposure to infection would have been particularly interesting and useful. As a result of editorial policy, the book contains remarkably few references to original published work on disease resistance. A less restricted bibliography would have made this a much more useful reference book.

In spite of its limitations and shortcomings, this book is an up-to-date, authoritative account of breeding for resistance to diseases. As such it deserves to be read by all those concerned with plant pathology and plant breeding.

G. E. RUSSELL

Ions into solids

Ion Implantation. By G. Dearnley, J. H. Freeman, R. S. Nelson, and J. Stephen. P. xv+802. (Defects in Crystalline Solids, vol. 8.) (North-Holland: Amsterdam and London; Elsevier: New York, 1973.) Dfl. 225; \$79.

EVER since it was found possible to generate beams of energetic ions, investigators have been directing them into solids, and noting the effects. As expected, a set of complex but interesting phenomena was to be found in the interaction of the beam with the solid; moreover, it was soon realised that implantation was a useful method of modifying solids, while ion channelling was a unique diagnostic tool. A mushroom of literature on the subject developed in the late 1960s.

An anticipatory thrill is thus in order when one opens a book in which four authorities at a centre like AERE, Harwell, have combined to try and review the field, especially when the weight and thickness of the book indicate that the coverage is likely to be broad and the treatment detailed. The main question then is not of accuracy or authority—these are taken for granted—but whether the authors have done their reading public the maximum service in digesting and explaining this great expanse of literature as well as bringing out salient points of their own research. My impression as a user of ion-implanted specimens is that they have certainly made a very good attempt at dealing at some depth with the physics and engineering aspects which relate to the uses of implantation. What failures there are will be felt more by those looking for insight and some are discussed later.

The book consists of four main sections, each attributed to one of the authors, followed by a shorter chapter on the less usual applications. Apart from the unavoidable lack of unity which this method gives, the sections have been well coordinated as to subject matter, level of discussion, graphics and so on, although there is little cross referencing. Only a few things fall between the cracks; for example all of the authors are mainly interested in ion implantation into metals or semiconductors, so that insulators are given very little space. The fact that each author is treating his special field also means that he writes at a rather high intellectual level and pace and (with the exceptions described below) rarely catches his breath to explain to lesser mortals what the inwardness of some statement may be. For example, one author remarks that metals, unlike semiconductors, remain crystalline under bombardment even up to several hundred

displacements per atom, and that the reason for this fundamental division in ion-bombardment effects is not known. Surely, between the four authors, is not some little speculation on the reasons desirable? One can certainly form some hypothesis on the question in terms of the kinetics and type of vacancy/interstitial recombination available in the two forms of solids. Indeed, throughout, few panoramic views of the subject are essayed.

The author of the first main section, on ion penetration and channelling, is both the most concise and the most willing to comment intelligently on the meaning and prospects of the research work discussed. For example, the very useful phenomenon of radiation-enhanced impurity diffusion is made more than usually fascinating by useful asides. The discussion of channelling is, as expected, masterly. The next section, on the physical state of ion-implanted solids, is also a good exercise in compression; perhaps too compressed, since some omissions can be felt. Here was the place for a discussion of the annealing of clustered defects, with all the physical insight which this technique gives. The annealing of metals, bombarded, say, with deuterons at low temperature, yields an important insight into ion damage. Yet the whole topic of annealing of metals is dismissed in one page. This author indeed explicitly limited his writing to the effects observed from irradiation at or above room temperature, and this artificial barrier itself depreciates the value of the section in giving physical insight into the structure of implanted solids.

The third section is a detailed description of the art of implanting. Ion sources, analysers, accelerators and targets are described by a past master of the art and a tabular appendix lists the practical needs of each element in the periodic table. This section is a handbook in itself, unique in that many engineering aspects of ion beam production are only to be found in theses or specialist reports. With its high content of unpublished Harwell data, it probably merits its 200 pages but its length, of course, contributes to the horrendous price of the book.

The fourth part is of similar length, and is on the use of implantation in semiconductor device fabrication. This application has dominated all others to date and it can be claimed that a comprehensive review here is justified on those grounds. But the argument of rarity, used above, does not apply in this case. Should this section perhaps have been clipped to 120 pages? Coming after many published research reviews, it probably should have; however, in itself, this section is still a very well informed and complete ac-

count and can be thought of as the United Kingdom's brief on the subject of implantation in silicon. The final chapter, on other applications of implantation, is a useful compilation.

When a work so significant appears at such a price the reviewer must also try to advise librarians as to whether it is a good buy. I have already told my librarian that it is, and to others would say that, like Harris tweed, the price may hurt but the goods will give long and unwilting service.

A. G. HOLMES-SIEDLE

Nature and nurture

Introduction to Behavioral Genetics. By G. E. McClearn and J. C. Defries. Pp. 349. (Freeman: San Francisco, 1973.) \$10.

SOME of the most important developments in the interdisciplinary field of behaviour genetics have occurred in the thirteen years since Fuller and Thompson's *Behavior Genetics* provided the first and, until now, the only comprehensive coverage of the subject. The publication of *Introduction to Behavioral Genetics* under the joint authorship of a geneticist and a psychologist is, therefore, particularly welcome.

The introduction puts the emergence of the central problem of behaviour genetics, the nature-nurture controversy into historical perspective and the final chapter reviews the current status of this controversy as it relates to human behavioural differences that contribute to social problems. In the intervening pages the basic principles of genetics are introduced and these are elaborated as necessary throughout the rest of the book. A brief account of basic statistical principles that stops short of the analysis of variance precedes the presentation of examples from the behavioural genetics literature. These are grouped under a number of headings according to whether one or many genes are involved, whether experimental breeding or cytological observation were the means of investigation, and whether the main interest of the results obtained relates to the biochemistry of gene action, the processes of development or the forces acting on natural populations. A chapter wholly devoted to quantitative genetics, which in content and approach owes much to Falconer's *Introduction to Quantitative Genetics* reflects the present day emphasis on quantitative differences in behaviour.

Though the authors can rightly claim to have produced an introduction to the entire range of behaviour genetics, they have done so, to some extent, by offering *ad hoc* solutions to the immediate issues raised by the

behavioural examples rather than using well-chosen examples to develop and illustrate the experimental methodology and the principles underlying the analytical procedures and interpretations. It is difficult to believe that more appropriate examples could not have been found or that they could not have been used more effectively to illustrate the principles of experimental design, model building, hypothesis testing, interpretation and prediction. These are, however, relatively minor deficiencies compared with the advantages of having a concise, up to date account of the subject.

J. L. JINKS

Deformed embryos

Environment and Birth Defects. By James G. Wilson. Pp. xiv+305. (Environmental Studies: an Interdisciplinary Monograph Series.) (Academic: New York and London, December 1973.) £9.10.

THOUGH mortality at all ages in the period of childhood and adolescence has fallen dramatically during the past few decades mortality from congenital defects has shown little change. The relative importance of congenital defects has increased and continues to increase. This applies both to mortality and morbidity. Knowledge on the aetiology and underlying pathogenesis of congenital defects is very limited and much of the information available is widely dispersed in the literature. A book which brings together and unifies much of the existing information on teratology—defined as the adverse effect of environment on developing systems—is to be welcomed and fills a gap in the medical and biological literature.

The book begins by identifying the broad issues in teratology including the basis on which the conceptus becomes susceptible to adverse effects; the disturbance of cellular tissue and developmental processes which constitute the basic pathology of teratology; the importance of accessibility of an agent to the conceptus in determining possible teratogenic effects; dose effects; and the possible range of manifestations of deviant development. The causes of developmental anomalies are discussed in terms of genetic influences, chromosomal aberrations, radiation, chemicals and drugs, dietary imbalance, infections, respiratory gas levels, extremes of temperature, metabolic or endocrine imbalance, physical trauma, mechanical factors, placental factors, antibodies and combined effects. A review of the various drugs known or suspected to cause congenital malformations (not entirely complete) is relevant to clinical pharmacology and it touches on the

possible teratogenic effects of environmental chemicals used in industry and in agriculture.

The book then goes on to discuss the means by which developmental defects occur—the mechanisms of teratogenesis—under the headings of mutation; chromosomal non-disjunction and breaks; mitotic interference; altered nucleic acid integrity or function; deficiency of precursors, substrate and enzymes; altered energy sources; enzyme inhibition; osmolar imbalance; changed membrane characteristics; excessive cell death; reduced cell proliferation; disturbed interaction between cells; and reduced migratory movement of cells. Figures are given for the general incidence of developmental defects in man based on differing assessments made on embryos, stillbirths, neonatal deaths and later deaths. The effect of teratogenic processes is classified into four categories, namely, intra-uterine death, malformation, growth retardation or functional defect.

There is a useful account of the early developmental stages of the human ovary and foetus with pictorial representation of 23 different stages from fertilisation to the 53rd day of gestation, and a discussion on the concept of critical periods of susceptibility.

Further chapters deal more specifically with the access of teratogenic agents to the foetus and the foetal defence mechanism against them, and with the means of detection of teratogenic effects in man including recognition based on observation of clusters of cases, epidemiological surveys and surveys of high risk populations.

The last quarter of the book deals largely with the contribution of animal experiments to the assessment of human teratogenic risk and to the problems which arise from such experiments. The latter include the differing effect caused by the timing of any insult, dose effect in respect of quantity of teratogen and duration of administration, the masking of teratogenesis by embryolethal effect and standards of recognition of defects. There is a section on the various animals which can be used in teratology testing. The author puts forward a suggested scheme of teratogenic testing based on the graded use of animals of different availability and cost. Lastly there are special sections on appropriate literature relative to a number of individual animals and there is a valuable general bibliography.

This book ranges over the field of teratogenic response to the environment as revealed by animals and human studies. It concentrates primarily on the broader biological issues of teratogenesis approaching the subject from a morphological rather than a biochemical standpoint. The principles of

teratological testing are discussed and the author's own recommendations regarding his scheme for animal testing of suspect drugs provides a special component to the discourse. The book is clearly and concisely written and will prove a very valuable source of information to a wide range of those who are concerned with the environment and its possible effect on human development. These would include environmental scientists, hygienists, developmental biologists, obstetricians and paediatricians, toxicologists and pharmacologists. JOHN O. FORFAR

Mathematical brainwaves

Automation of Clinical Electroencephalography. Edited by Peter Kellaway and Ingemar Petersén. Pp. viii+318. (Raven: New York; North-Holland: Amsterdam, 1973.) \$22.70; Dfl.59.

THIS book provides an excellent guide to the quantitative methods of analysis currently being employed in EEG research. In the opening chapters, the editors draw attention to the major problem involved, namely that of defining the standard method of visual analysis in mathematical terms. For the non-clinician, a comprehensive description of standard EEG analysis is included, together with examples of typical wave forms and commoner abnormalities.

The case is presented for regional centres for automatic EEG analysis, so that research methods described can be widely applied to clinical practice. To implement this programme, a telemetry system capable of carrying at least eight channels of information would be required, a suitable system operating by public telephone being described later in the book. Continuing this theme, methods are described enabling multichannel EEG data to be presented in a simplified form on a single page, either by bar graphs for the principal frequency bands (delta, theta, alpha, beta), or by compressed spectral array, neither technique requiring as experienced an interpreter as conventional paper records. Extensive data reduction is involved, and although the two methods are complementary, they are suitable only as screening tests, and not as replacements for conventional EEG techniques. The method and application of Fast Fourier Transform are considered, and among many other topics included are pattern recognition techniques and EEG analysis in sleep.

The book is well presented and clearly illustrated throughout, and should prove a valuable reference work for those with interests in this expanding field. L. M. BRANCH

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AN EQUAL OPPORTUNITY EMPLOYER. (3)

INFORMATION SCIENTIST

required to prepare input and abstracts for the monthly abstracting journal "Aquatic Sciences and Fisheries Abstracts", and to assist in the general running and development of the Marine Pollution Information Centre. Applicants should be science graduates with relevant qualifications or experience in information work. Salary on the Civil Service scale for Scientific Officers, £1,435 to £2,329 (max) (under review). Applications, giving full relevant details and naming two referees, or requests for further information, should be sent to: The Librarian, Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth PL1 2PB, Devon. (294)

STAFF VACANCY OXFORD ENGLISH DICTIONARY

Applications are invited for a new full-time appointment as:

RESEARCH ASSISTANT (Science)

for A SUPPLEMENT TO THE OXFORD ENGLISH DICTIONARY, now in preparation at Oxford (Volume 1, A-G, published in 1972). The successful applicant will be required to investigate the history and usage of terms from all branches of science, using the resources of the Oxford University libraries. A degree in science is essential. Salary in accordance with experience and qualifications, but not normally less than £1,667 per annum, with participation in Clarendon Press superannuation scheme.

Further particulars from:

The Personnel Department (KB),
The Clarendon Press,
Walton Street,
Oxford OX2 6DP

to whom applications should be made in writing, with curriculum vitae and the names and addresses of two referees, by August 5. (293)

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF ZOOLOGY

Applications are invited for the post of EXPERIMENTAL OFFICER IN ZOOLOGY tenable from September 1, 1974, to assist in research (directed by Dr K. C. Highnam) into the endocrine control of development and reproduction in insects. Salary Range: £1,596 to £2,340 (£1,707 to £2,505 from October 1, 1974). Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications (3 copies) should be sent by July 31, 1974. Please quote reference R110/G. (269)

E.E.C. RESEARCH CENTRE ITALY

The E.E.C. Research Centre at Ispra (near Lake Maggiore in Italy) is seeking to fill the following posts:

Head of Engineering Service

This service, totalling about 20 persons, is comprised of university graduates and design draughtsmen divided into groups dealing with reactor physics, instrumentation, mechanical design and project engineering.

Candidates must have a good university degree, preferably in engineering, but a physicist or chemist with suitable experience would also be considered.

At least 10 years' experience in nuclear engineering is required, preferably with water reactors and, if possible, with in-pile loops. Approximately half of this time should have been spent in a position of responsibility with resultant capability to lead a group of qualified engineering personnel.

Good knowledge of French, Italian or English is required with a fair knowledge of at least one of the other two languages.

Radiochemist

for work involving research in the field of transuranium and transplutonium elements separation by solvent extraction or other methods.

Candidates should be under 35 years old and university graduates or postgraduates with at least two years' radiochemistry experience.

Senior Technical Assistant (Engineering Technical Assistant)

for the Fracture Mechanics Group (Reactor Safety Programme) of the Technology Division, to be responsible for the standard destructive mechanical tests that are carried out on specimens (tensile strength, hardness, impact strength, Pellini, etc.); to devise and carry out fracture-mechanics tests (COD, K_{IC}, I, etc.) and to devise non-destructive methods of stress-strain measurement (Moiré, photo-elasticity, etc.).

Candidates should have a Higher National Diploma, Higher National Certificate or equivalent, in mechanical engineering and at least five years' experience of destructive mechanical testing and/or stress-strain measurement. Additional experience of data processing would be an asset.

Radiochemical Technician

for radiochemical work in glove-boxes on transuranium and transplutonium elements.

Candidates should be HND graduates in applied chemistry with radiochemistry elements and have achieved practical experience preferably in a radiochemical laboratory.

In all cases, contract will be initially for two years, probably renewable for a further year.

Application forms and further details of the terms and conditions of employment, which compare well with national scientific institutions, may be obtained from: Mr. P. Blaes, Personnel and Administration Division, Commission of the European Communities (Euratom), Ispra (Varese), Italy.

Completed application forms, indicating the post for which you wish to be considered, should be received at the above address by 19th August, 1974.

(267)

KINGSTON POLYTECHNIC SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES RESEARCH ASSISTANT

required to study ultrasonically-induced damage in biological material and the mechanisms by which this occurs. You will be encouraged to register for a higher degree. Applicants should be honours graduates in a physical science or biology. Salary £1,427 to £1,537 (under review). Further details and application forms from Appointments Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. (287)

UNIVERSITY OF LEICESTER DEPARTMENT OF CHEMISTRY

Applications are invited for the post of RESEARCH TECHNICIAN (Grade 3) to carry out manipulation on a high vacuum line and to operate an Electron Spin Resonance spectrometer. H.N.C. or a degree would be an advantage but other applicants with appropriate experience will be considered. Apply to Dr J. B. Raynor, Chemistry Department, The University, Leicester LE1 7RH, naming two referees. Salary in range £1,650 to £1,920 with threshold agreement. (275)

NEW ZEALAND
Ministry of Agriculture & Fisheries

Applications are invited for the undermentioned vacancy:

SCIENTIST

(BEEF PRODUCTION)

**RUAKURA ANIMAL RESEARCH STATION
 HAMILTON, NEW ZEALAND**

SALARY:

Payable up to NZ\$12,653 depending on qualifications and experience.

DUTIES:

To carry out feeding and management studies with grazed and stall fed cattle. Comparisons are required between the performance of grass fattened and feed-lot finished stock, both relative to physical rates of live weight and carcass gain, and to eating quality of the meat. The influence of rearing systems and the contributions of compensatory growth of beef cattle performance require examination under a wide range of circumstances.

QUALIFICATIONS DESIRED:

Masters degree or higher in agricultural science.

PASSAGES:

Fares for appointee and his wife and family, if married, will be paid.

INCIDENTAL EXPENSES:

Up to NZ\$120 for a single man and NZ\$800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London SW1Y 4TQ, with whom applications will close on 22 August 1974.

Please quote reference P/T 104 when enquiring.

(274)

**UNIVERSITY COLLEGE CARDIFF
 TEMPORARY RESEARCH
 TECHNICIAN**

IN THE DEPARTMENT OF ZOOLOGY

Applications are invited for the above position concerned with work on the distribution of animals on the Severn Estuary. Candidates must possess some zoological training, and familiarity with two stroke motors would be an advantage. Duties to commence August 1, 1974. Closing date is one week from the appearance of this advertisement. Salary range £1,524 to £1,794.

Applications, together with the names and addresses of two referees, should be forwarded to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Please quote ref. 0605. (290)

**UNIVERSITY OF GLASGOW
 RESEARCH ASSISTANT**

Applications are invited from medical graduates, honours or postdoctoral graduates in physiology, pharmacology or biology to undertake an M.R.C. grant-aided project on Neurotransmitter Metabolism in the Cerebral Cortex in the Institute of Physiology. The post is tenable for 3 years from October 1, 1974 and salary range will be £2,118 to £2,412 per annum.

Applications should be lodged with Professor I. A. Boyd, Institute of Physiology, University of Glasgow by August 16, 1974. Further information can be obtained from Dr J. A. G. Watt, Gartnavel Royal Hospital, 1055 Great Western Road, Glasgow G12 0XH.

In reply please quote Ref. No. 3511M. (281)

UNIVERSITY OF EAST ANGLIA

Applications are invited from suitably qualified persons for a SENIOR RESEARCH ASSOCIATE-SHIP in the Climatic Research Unit (associated with the School of Environmental Sciences). A good degree in physics, meteorology or geophysics and previous experience of research and practical work in climatology and/or the global circulation of the atmosphere or oceans is desirable. The work of the Unit is concerned with improving knowledge of the long record of climate, and of global relationships, with a view to establishing predictive techniques and statistical rules based on understanding of the physical processes of climatic variation. Salary on the scale £2,118 to £4,896 according to age and experience. The appointment will be for 3 years in the first instance.

Applications (one copy only), with a curriculum vitae and list of publications, together with the names and addresses of two persons to whom reference may be made, should be sent to the Establishment Officer, University of East Anglia, Norwich NOR 88C, England, and must be received before August 31, 1974. (276)

**UNIVERSITY OF LONDON
 GOLDSMITHS' COLLEGE
 NEW CROSS, LONDON SE14 6NW**

Applications are invited for the post of Technician (Grade 3) in the Geology Department to start as soon as possible.

The person appointed should have a keen interest in geology and preferably be studying for an academic qualification in geology, if not already qualified.

Duties would include:

1. Curating the Departmental map and specimen collection.
2. Thin section making.
3. Photographic and reprographic work.
4. Display work.
5. Attendance at field-classes.

Salary, according to qualifications and experience, within the following range: £1,740 to £2,010 p.a. including £90 London Weighting Allowance.

For further details write, enclosing a stamped addressed A4 envelope, to the Personnel Officer, to whom applications should be sent by August 16, 1974. (272)

**MEDICAL LABORATORY
 TECHNICIAN**

required for expanding diagnostic radioimmuno-assay/saturation assay department. Experience in radioisotopic methods is desirable though not essential, but attention to detail and a high standard of orderliness in laboratory work are very important requirements. Successful candidates will be expected to display initiative and to contribute to the development of new techniques in this expanding and important field. Salary on Whitley Council scale according to age and experience (maximum £2,577 p.a.). Applications including a curriculum vitae and the names and addresses of two referees should be submitted to: Professor R. P. Ekins, Department of Nuclear Medicine, The Middlesex Hospital School, London. W1N 7RL by July 31, 1974. (266)

Find your place in British Gas

SPECTROSCOPIST

A scientist with post-graduate research experience in spectroscopy is required to join a group engaged on R and D in combustion. Work in the group would include the development of laser diagnostic techniques, including Raman spectroscopy, for the study of high temperature flames.

Candidates should be graduates in one of the physical sciences. Starting salary will depend on qualifications and experience, but will be within a scale rising to £3,192 or £3,510 (currently under review).

For further details and an application form, write to Research Secretary, British Gas Corporation, London Research Station, Michael Road, London SW6 2AD, quoting reference 4010/12/NA. (327)

BRITISH GAS



UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post of Post-doctoral Research Assistant, financed by the S.R.C., to develop some aspects of Affinity Chromatography. Candidates should have an interest in Enzymology.

Applications, stating age, qualifications and experience, together with the names of two academic referees should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276131. (271)

UNIVERSITY OF ALBERTA
DEPARTMENT OF ENTOMOLOGY

Applications are invited for the position of ASSISTANT PROFESSOR, effective April 1, 1975. Qualifications are Ph.D. with postdoctoral experience in insect physiology and interest in aspects of this field applicable to agricultural or forest entomology. Duties include teaching courses in general or applied entomology and insect physiology, the development and direction of a research program and supervision of graduate students in insect physiology and in application of some aspect of physiology to agricultural or forest entomology. Maximum starting salary \$14,043.

Please send full curriculum vitae and names of 3 referees by October 31, 1974 to: Dr George E. Ball, Chairman, Department of Entomology, 260 Agriculture Building, University of Alberta, Edmonton, Alberta T6G 2E3. (297)

UNIVERSITY COLLEGE GALWAY

JUNIOR LECTURESHIP
IN ZOOLOGY

Applications are invited for the above post. Salary scale £2,478 by 99 (10) to £3,468, plus Family Allowances. The closing date for receipt of applications is **August 8, 1974**. Prior to application, further information should be obtained from the Registrar of the College. (308)

UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF BOTANY AND
CHEMISTRY
EXPERIMENTAL OFFICER

Applications are invited for the post of Experimental Officer to assist in a research project concerned with the biosynthesis of secondary metabolites in plant tissue cultures. The project is financed by the S.R.C. for a period of two years. Candidates should be graduates in a biological science or chemistry. Salary on a scale rising to £1,647 pds F.S.S.U. depending on experience and qualifications.

Further particulars and forms of application which should be returned immediately, can be obtained from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham. Ref. No. 385. (301)

UNIVERSITY OF KEELE
DEPARTMENT OF PHYSICS

Applications invited for post of Demonstrator to assist with laboratory and tutorial work. Post on year to year basis renewable up to total of three years. Salary £1,815 per annum. Average 12 hours formal teaching per week. Rest of time available for research. Candidates should have good honours degrees. Application forms and further particulars from the Registrar, The University, Keele, Staffs., ST5 5BG, to whom completed forms should be returned by August 6, 1974. (302)

RESEARCH BIOCHEMIST

A vacancy exists in the Lipid Research Laboratory at St Bartholomew's Hospital, London, studying lipoprotein metabolism in relation to Diabetes and Hyperlipaemia. The post would be suitable for a higher qualification (Ph.D. or M.D.). Previous experience in lipoproteins or lipid metabolism would be an advantage. Salary according to age and experience.

Applications with full curriculum vitae to the Personnel Department, St Bartholomew's Hospital, West Smithfield, LONDON EC1A 7BE quoting ref R/4603/N. Closing date August 8, 1974. (312)



Wellcome

Research Technicians

Pirbright, Surrey

We require Technicians or Senior Technicians for our Laboratory at Pirbright, Surrey, to work mainly on the research aspects of the immune response to foot-and-mouth disease vaccine. Candidates should be between 20-25 with ONC, HNC or equivalent qualification and have a biochemical background. Some experience in chromatography electrophoresis and precipitation techniques is desirable.

These posts are permanent and offer a good salary together with excellent conditions of employment.

Please write quoting ref: U/466 and giving brief details of qualifications and experience to the
Personnel Officer,
The Wellcome Research Laboratories,
Langley Court, Beckenham,
Kent BR3 3BS.



(303)

NEW ZEALAND

Department of Scientific and Industrial Research

Applications are invited for the undermentioned vacancy:

SCIENTIST

The New Zealand Oceanographic Institute, Department of Scientific and Industrial Research, Wellington, has a vacancy for a Marine Zoologist to work on shallow water invertebrates in fluctuating environmental situations.

SALARY:

According to qualifications and experience.

DUTIES:

It is envisaged that following some initial survey work, the appointee could concentrate on ecology and/or physiology of selected species which appear indicators of environmental changes due to human activity.

QUALIFICATIONS DESIRED:

Ph.D with good research background as evidenced by published papers, and preferably some post-graduate experience.

PASSAGES:

Fares for appointee and his wife and family, will be paid.

INCIDENTAL EXPENSES:

Up to NZ\$120 for a single man and NZ\$800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, SW1Y 4TQ, with whom applications will close on 22 August 1974.

Please quote reference P/T 108 when enquiring.

(277)

Immunology Research

G. D. Searle are one of the world's leaders in medical research. A vacancy has arisen in the Research Division based at High Wycombe for a Technician/Research Assistant to join a group working on the experimental aspects of tumour immunology.

The position will probably suit someone with laboratory experience in the field of immunology, but recent graduates in biological science who are interested in this area of work should also apply. In addition to an attractive commencing salary, company

benefits include a contributory Pension Fund, BUPA Scheme, excellent canteen and an active sports and social club. If necessary, assistance with relocation expenses will be given in appropriate circumstances. Please apply to H. W. Cooke, Personnel Manager, Research and Development Division, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe, Bucks, or telephone him on High Wycombe 21124 ext. 130 quoting reference IR/113.

SEARLE

(279)

TECHNICAL DEVELOPMENT MANAGER HIGH VACUUM TECHNOLOGY

A Physics Graduate, aged about 40, is required to head a technical group engaged in high voltage, high vacuum technology.

The Group will evaluate developments and as appropriate will introduce innovations to manufacturing processes which have, in part, being current for some 25 years. The Manager may initially secure a detailed appreciation of the problem areas through secondment for a period of six months to an associated U.S. Company.

This is a challenging appointment, and

there are opportunities for further advancement within the organisation. Location is North West London.

Please reply giving full personal and career details to Position Number BKT 4495 Austin Knight Limited, London, W1A 1DS.

Applications are forwarded to the client concerned, therefore companies in which you are not interested should be listed in a covering letter to the Position Number Supervisor.

(321)

MANCHESTER AREA HEALTH AUTHORITY (TEACHING)
SOUTH DISTRICT, WITHINGTON HOSPITAL
MANCHESTER M20 8LR

The National Reference Laboratory for Anticoagulant Control Reagents

MEDICAL LABORATORY TECHNICIAN

required. A.I.M.L.T. qualifications or good honours degree in applied science necessary. Appointee will be trained in basic routine blood coagulation procedure and will participate in routine work, research and development. Good career prospects.

Applications including names of two referees to the Hospital Secretary quoting reference B29. (343)

ROYAL PERTH HOSPITAL (WESTERN AUSTRALIA)

Applications are invited for the position of:
**SENIOR TECHNOLOGIST
(ELECTRON MICROSCOPY)**

Applicants should possess an appropriate qualification such as A.A.I.M.T., F.A.I.M.T., B.Sc., B. App.Sc. (M.T.) or equivalent, and should be experienced in both Transmission and Scanning Microscopy techniques and equipment.

Supplementary trading at the manufacturer's works in Germany or America may be offered according to the needs of the appointee.

The appointee will be responsible for the technical development and function of the newly established Electron Microscopy Unit at this 1,004 bed General Hospital.

Duties will involve the preparation, examination and photomicrography of predominantly biological specimens in collaboration with various hospital departments; instruction and supervision of technical staff; adjustment and maintenance of equipment; records, filing etc.. Salary range \$A10,039 to \$A11,033 per annum.

Further information is available on request.

Applications stating name, age, qualifications, experience, Ref. No. 140a, together with names and addresses of three referees and accompanied by a recent photograph should reach the Administrator by August 19, 1974.

Box X 2213, GPO, Perth, Western Australia, 6001. (319)

MAX-PLANCK INSTITUT FÜR MOLEKULARE GENETIK

BERLIN (WEST)

has openings for

QUALIFIED TECHNICIANS

to aid with research in the following areas: electron microscopy, microbial genetics, biochemistry of microorganisms, biochemistry of nucleic acids. Salary according to age and experience between DM 19,000 and 24,000 p.a. Travel expenses to Berlin after employment can be met. Speaking knowledge of German would be helpful but not required in the laboratory. Applications giving names of two referees should be sent to:

Professor T. A. Trautner
Max-Planck-Institut für molekulare Genetik
D-1000 Berlin 33
Innestrasse 63/73 (316)

UNIVERSITY OF NATAL DEPARTMENT OF CHEMISTRY PIETERMARITZBURG

Applications are invited from suitably qualified persons for the appointment to the post of

JUNIOR LECTURER IN CHEMISTRY

The salary scale attached to the post is R3 600 by 150 to R4 500 plus a 15% pensionable allowance. The position is tenable for three years in the first instance.

The duties are teaching and research in Organic Chemistry. The post is suitable for a person with a B.Sc.(Hons.) or higher degree. The research will be in the field of insulin chemistry.

Application forms are obtainable from the Registrar, University of Natal, King George V Avenue, Durban, 4001, Natal, South Africa, with whom applications on the prescribed form should be lodged not later than August 31, 1974, quoting Ref. Adv. 78/74.

Further particulars are obtainable from Professor D. A. Sutton, Head of the Department of Chemistry, "Middle Cottage", Sutton End, Crockerton, Warminster, Wiltshire, England, from July 24 to 31, 1974. (296)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF PARASITOLOGY

Applications are invited for the post of Lecturer in the Department of Parasitology. This post is to be filled in January 1975. Candidates should hold a good science degree and doctorate in relevant subjects. Practical experience in the parasitology of tropical diseases is essential and preference will be given to candidates who already have experience in teaching and/or research on parasitic diseases.

The successful applicant may have the opportunity to spend periods of service on secondment overseas at teaching and/or research centres but will be based in the Department of Parasitology.

The initial salary will be within the range £2,118 to £4,896 per annum according to qualifications and experience.

Applications, together with the names of three referees should be received not later than August 15, 1974 by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref. RV/N/276127. (270)

ENTOMOLOGIST—**Agrochemical Research**

Fisons Agrochemical Division, one of the major manufacturers of chemicals for crop protection, are expanding their programme of Research and Development. To satisfy part of our needs, we now wish to appoint a Senior Research Assistant in the Biological Screening Department of our Chesterford Park Research Station, near Saffron Walden, Essex.

Ideally, we are seeking either a young graduate in the Biological Sciences, or someone who expects to graduate in 1974, as experience is less important than the ability to fit into an existing team.

As part of that team, the person appointed would be required to carry out insecticide and nematocide screening tests to evaluate the effectiveness of novel compounds, and to assist in the culture of the insect colonies

including the development of methods for new species.

We can offer a competitive starting salary and our other Company conditions of employment are amongst the market leaders.

For further details and an application form, please write, quoting reference 633/to:

Personnel Department,
Fisons Limited—Agrochemical Division,
Chesterford Park Research Station,
Near Saffron Walden,
Essex, CB10 1XL.



(284)

UNIVERSITY OF
NEW SOUTH WALES
SCHOOL OF PHYSICS
LECTURER

High academic qualifications required. Experience in teaching at tertiary level an advantage. Appointment from February 1975.

Salary \$A9,002 range \$A12,352 per annum. Commencing salary according to qualifications and experience.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on **September 13, 1974.** (304)

UNIVERSITY OF QUEENSLAND
Australia
READER IN VETERINARY
ANATOMY

This is the most senior departmental position and it is likely that the successful applicant will rapidly assume the Headship of the Department. Applicants should preferably hold both a veterinary and a higher qualification; they should be able, experienced teachers, with a particular interest in gross anatomy. Research skills, a flair for organisation and originality will all be considered favourably.

The salary is \$A16,389 per annum, and the University provides travelling and removal expenses, superannuation similar to F.S.S.U., housing assistance and study leave.

Additional information and application forms are obtainable from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in London and in Brisbane on **September 30, 1974.** (305)

DALHOUSIE UNIVERSITY
DEPARTMENT OF MICROBIOLOGY

Applications are invited for a Faculty position at the level of **Lecturer/Assistant Professor**. Applicants should have an M.D. or Ph.D. with research experience and a major interest in medical microbiology. Salary according to qualifications and experience.

Applications including Curriculum Vitae and the names of three referees to:

Dr K. R. Rozee,
Department of Microbiology
Dalhousie University
Halifax, Nova Scotia, Canada.

(307)

Glaxo

GLAXO LABORATORIES LIMITED

Fermentation Microbiologist

Ulverston, Cumbria

We require an experienced microbial physiologist to join our Fermentation Development Department. The department consists of microbiologists, biochemists, geneticists and chemical engineers in multi-disciplinary project teams. Each team is responsible for the R & D in one of our major product areas, which includes antibiotics, vitamin B12 and industrial enzymes. The successful candidate would be appointed as a member of a team, seniority depending on his or her proven ability and experience.

The job involves the origination and execution of experimental programmes from the laboratory to the production scale. Although experience of strain selection and the biosynthesis of extracellular products would be an advantage, the main qualities we are looking for are an understanding of the physiology of microbial growth and product formation and experience in the use of fermenters.

Ideally candidates should have some postgraduate experience either in academic or industrial research. The salary will be according to age and experience.

The factory is one of the largest fermentation factories in the world. Ulverston is a pleasant market town situated on the southern fringe of the Lake District National Park. Relocation expenses will be reimbursed where appropriate.



Applications in writing to:

**The Senior Personnel Officer (EWM), Glaxo Laboratories Limited,
North Lonsdale Road, Ulverston, Cumbria, LA12 9DR.**

(322)

Republic of South Africa

PROVINCIAL ADMINISTRATION OF THE CAPE OF GOOD HOPE

Hospitals Department

Principal Professional Officer (Medical Biochemist)

The successful applicant will hold a responsible position in diagnostic Chemical Pathology at the Groote Schuur Hospital and other associated Teaching Hospitals in the environment of Cape Town.

Essential requirements are a Bachelor's degree, Honours degree, or a Master's degree plus 3, 2 or 1 years' training as a Professional Officer, respectively. Additionally, a Ph.D in Medical Bio-Chemistry or Chemical Pathology will be a strong recommendation. A medical qualification is not a necessity.

Salary will be in the range £4523 x £188 — £5089, approximately. In addition, a pensionable allowance of 15% of basic salary (excluding allowance) is payable as well as an annual vacation savings bonus, subject to certain conditions.

Applications must be made in duplicate on the prescribed form (Staff 23) which is obtainable from the Chief Migration Officer, South African Embassy, Trafalgar Square, London WC2N 5DP and should be forwarded, by airmail, to the Director of Hospital Services, PO Box 2060, Cape Town 8000, South Africa, as soon as possible.

CHROMATOGRAPHY SPECIALIST

The role of the Analytical Chemistry Department within the Central Research Division is to devise and develop methods to control potential new drugs from the discovery to marketing phase. A principal aim is the generation of technical data for submissions to world-wide regulatory agencies.

To maintain the technical support currently required, we now need an additional graduate Chemist to work as a Chromatography Specialist. The work of the Analytical Chemistry Department is varied and includes the application of GLC, HPLC and TLC to a wide variety of problems including homogeneity assessment, the quantitative control of impurities and stability studies. The job involves working with a large number of Research Staff and provides an excellent opportunity to gain a wide experience of

separation science in a pharmaceutical research environment.

The successful applicant will have a BSc or GRIC qualification plus up to four years industrial experience in one or more branches of Chromatography. He/she will have a keen interest in the qualitative and quantitative analysis of mixtures of organic compounds. A knowledge of other instrumental techniques will be an added recommendation.

Our laboratories are situated in a pleasant rural/coastal area at Sandwich, Kent.

Our conditions of employment include a free pension and death benefit scheme, assistance with removal costs (where appropriate), bonus and flexible working hours.

Pfizer

Applications, giving brief details of age and experience should be addressed to:—

D. W. Sells
Personnel Manager
Central Research
Pfizer Limited
Sandwich
Kent

(300)

Agricultural Research Council UNIT OF MUSCLE MECHANISMS AND INSECT PHYSIOLOGY OXFORD

This Unit is investigating the molecular mechanism of muscular contraction and requires:

1) **RESEARCH ASSISTANT**
with degree in physics or chemistry for research programme involving measurements of electrical conductivity

2) **SCIENTIFIC OFFICER**
with qualification in entomology and practical ability to supervise colonies of unusual tropical insects

3) **RESEARCH TECHNICIAN**
for work on protein biochemistry

4) **RESEARCH STUDENTS**
with good degree in biochemistry or physical sciences for research on contractile systems

Further details from Professor J. W. S. Pringle,
Department of Zoology, Oxford OX1 3PS.

(310)

UNIVERSITY OF WATERLOO DEPARTMENT OF APPLIED MATHEMATICS

invites applications from candidates qualified for a position at the

ASSISTANT OR ASSOCIATE PROFESSOR

level with research interests in the area of Partial Differential Equations. Candidates will be expected to have at least three years post Ph.D. experience and a background in applied mathematics. The lecturing load is not heavy but considerable emphasis will be placed on teaching ability. Present minimum salary for Assistant Professors is \$13,000 and for Associate Professors is \$17,000. Closing date for applications November 30, 1974. Starting date to be arranged but not later than July 1, 1975. Send curriculum vitae, details of research and teaching experience and names of three referees to Dr D. G. Wertheim, Chairman, Department of Applied Mathematics, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

(311)

IRELAND

(a) SENIOR GEOLOGISTS (5) (b) GEOLOGISTS (II)

required in the Geological Survey.

The Government is continuing the implementation of its programme for the modernisation and expansion of the Geological Survey of Ireland to ensure that it is able to contribute in full to the economic development of the country.

The present recruitment is in the second phase of expansion which includes the provision of additional office and laboratory accommodation and the necessary modern equipment.

It is proposed to continue the re-mapping of the entire area of the Republic and to carry out the necessary regional studies both onshore and offshore. Applied research projects are also being undertaken to improve prospecting and mapping methods in Ireland.

It is hoped to assemble a team of earth scientists of high calibre who will have a practical bias and who will be given every encouragement to carry out applied research and to publish the results.

Senior Geologist:—There is one vacancy in each of the following fields:—

Economic Geology, Marine Geology, Palaeontology, Engineering Geology, Hydrogeology. Applicants must hold a PhD and have had at least 6 years relevant experience *or* hold a 1st or 2nd Class Honours degree and have had at least 8 years relevant experience and be under 50 years of age.

Commencing salary up to £3952 (man) or £3359 (women) possible
Maximum £4095 (Man) £3481 (Woman)

Geologist: There are 11 vacancies.

Applicants must hold a 1st or 2nd Class Honours Degree in Geology *or* in Geography with Geomorphology as a major subject and be under 40 years of age. One of the appointees must have experience in mineral resources, data collection and prospecting, one must have training in hydrogeology and a knowledge of water supply problems and one must have a knowledge of geochemical techniques.

Commencing salary up to £3041 (Man) or £2699 (Woman) possible
Maximum £3736 (Man) £3183 (Woman)

Non-contributory pension scheme and contributory Widows and Orphans pension scheme apply to all appointees.

Application forms, and further details of these posts may be obtained from the Secretary, Civil Service Commission, 45 Upper O'Connell Street, Dublin 1.

CLOSING DATE: 15th AUGUST 1974

(342)

CSIRO AUSTRALIA

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

RESEARCH SCIENTIST DIVISION OF ANIMAL HEALTH

Melbourne, Victoria

FIELD

MICROBIOLOGICAL SECURITY

LOCATION: The position is initially located at the CSIRO, East Melbourne, but some time will be spent at other locations in Melbourne. After a period of 5–6 years the position will be transferred to the Animal Health Laboratory at Geelong, Victoria.

GENERAL: A major biological laboratory for the diagnosis of and research on exotic and indigenous diseases of livestock will be built in Geelong. The microbiological performance of the facilities of great importance and will be the responsibility of the Officer-in-Charge of the Laboratory. The successful applicant will be working closely with and be responsible to the Officer-in-Charge.

DUTIES: Research into the behaviour of micro-organisms in non-biological environments and specifically into engineering, mechanical, hydraulic and chemical systems for handling these agents.

QUALIFICATIONS: A Ph.D. degree in microbiology or equivalent qualifications; extensive experience in the achievement, maintenance and control of microbiological security, with demonstrable research ability.

SALARY: Appointment will be made within the salary ranges of Senior Research Scientist or Principal Research Scientist \$A12,674—\$A17,549 p.a.

TENURE: The position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional particulars, the names of at least two professional referees and quoting Reference Number 201/455, should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64–78, Kingsway,
LONDON, WC2B 6BD.
by the 16th August, 1974

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(346)

SHEFFIELD AREA HEALTH AUTHORITY (TEACHING)

Sheffield Central District (Teaching) NON-MEDICAL SCIENTIFIC OFFICER

required until October, 1975, to take part in a research project investigating into the Excretion of Sulphated Corticosteroids in Breast Cancer.

Applicants should possess a first or second class Honours degree. Whitley Council Terms and Conditions of Service apply. Salary: £1,797 rising to £2,259 p.a.

Applications stating age, qualifications, experience and the names and addresses of two referees to the District Administrator, Sheffield Area Health Authority (Teaching), Central District (T), 10 Beech Hill Road, Sheffield S10 2RZ, by August 3, 1974. Further details supplied on request.

(376)

Fermentation Technologist CAWTHORN INSTITUTE NELSON NEW ZEALAND

Application are invited for the position of **FERMENTATION TECHNOLOGIST** in the Research Section of the Cawthorn Institute. University degrees are not a prime consideration.

A proven record of accomplishment in carrying out both batch and continuous fermentations is required. Skill in assembling and operating modern control equipment for pH control, dissolved oxygen control, etc. is essential.

A new and well equipped laboratory is available for this work.

Salary will be commensurate with qualifications and experience within the range of \$6,000 to \$9,000 N.Z. a year.

Nelson combines a relatively small community with a very favourable climate and ready access to three National Parks and many recreations eg. swimming, boating, tramping, skiing, climbing, fishing, shooting.

Please submit applications with full details of educational and experience qualifications and the names of at least 2 referees to the Director, P.O. Box 175, Nelson, New Zealand by August 1, 1974. (317)

MOLECULAR BIOLOGIST

Faculty position for Ph.D. molecular biologist, 2 or more years of experience desirable. Position involves collaborative research on herpesviruses and oncornaviruses with light teaching responsibility to medical and graduate students. Send applications and curriculum vitae to Dr Meihan Nonoyama, Department of Microbiology, Rush Medical College, 1753 West Congress Parkway, Chicago, Illinois, U.S.A. 60612. An equal opportunity employer. (318)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF BOTANY AND MICROBIOLOGY

Applications invited for the post of postdoctoral **RESEARCH ASSISTANT** in the Department of Botany and Microbiology, to work on the differentiation and ultrastructure of spermatozoid nuclei. Familiarity with the basic techniques of light and electron microscopy is essential, and some experience of fluorescence microscopy and autoradiography would be desirable. Applications, giving the names of two referees, to Assistant Secretary (Personnel) University College London, Gower St. London WC1E 6RT. (323)

UNIVERSITY OF BRADFORD POSTGRADUATE SCHOOL OF STUDIES IN BIOLOGICAL SCIENCES

Applications are invited from graduates in biochemistry, biology or suitable related subjects for a research assistant to work on the carbohydrate of x-lactalbumin and their role in lactose synthetase activity. The appointment is for a period of one year and the successful applicant will have the opportunity of registering for an MSc. Informal interview can be arranged by contacting Dr J. V. Wheelock, Tel. No. Bradford 33466, Ext. 579. Further particulars and application forms from the Registrar, University of Bradford, Bradford, BD7 1DP. (291)

Histology Technician

required in the Department of Neurology to take charge of nerve and muscle biopsy work. Applicants must be state registered and have had a minimum of 3 years experience in basic histological techniques. Experience in electron microscopy an advantage, but not essential. Salary scale £1,566 to £2,418.

Job description and application form (to be returned by July 31), from Personnel Officer, The Royal Free Hospital, 21 Pond Street, Hampstead NW3 2PN. Tel: 01-794 0431 Ext. 12. (320)

UNIVERSITY OF RIYADH
MEDICAL SCHOOL

Saudi Arabia

(in association with the University of London)

Applications are invited from male candidates only for the following posts in the Medical School at the University of Riyadh. The vacancies have arisen as a result of the rapid expansion of the School.

TECHNICIAN IN CHEMISTRY
TECHNICIAN IN BIOLOGY
TECHNICIAN IN HISTOLOGY
TECHNICIAN IN ELECTRONIC
PHYSIOLOGY
TECHNICIAN IN PATHOLOGY
TECHNICIAN IN PHARMACOLOGY

Successful candidates will possess an O.N.C., City and Guild certificate or equivalent qualification and considerable experience.

The University of Riyadh is an independent University established in 1957. In 1968 the University established a Medical School in association with the University of London. The request by the University of Riyadh for assistance in this project is covered by a sponsorship agreement. The University of London advises on the curriculum, the form and conduct of examinations, the physical facilities for teaching, the appointment of academic staff and other matters. From the outset it has been anticipated that this assistance would continue over a period of at least 10 years possibly extending to 15 years. All teaching of medical undergraduates is in the English language.

Appointments are for 1 year or longer; renewable. Secondments would be considered.

Salary will be negotiable in accordance with qualifications and experience. An attractive housing allowance will be granted.

Detailed applications (three copies) including a curriculum vitae and naming three referees should be sent not later than July 26 to the Inter-University Council for Higher Education Overseas, 90-91 Tottenham Court Road, London, W1P 0DT from whom further particulars are available.

(336)

IMPERIAL COLLEGE
DEPARTMENT OF BIOCHEMISTRY**Microbial
Biochemist**

Applications are invited for a S.R.C. Postdoctoral Assistantship for work on the Biosynthesis of Ergot Alkaloids in collaboration with Dr P. G. Mantle and Dr G. Mellows.

Candidates should have research experience in Microbial Biochemistry. Experience in the culture of Filamentous Fungi and in Biosynthetic Techniques would be an advantage.

The appointment is tenable for two years, commencing October 1, 1974. Salary range £2,118 to £2,247 p.a. plus £162 London Allowance (under review) (with F.S.S.U.).

Applications, including curriculum vitae and the names of two referees should be sent as soon as possible to either Dr P. G. Mantle or Dr G. Mellows, Department of Biochemistry, Imperial College, London SW7 2AZ.

(337)

POSTDOCTORAL BIOCHEMIST

required with experience in enzymology to investigate certain aspects of immunology in dermatology in the dermatology laboratories at St Helier Hospital. The post is for a period of three years in the first instance on a Senior Biochemist grade. Subject to Whitley Council conditions of service. Salary £2,964 to £3,843 plus London Weighting, depending on experience. Other staff in the laboratory include a graduate research worker and a research registrar. Further information may be obtained from Dr E. L. Rhodes, St Helier Hospital, Carshalton, Surrey. Closing date for receipt of applications September 1.

(338)

**CSIRO
AUSTRALIA****APPOINTMENT OF
RESEARCH SCIENTIST**DIVISION OF PLANT INDUSTRY
CANBERRA A.C.T.

FIELD:

**MOLECULAR
GENETICS**

GENERAL: The Organisation's Division of Plant Industry, with Headquarters situated in Canberra, A.C.T., has a staff of 120 scientists, conducting pure and applied research in genetics, plant breeding, biochemistry, physiology, microbiology, mineral nutrition, ecology, crop adaptation and plant introduction. It is well equipped with instruments for the characterisation of macromolecules.

The Genetics Group consists of 13 Research Scientists. The work in progress includes the study of chromosome structure and replication, the evolution of enzymes and the genetic mapping of enzyme structure, molecular mechanisms of mutation and adaptation and the genetics of regulatory processes.

DUTIES: To participate in a research programme concerned with studies of the molecular structure and function of eukaryote chromosomes.

QUALIFICATIONS: Applicants should have a Ph.D. degree in an appropriate field or postgraduate research experience of equivalent standard or duration supported by satisfactory evidence of research ability.

SALARY: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a. In addition to salary, a reasonable rental subsidy on furnished accommodation may be paid to a married male appointee.

TENURE: The duration of the appointment will be for a fixed term of either two or three years, after which, if mutually desired, either an extension of tenure or appointment for an indefinite period will be considered. An indefinite appointment carries Australian Government Superannuation benefit.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 130/1270 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64-78 Kingsway,
LONDON WC2B 6BD

by August 9, 1974.

Applications in U.S.A. and Canada should be sent to:
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(351)

UNIVERSITY OF MELBOURNE
LECTURESHIP (LIMITED TENURE)

in the

DEPARTMENT OF MATHEMATICS

Applications are invited for this position, which is for a Limited Tenure period of 3 years. The starting date is January 1, 1975, or as soon as possible thereafter. Preference will be given to applicants whose field of interest is analysis, algebra, topology or some other branch of pure mathematics.

Salary: A\$9,002 to A\$12,352 per annum. Initial salary will be in the range depending on qualifications and experience.

Further information, including conditions of appointment, is available from The Registrar. All correspondence should be addressed to The Registrar, referring to Position No. 618033, University of Melbourne, Parkville, Victoria, 3052, Australia. Applications close on August 23, 1974.

(347)

PUBLIC HEALTH LABORATORY
SERVICE

LEEDS REGIONAL LABORATORY

Vacancies for

SCIENCE GRADUATES

Applications are invited from science graduates with Class I or II honours degrees in microbiology.

One post is in general microbiology including reference, hospital and public health work and one is particularly in the microbiology of food, milk, water and the environment.

Initial appointment will be to the Senior or Basic Grade, depending on qualifications and experience. N.H.S. terms and conditions of service.

Applications, including the names of two referees to the Director, Leeds Regional Public Health Laboratory, Bridle Path, York Road, Leeds LS15 7TR, by August 15, 1974.

(361)

SOUTH AFRICAN SUGAR ASSOCIATION
EXPERIMENT STATION
MOUNT EDGECOMBE, NATAL
REPUBLIC OF SOUTH AFRICA

CHIEF RESEARCH OFFICER

The Experiment Station of the South African Sugar Association at Mount Edgecombe, Natal, requires the services of a Chief Research Officer. The successful applicant will be responsible to the Director for the operations of the following departments: Agricultural Engineering; Agronomy; Biometry; Chemistry and Soils; Entomology and Nematology; and Land and Water Management. His duties will include the preparation and supervision of annual programmes of work, the administration of a specialist advisory service to all sugarcane growers and the motivation and co-ordination of special research projects. Applicants should preferably hold a Ph.D. or an equivalent degree in science or agriculture. Extensive experience in research will be an essential pre-requisite.

This appointment holds good prospects for an outstanding scientist with organizing ability. It will also include opportunities for overseas travel when visits to other institutes are warranted.

Salary will be commensurate with qualifications, experience and the senior grading of the appointment.

Application forms, a list of benefits (which include a 10% annual bonus) and conditions of service are obtainable from:

South African Sugar Association,
Fountain House,
125-135 Fenchurch Street,
London,
England EC3M 5EH.

Completed forms, together with curriculum vitae, should be air-mailed directly to:

The Director,
S.A.S.A. Experiment Station,
P.O. Mount Edgecombe, 4300,
Natal,
Republic of South Africa.

(332)

ROYAL MARSDEN HOSPITAL
SUTTON, SURREY

Pharmaceutical Scientist

Graduate scientist, required for responsible position in radioisotope section of Physics Department. Experience with radioactive materials or qualification in pharmacy essential. Duties involve all aspects of preparation, dispensing and testing of radiopharmaceuticals used mainly by a large Nuclear Medicine Department. Participation in development of new radiopharmaceuticals and techniques will also be expected. Salary and grading will depend on qualifications and experience but is expected to be in range approximately £2,000 to £3,000 per annum.

Application forms and further information can be obtained from The Administrator, Royal Marsden Hospital, Downs Road, Sutton, Surrey. (01-642 6011 Ext. 201). (339)

INSTITUTE OF NEUROLOGY

requires TECHNICIAN to assist in Muscular Dystrophy Dept. in biochemical studies of muscle. Qualifications: H.N.C. preferable but not essential. Salary: University scale from £1,440 to £1,845 plus £126 L.A. Applications in writing giving qualifications, experience and names of two referees to: Dr R. Yasin, Muscular Dystrophy Research Laboratories, National Hospital, Queen Square, London WC1N 5BG. (333)

UNIVERSITY OF OXFORD NUFFIELD INSTITUTE FOR MEDICAL RESEARCH

Applications are invited for a Graduate Research Assistant to participate in a programme of research (supported by the M.R.C.) into the regulation of glucagon and insulin release in foetal and newborn lambs. Applicants should have a Ph.D. or an honours degree in biochemistry and physiology with some postgraduate experience. Experience in radio-immunoassay techniques or foetal studies is desirable. The post would be for a period of two years. Salary in the range £1,833 to £2,580 p.a. Applications, together with a curriculum vitae and the names of two referees should be sent to Dr J. M. Bassett, Nuffield Institute for Medical Research, Headley Way, Headington, Oxford OX3 9DS. (367)

INSTITUTE OF ORTHOPAEDICS

(University of London)

ROYAL NATIONAL ORTHOPAEDIC HOSPITAL
Brockley Hill, Stanmore, Middlesex HA7 4LP

Applications are invited for the post of

Lecturer in Cell Science

within the Professorial Research Unit of the Institute.

Applicants should preferably have a higher degree, some experience in electron microscopy and an interest in cell biology. This post, which is renewable annually for the first three years, will carry a salary within the Lecturer scale £2,118 to £4,896 plus £213 London Allowance; F.S.S.U. superannuation. Placing on the scale will be according to qualifications and experience. Further details may be obtained from Dr M. W. Elves, Professorial Research Unit, Stanmore.

Applications, together with names of three referees, should be received by the Secretary, Institute of Orthopaedics, 234 Great Portland Street, London W1N 6AD, not later than August 30, 1974.

(365)

THE QUEEN'S UNIVERSITY OF BELFAST

Research Assistant

Applications are invited from graduates in botany, biochemistry or biology for an S.R.C. research assistantship in the Department of Botany to work on the proteins and lipids of envelope membranes of differentiating chloroplasts. The post is tenable initially for a period of one year, but it may be renewed for a further period of two years.

The salary scale is £1,239 to £1,818; initial placing on this scale will depend on age and qualifications.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, Belfast BT7 1NN, Northern Ireland. (Please quote Ref. 74/N).

Applications, giving names and addresses of two referees, should be received by August 23, 1974. (349)

**PARKINSON'S DISEASE SOCIETY
OF THE U.K. LTD.
RESEARCH FELLOWSHIP**

Applications are invited for the appointment of a full-time **RESEARCH FELLOW** into subjects related to the cause and treatment of parkinsonism. The work must be conducted at a recognised institution and the tenure of support will be one year in the first instance renewable for a maximum of three years. Salary will be determined according to seniority and experience. Application forms are not issued but applicants are invited to write to The Executive Director, Parkinson's Disease Society, 81 Queens Road, London SW19 8NR giving curriculum vitae, proposed place of work, subject of research and tenure of grant and names of two or three referees. (356)

**CHAIRMAN
UNIVERSITY OF CALIFORNIA
SCHOOL OF MEDICINE**

Applications are invited for the position of Chairman, Department of Human Anatomy, University of California School of Medicine, Davis.

We are seeking applicants with an active interest in teaching and research. Successful applicant will assume leadership in the department's rôle in an integrated medical school curriculum and in graduate training. Send resumé and references to Dr Edwin G. Krebs, Chairman of Search Committee, Department of Biological Chemistry, School of Medicine, University of California, Davis, Ca. 95616. An affirmative Action-Equal Opportunity Employer. (357)

THE UNIVERSITY OF ADELAIDE

proposes to appoint one or more

DEPUTY VICE-CHANCELLORS

from the beginning of 1975.

Persons with appropriate academic and/or administrative qualifications and experience who may be interested in such an appointment are invited to write as soon as possible, in confidence, to the Vice-Chancellor, the University of Adelaide, G.P.O. Box 498, Adelaide 5001, South Australia, from whom further information may be obtained.

The Calendar of the University may be seen in the office of the Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF, or in the library of any university that is a member of the Association. An outline description of the University may be found in the A.C.U. Yearbook, which is similarly available. (358)

**UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF EXPERIMENTAL
PATHOLOGY
GRADUATE BIOCHEMIST**

required for M.R.C. supported project on Characterisation of urokinase. Two year appointment from October 1, 1974.

Salary according to age, qualification and experience in the range either for Research Assistants (if no previous experience) £990 to £1,404, or for Research Associates £1,758 to £2,404 plus F.S.S.U.

Further particulars from Dr P. Wolf, Department of Experimental Pathology (Rheumatism Research Wing), the Medical School, University of Birmingham, Birmingham B15 2TJ, to whom applications (six copies) naming two referees should be sent by August 10, 1974. (363)

**UNIVERSITY COLLEGE GALWAY
DEPARTMENT OF MICROBIOLOGY
RESEARCH ASSISTANT**

Applications are invited for the post of post-graduate research assistant to participate in a Euratom sponsored project on the effects of radiation on blue-green algae. Candidate should be qualified in Microbiology or Genetics or a related field. Full particulars from Dr J. A. Houghton, Department of Microbiology, University College, Galway, Ireland. (369)

**UNIVERSITY OF ABERDEEN
DEPARTMENT OF BIOCHEMISTRY**

A postdoctoral research assistant with experience of enzyme isolation is required to work under the direction of Dr J. Jeffery on the isolation and study of certain dehydrogenases. Initial appointment for one year at a salary of about £2,000 per annum, depending on experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by August 31, 1974. (374)

University of Amsterdam

In the Chemistry Subfaculty a position is available for a

**(senior) lecturer
(wetenschappelijk
(hoofd) medewerker)**

within the group Separation Methods of the Department of Analytical Chemistry. This group works on chromatographic methods of separation, especially on advanced (high) pressure liquid chromatography.

The applicant is expected after some time to make an active contribution to the further development of this field of chromatography and he (she) is expected to guide scientific research of doctoral candidates and students and to partake in instruction.

Applications in writing within 3 weeks after publication are to be addressed under number 74-1 to

Prof. Dr. J. A. A. Ketelaar, chairman of the nomination committee, Laboratory for Electrochemistry, Nieuwe Achtergracht 166, Amsterdam-C., Netherlands.

Information can be obtained from Dr. H. Poppe, of the Department of Analytical Chemistry, Nieuwe Achtergracht 166, Amsterdam. (Tel. (020) 522.3540).

(309)

**FLINDERS UNIVERSITY OF SOUTH AUSTRALIA
SCHOOL OF MEDICINE
LECTURERS, SENIOR DEMONSTRATORS AND DEMONSTRATORS
IN CLINICAL BIOCHEMISTRY, HUMAN MORPHOLOGY
AND HUMAN PHYSIOLOGY**

Applications are invited for lectureships and demonstratorships in the new School of Medicine currently being established in Adelaide at the Flinders University of South Australia in each of the following fields: Clinical Biochemistry, Human Morphology and Human Physiology.

The School of Medicine will form an integral part of the Flinders Medical Centre, a 710 bed teaching hospital located on campus. The first medical students began their course this year and will enter the Centre in February 1975. It is hoped that appointees will take up their positions by October 1974 in order to help plan the integrated curriculum.

Suitably qualified appointees will have responsibility in the areas of teaching, research and clinical service. Lecturers with medical qualifications registrable in South Australia will receive a clinical loading, the amount depending on their qualifications and the degree of clinical responsibility undertaken. Conditions of service include provision for transportation expenses, superannuation tenure, study and long service leave.

Present salary scales are: Lecturer \$A8,698 to \$A11,982; Senior Demonstrator \$A7,270 to \$A8,698; Demonstrator \$A5,332 to \$A7,015.

Further information may be obtained from the Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF.

Applications should be lodged with the Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042, by August 16, 1974. (379)

EXPERIENCED PHARMACOLOGIST? PHYSIOLOGIST? BIOCHEMIST?

...lead a team with ICI

ICI Pharmaceuticals Division seek an experienced Pharmacologist, Physiologist or Biochemist with training or interest in gastroenterology to lead a biological team in a multi-disciplinary group concerned with therapy for peptic ulcer disease. It is envisaged that the work will involve fundamental research into factors influencing gastric secretion mucosal 'barrier'.

This is a challenging opportunity to work in modern well-equipped laboratories situated in rural North Cheshire but within easy reach of Manchester and other important centres in the area. The area offers a good range of houses suitable for most price ranges.

Write, giving brief details of experience and qualifications, to:

M. F. Losse, Personnel Department, Imperial Chemical Industries Limited, Pharmaceuticals Division, Mereside, Alderley Park, Nr. Macclesfield, Cheshire.



**Pharmaceuticals
Division**

(378)

LEICESTERSHIRE AREA HEALTH AUTHORITY (T) AREA MEDICAL PHYSICS DEPARTMENT

Applications are invited from Graduates for three newly established posts in the Leicestershire Medical Physics Department. This department, which is undergoing considerable expansion, provides a service for the hospitals in the Leicester Area.

PHYSICIST SENIOR OR BASIC GRADE

Physicist is required with experience of Physiological Measurement. The successful applicant will be expected to become involved in various aspects of medical measurement. Within a developing medical school, these will initially include the measurement of blood flow and pressure, particularly in the limbs. Isotope work will also be undertaken at a later stage. The successful candidate should be prepared to become involved in both developmental and routine aspects of this programme.

ELECTRONIC ENGINEER or PHYSICIST SENIOR OR BASIC GRADE

A vacancy exists for an experienced Physicist or Electronic Engineer to set up an Audiological testing section involving clinical work.

This post will provide scope for developmental work in which the successful applicant will be encouraged to participate. Opportunities will occur for him to be engaged also in other aspects of Medical Physics at the Leicester Royal Infirmary. Applicants should have a good honours degree in electronic engineering or physics with a special interest in electronics and experience in this field would be desirable, though not essential.

PHYSICIST BASIC GRADE

Applications are invited for the post of basic grade physicist in the Leicestershire Area Medical Physics Department. The successful candidate will participate in all aspects of the work of the department including Nuclear Medicine, Radiotherapy, Electronics and Instrumentation. This post would be suitable for candidates graduating this summer. Candidates should possess a good honours degree in Physics/Electronic Engineering.

Further information from the **Principal Physicist in Charge.**

Applications, etc. to the Secretary, The Leicester Royal Infirmary, Infirmary Square, Leicester. LE1 5WW.

(326)

CHARING CROSS HOSPITAL MEDICAL SCHOOL

Applications are invited from persons with some experience of electron microscopy or graduates considering entering this field for a new appointment of technician in the Department of Histopathology of Charing Cross Hospital Medical School. Duties commencing September/October 1974 will include the maintenance and operation of transmission and scanning electron microscopes. Some initial training will be given which the successful applicant will be encouraged to supplement by external study. Whitley Council terms and conditions of service. Apply to Dr J. G. Jackson, Department of Histopathology, Charing Cross Hospital Medical School, Brandenburgh House, Fulham Palace Road, London W6 9HH. (352)

Guy's Hospital Medical School

Applications are invited for appointment of
JUNIOR LECTURER/LECTURER
in the Department of Microbiology.

Preference will be given to candidates with research experience in the general area of host-microbe interactions or virology.

The Department's interests include pathogenesis and immunology in herpes virus (CMV) infections and cell membrane changes in viral transformation.

Further particulars are available from Professor Mims, Department of Microbiology.

Salary in range £2,553 to £2,787 or £3,012 to £4,668 (both currently under revision) plus superannuation if medically qualified; otherwise £2,580 to £3,285 or £3,285 to £4,896 plus £162 London Allowance and superannuation.

Application forms obtainable from the Dean, Guy's Hospital Medical School, London Bridge SE1 9RT. Closing date August, 21, 1974. (355)

UNIVERSITY OF STIRLING
DEPARTMENT OF BIOLOGY

Research Assistant

Applications are invited for the above S.R.C. financed post to work on carbon metabolism of leaf epidermal tissue with particular reference to stomatal functioning. The appointment is for 3 years with a starting salary of £1,350.

Applications, including the names of two referees, should be sent by August 10, 1974 to Dr C. M. Willmer, Department of Biology, University of Stirling, Stirling. (370)

NEW ZEALAND
UNIVERSITY OF CANTERBURY
CHRISTCHURCH

The Council of the University invites applications for the following vacancy:

**SENIOR LECTURER or
LECTURER IN ASTRONOMY**

Applicants must have a strong interest in the fundamental physical processes of importance in Astronomy, so that they are capable of teaching not only in Astronomy but also in Physics courses.

The salary for Lecturers is on a scale from NZ\$7,361 to NZ\$9,339 per annum; for Senior Lecturers NZ\$9,503 to NZ\$11,153 (bar) NZ\$11,484 to NZ\$12,142 per annum.

Particulars, including information on travel and removal allowances, study leave, housing and superannuation may be obtained from the Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications close on **August 31, 1974.** (371)

UNIVERSITY OF EDINBURGH
LECTURESHIP IN APPLIED
GEOLOGY

Applications are invited for the post of Lecturer in Applied Geology. Candidates should be experienced in some aspect of Applied Geology, for example, fossil fuels.

Salary scale is £2,118 to £4,896 per annum. Superannuation under F.S.S.U.

The successful candidate will be expected to take up the post on October 1, 1974 or as soon thereafter as may be mutually convenient.

Applications (six copies) should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL by August 31, 1974. Please quote reference number 1034. (372)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF GEOLOGY
UNIVERSITY DEMONSTRATOR
IN OCEANOGRAPHY

Applications are invited for the post of University Demonstrator in Oceanography. Candidates, preferably, should have experience in the field of Marine Chemistry.

Salary scale is £1,848 to £2,538. Superannuation under F.S.S.U.

The successful candidate will be required to take up his or her post on October 1, 1974 or as soon thereafter as may be mutually convenient.

Applications (three copies) should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh EH8 9YL by August 31, 1974. Please quote reference number 7007. (373)

TECHNICIAN for POLIO UNIT

Technician required for work involving safety and animal testing of human vaccines at a small unit based at Mill Hill, N.W.7.

Duties consist of preparation of equipment and care of laboratories, clinical examinations, some post mortem work and preparation of pathological tissues. The successful candidate, whilst having practical bias, would be involved in the research work carried out by the scientist. Relevant laboratory experience would be an advantage. He or she should hold at least a relevant H.N.D. or H.N.C.

Salary: On the Technician scale £1,566 to £2,418 per annum, depending upon experience. (Salary award pending).

be addressed to Robin Dunn, Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232. Applications quoting reference 0021 should (350)

NIBSC

National Institute for Biological Standards and Control



Reckitt & Colman

PHARMACEUTICAL DIVISION

Psychopharmacological Research

Applications are invited from graduates with 2 or 3 years experience in CNS research or from candidates completing a Ph.D. in psychopharmacology.

The successful candidate will be engaged in the evaluation of the actions of psychotropic drugs on animal behaviour, particularly rodent operant behaviour, and in innovative experimental work.

Reckitt and Colman operates internationally and its pharmaceutical interests are based at Hull where first class facilities are available. Over the next few years major investment is planned in an expansion of the Company's pharmaceutical research activities.

Salary is negotiable according to experience and qualifications. Fringe benefits include annual bonus and relocation assistance.

Please write quoting ref. U.85 and giving relevant details of experience and qualifications to: **R. A. Sandow, Divisional Personnel Manager, Pharmaceutical Division, Reckitt & Colman, Dansom Lane, Hull HU8 7DS.**

(359)

Food Research

H. J. Heinz Company Limited require a Technical Assistant to work in their Food Research Department situated in the company's modern headquarters at Hayes Park.

The vacancy will probably be filled by an experienced person, aged 30-50, with a sound technical education to ONC/HNC level.

Primary duties will be concerned with the shelf life assessment of various products, and the observation of changes in the chemical/physical characteristics during storage. Previous experience in the food packaging industry is an obvious advantage. The person appointed to this post will enjoy excellent conditions of work, including a subsidised canteen, annual bonus, staff sales and good pension/free life assurance schemes.

Write in the first instance, giving brief details of career to date to: **I. Wason, HO**
Personnel Services, H. J. Heinz Co. Ltd.,
Hayes Park, Hayes,
Middlesex UB4 8AL.



(377)



Wellcome

Pharmacology Technicians

We have opportunities for Senior and Junior Technicians in our Pharmacology Laboratory. Using modern instrumentation they will work with a team concerned with the discovery and evaluation of new drugs. Candidates should have a keen interest and preferably experience in biochemical or physiology/pharmacology work.

Our laboratories are situated in pleasant parkland surroundings, about 12 miles from Charing Cross, within easy reach of Bromley and Beckenham.

Please write quoting reference U.467 and giving brief details of qualifications and experience to the Personnel Officer,

THE WELLCOME RESEARCH LABORATORIES,
Langley Court,
Beckenham,
Kent BR3 3BS.



(375)

THE CITY UNIVERSITY Department of Chemistry

Applications are invited for a TEMPORARY LECTURESHIP IN PHYSICAL CHEMISTRY

The post will be tenable for up to two years from October 1, 1974 (or as soon as possible thereafter) and will involve both undergraduate teaching and supervision of research students in a group under the general direction of Professor C. F. Cullis. Candidates should preferably have an interest in some branch of chemical kinetics. The salary will be in the range £2,118 to £4,896 per annum plus £213 per annum London Allowance.

For further particulars and application forms please write to the Deputy Academic Registrar, The City University, St. John Street, London EC1V 4PB, quoting reference 143/L/N. The closing date for applications is August 19, 1974. (AK 354)

ROYAL POSTGRADUATE MEDICAL SCHOOL JUNIOR TECHNICIAN/TECHNICIAN

required in Department of Clinical Cardiology. This post will involve dealing with patients and some research work. Qualifications GCE "O" level passes in English, Maths and two Science subjects/ O.N.C. or equivalent. Salary according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting ref. no. 2/354N. (380)

SUNDERLAND POLYTECHNIC

- a. Lecturer I in Ecology
- b. Lecturer II in Geography

Applications are invited for appointment to the above posts as soon as possible.

For post (a) applicants should have a good honours degree and preferably a higher degree with special interest in aspects of applied Ecology. The successful candidate will be required to contribute to a B.Sc. Environmental Studies degree beginning in September 1974, and to degree and H.N.D. Applied Biology.

For post (b) applicants should have a good honours degree. An interest in planning and other aspects of applied geography would be advantageous. The successful candidate will be required to contribute to a B.Sc. Environmental Studies degree beginning in September 1974, Teachers Certificate, B.Ed., and B.A. Social Sciences degree.

Salary in accordance with Burnham Technical scale, viz:—

Lecturer I: £1,800 to £3,045 (Subject to confirmation);

Lecturer II: £2,700 to £3,474 (Subject to confirmation).

Application form and further particulars may be obtained from: Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD, Co. Durham.

Applications should be returned within 14 days of the appearance of this advertisement. (362)

British Museum (Natural History)

Biometrician

- Set up library of computer programs for multivariate analysis and numerical taxonomy ■ Process data using library programs ■ Carry out statistical analysis on a calculator.

□ Degree or equivalent in mathematics including statistics □ Experience in computer programming and handling biological data desirable □ Age under 30 □ Appointment as Scientific Officer (£1900-£3000) or Higher Scientific Officer (around £2800-£3700) according to age and experience □ Ref: SB/30/DK. □ Application forms (for return by 12 August 1974), from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

Tropical Products Institute, Slough

Crop Storage Engineers

- R & D and advisory work on structures and systems for handling and storage of cereal grains and other crops in developing countries ■ Assist in training of storage technologists from overseas ■ Overseas assignments may be required.

□ Degree or equivalent in Agricultural or Civil Engineering □ Knowledge of agricultural crop production, storage systems and simple structural design work desirable □ Experience in tropical or sub-tropical countries an advantage □ Age under 30 □ Appointments as Scientific Officer (£1700-around £2800) or Higher Scientific Officer (over £2550-around £3500), according to age and experience □ Ref: SA/27/JD. □ Application forms (for return by 12 August 1974), from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

Tropical Products Institute
London**Pulp and Paper Technologist**

- Assess potential of fast-growing materials for paper pulp in developing countries ■ Chemical analysis, chemical, semi-chemical and mechanical pulping trials, pulp evaluation and consideration of economics involved.

□ HNC or equivalent in relevant subject □ Broad knowledge of paper-making an advantage □ Age normally under 30 □ Appointment as Higher Scientific Officer (around £2800-£3700) or Scientific Officer (£1900-£3000) □ Ref: SA/25/JD. □ Application forms (for return by 9 August 1974) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London, EC1R 5DB.

**Science
group**
CIVIL SERVICE

(360)

THE UNIVERSITY OF ASTON
IN BIRMINGHAM
DEPARTMENT OF PHARMACY
RESEARCH STUDENTSHIP

Applications are invited from candidates with a good degree in Chemistry (G.R.I.C.), Pharmacy or Biochemistry to work on the isolation and identification of biologically active compounds in selected coal-tar fractions.

The studentship, sponsored by the N.C.B., of not less than £700 p.a. (under review) plus demonstrating fees is tenable at the University of Aston. The successful candidate will be registered for the Ph.D. degree.

Applications, together with the names of two referees, should be sent as soon as possible to Dr A. Z. Britten, Department of Pharmacy, University of Aston, Gosta Green, Birmingham B4 7ET.

(286)

CRYOGENICS RESEARCH
UNIVERSITY OF SOUTHAMPTON
POSTDOCTORAL FELLOWSHIPS

Applications are invited for the following posts commencing October 1, 1974.

DEWAR DESIGN : 1 year

To evaluate use of plastics in portable containers for cryogenic liquids.

LNG HEAT TRANSFER : 2 years

To measure 2 phase condensing heat transfer coefficients on large rig at A.E.R.E., Harwell for LNG heat exchangers.

Applicants should have experience in mechanical/chemical engineering or low temperature physics.

Salaries in range £2,100 to £2,400 (plus F.S.S.U.). Applications with names of two referees to the Deputy Secretary, University of Southampton, Southampton SO9 5NH. Please quote reference Na/254/R.

(278)

UNIVERSITY OF DUNDEE
DEPARTMENT OF BIOLOGICAL
SCIENCES

Applications are invited from suitably qualified candidates for the post of

POSTDOCTORAL RESEARCH
FELLOW

to work on a project on the transport of hydrogen ions in algal cells.

The post is available for up to 3 years from October 1, 1974 at a starting salary of £2,118 p.a.

Applications, quoting Ref. Est/47/74J and naming 2 referees, should be sent by August 9, 1974 to The Secretary, The University, Dundee from whom further information is available.

(306)

The Hatfield Polytechnic
Biological Sciences
SRC STUDENTSHIP

Applications are invited for a studentship in one of the following areas:-

Teratology of anti-tumour agents
Neuronal uptake of catecholamines
Physiology of vascular smooth muscle
Lipid metabolism in plant tissue cultures
Algal-biocide interactions
Stored product acarology

Leaf microfilm and disease incidence
Applicants should possess a good honours degree and will be expected to register for a higher degree.

Full details of the projects and application forms may be obtained from: Dr K. Wilson, Department of Biological Sciences, P.O. Box 109, Hatfield, Herts. AL10 9AB.

(298)

UNIVERSITY OF ST ANDREWS
S.R.C. Research Studentship

The successful candidate will register for a Ph.D. and investigate the conditions necessary for in vitro breakdown and re-assembly of microtubule bundles isolated from the ciliate *Nassula*. Electrophoresis and dialysis on a micro-scale, and electron microscopy will be involved. Applicants, preferably graduates in biochemistry with an interest in cell biology and fine structure, should send a curriculum vitae with the names, addresses, and phone numbers of two referees to Dr J. B. Tucker, Department of Zoology, The University, St Andrews, Fife KY16 9TS, not later than July 30, 1974.

(280)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF WARWICK
POSTDOCTORAL FELLOWSHIP
IN BIO-ORGANIC CHEMISTRY

Applications are invited for Postdoctoral Fellowships to study (under the direction of Dr B. T. Golding) either reactions catalysed by coenzymes of vitamin B₁₂ or hydrocarbon oxidases. The appointments are for one year in the first instance (commencing as soon as possible) with the possibility of renewal. Salary will be in the range £2,118 to £2,412 p.a. plus threshold payment and participation in F.S.S.U. Further details may be obtained from the Academic Registrar, University of Warwick, Coventry CV4 7AL to whom applications should be returned by September 1, 1974. Please quote Ref. No.: 49/Q/74.

(292)

MEDICAL RESEARCH COUNCIL
RESEARCH STUDENTSHIP

Applications are invited from graduates with a first or upper second class honours degree in biochemistry or biology to study phospholipases in epidermal cells. The work is part of a programme of studies on the contribution of lipid components of subcellular and plasma membranes to the structure and function of the epidermis.

The appointment will be for three years and the successful candidate will register for a higher degree. Applications with curriculum vitae and names of two referees should be made to Dr G. M. Gray, M.R.C. Unit on the Experimental Pathology of Skin, The Medical School, Birmingham University, Birmingham 15.

(288)

university of wales
**university
college of
swansea**

Applications are invited for the following posts:—

Research Fellow

in the Department of Metallurgy and Materials Technology. Applicants should have a Ph.D. degree or equivalent experience and the successful candidate will be required to undertake a fundamental investigation into the elimination of heat treatment faults in general carbon systems in close collaboration with a local carbon manufacturing industry.

The appointment, which will be for one year in the first instance, will be on a scale up to £2,247 per annum, together with F.S.S.U. benefits. The closing date is **Friday, August 2, 1974.**

Technical Officer

in the Department of Metallurgy and Materials Technology, to work on a joint research programme with the Welsh National School of Medicine sponsored by the M.R.C., to develop new materials and material systems designed for medical implant. The successful applicant will be responsible for the fabrication of prostheses and should have experience in experimental work in materials technology, and will be required to collaborate closely with orthopaedic surgeons.

The appointment which will be for three years, will be on a scale up to £2,115 per annum.

The closing date is **Monday, August 12, 1974.**

Department of Zoology

Applications are invited for the vacancy of Research Assistant in the Department of Zoology. Applicants should be recent graduates with an interest in animal behaviour and/or endocrinology.

The appointment, which will be for up to three years from October 1, 1974, will be on the scale £1,425-£1,638 per annum.

The closing date is **Friday, August 2, 1974.**

Further particulars and application forms may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned by the appropriate date. (283)

**Dunstaffnage Marine
Research Laboratory**

Applications are invited for a three year studentship to work on the role of meiofauna, particularly protozoa, in processes originating from the breakdown of cellulose fibre in marine sediments. The studentship carries with it a substantial equipment allowance. The successful applicant would be expected to read for a Ph.D. and would be required to satisfy University regulations for a higher degree. Application forms and further information can be obtained from the Director, Dunstaffnage Marine Research Laboratory, P.O. Box 3, Oban Argyll PA34 4AD. (313)

**UNIVERSITY OF LEEDS
DEPARTMENT OF EARTH
SCIENCES**

Experimental studies at High Pressures and Temperatures

Applications are invited for the post of **RESEARCH FELLOW** to work in the solubility of water and carbon dioxide in silicate melts at high pressures (up to 10 kbars) and temperatures (up to 1400°C). Applicants should have prior post-graduate experience and qualifications in high-pressure techniques or some related field. The post, funded from a NERC grant, is for up to three years; salary on the scale £1,929 to £2,388 (under review).

Applications should be addressed to Professor P. G. Harris, Department of Earth Sciences, The University, Leeds LS2 9JT, from whom further particulars can be obtained. Closing date August 15. (314)

**THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF MICROBIOLOGY**

Applications are invited for a **S.R.C. POST-GRADUATE STUDENTSHIP** leading to the degree of Ph.D. Several research topics available, details of which can be obtained from Professor J. R. Quayle, Department of Microbiology, The University, Sheffield S10 2TN. Please quote ref R 109/G. (268)

**UNIVERSITY OF LEEDS
DEPARTMENT OF PHYSIOLOGY
M.R.C. Research Studentships**

Applications are invited for M.R.C. studentships in the Department of Physiology, University of Leeds. Applicants should have at least an upper Second Class degree in Physiology or associated subjects.

There is a wide range of research in the department which includes renal physiology, central nervous neurophysiology, electrophysiology of visceral receptors, several aspects of cardiovascular physiology, endocrinology and aspects of energy exchange and temperature regulation.

Application forms and further information on specific topics available from Professor G. R. Hervey, Department of Physiology, The University, Leeds LS2 9JT. Closing date for applications July 27. (315)

**THE UNIVERSITY OF ASTON
IN BIRMINGHAM
DEPARTMENT OF PHARMACY
RESEARCH FELLOW**

Applications are invited for the above post which will involve working in the Departments' Pharmacological Laboratories. The Fellow will be a member of a team, led by Professor C. B. Ferry, working on the physiology and pharmacology of normal and disordered neuromuscular transmission. Experience with electrophysiological techniques is essential.

Commencing salary will be within the range £1,929 to £2,223 per annum on a scale rising to £3,378 per annum. With effect from October 1, 1974 commencing salary will be within the range £2,118 to £2,412 per annum on a scale rising to £3,636 per annum.

Requests for application forms (which should be returned not later than August 5), should be sent, preferably on a postcard, quoting Ref. No. 982/6 to the Staff Officer, the University of Aston in Birmingham, Gosta Green, Birmingham, B4 7ET. (285)

**THE POLYTECHNIC OF NORTH LONDON
Faculty of Science and Technology
Research Opportunities**

A number of studentships, assistantships and research awards are available. Their value, and conditions of tenure, vary; further details will be sent on request. In most cases a first or second class honours degree (or equivalent qualification) is required, and the holder will be able to submit the results of the work for a higher degree.

The research projects are in the following fields:

Biology: genetics, biochemistry.

Chemistry: bio-inorganic, chemical analysis, heterocyclic compounds, natural products, reaction mechanisms and kinetics, fast reaction kinetics, nmr and mass spectroscopy, metal complexes, X-ray crystallography, waste utilization.

Physics: spectroscopy, magnetic properties of thin films, cosmic ray physics.

Polymer Science and Technology

Please reply, giving full details of experience and qualifications, with subject and class of degree, names and addresses of two referees, dates when available for interview, and area of research proposed, to the Academic Secretary of the Faculty Board:

**Dr. J. Charalambous, Chemistry Department
The Polytechnic of North London
Holloway Road, London N7 8DB**

(299)

UNIVERSITY COLLEGE DUBLIN

DEPARTMENT OF CHEMISTRY

Applications are invited for a Postdoctoral Fellowship in Organic Chemistry to investigate tautomerism in heterocyclic acyl enamines. The study will involve straightforward synthesis and stopped flow kinetic measurements. The fellowship will be supported by the National Science Council of Ireland. The appointment will initially be for one year, from October 1, 1974, with a possible renewal for one or two further years.

Salary will commence at £2,100 per annum.

Applications, accompanied by a curriculum vitae, and the names of two referees, should be sent to:

Dr R. A. More O'Ferrall
Department of Chemistry
University College
Belfield, Dublin 4.

(331)

POSTDOCTORAL FELLOWSHIP

Applications are invited for a postdoctoral fellowship for an investigation of the use of a cusped-Gaussian basis for the construction of molecular wave functions. Applicants should have a Ph.D. degree and experience in computational quantum chemistry or allied field.

The appointment will be for two years from October 1, 1974. Salary: £2,118 to £2,247 with F.S.S.U. benefits. Application with the names of two referees should be sent to Dr E. Steiner, Department of Chemistry, University of Exeter, Stocker Road, Exeter, EX4 4QD to reach him not later than August 16, 1974. Please quote ref. 1/12/7077.

(324)

RESEARCH STUDENTSHIP IN NEUROPHARMACOLOGY

University College London

A M.R.C. studentship, leading to a higher degree, is available for a graduate with a 2:1 or 1st class degree in pharmacology or physiology and biochemistry or a related science to join a unit studying the release of neurotransmitters in the mammalian central nervous system.

Applicants should send their c.v. and names and addresses of two referees by July 26, to Dr R. A. Webster, Dept. of Pharmacology, University College, Gower Street, London, WC1E 6BT.

(329)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF NEUROLOGY

RESEARCH STUDENTSHIP IN EXPERIMENTAL NEUROPATHOLOGY

Applications are invited for two Research Studentships, tenable from October 1, 1974, to work on histological, ultrastructural and histochemical aspects of experimental myopathies. Facilities are also available for collaborative work involving tissue culture and muscle physiology. Candidates should have a 1st or 2nd class honours degree in a biological science and will be eligible for registration for the degree of Ph.D. The award will be at the usual rate for Ph.D. students. Applications, including a full curriculum vitae and the names of two referees to:

Professor W. G. Bradley,
Muscular Dystrophy Research Laboratories,
Newcastle General Hospital,
Newcastle upon Tyne, NE4 6BE.

(330)

CHELSEA COLLEGE—UNIVERSITY OF LONDON

DEPARTMENT OF PHARMACY

RESEARCH STUDENTSHIP IN MEDICINAL CHEMISTRY

One S.R.C. (C.A.S.E.) studentship is available for a research project involving a study of structure/activity relationships in a series of bridgehead nitrogen compounds and the requirements of the analgesic receptor, to be carried out in co-operation with Allen & Hanburys Ltd. The studentship is subject to normal S.R.C. conditions and applications are invited from candidates with a good honours degree in Chemistry or Pharmacy.

The successful applicant will be expected to register for a higher degree.

Applications to:

Dr J. Walker,
Pharmacy Department,
Chelsea College,
University of London,
Manresa Road, London, SW3 6LX.

(335)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF PURE AND APPLIED CHEMISTRY

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from graduates in Physics, Chemistry, Chemical Physics or other relevant disciplines for an S.R.C. Postdoctoral Fellowship.

The successful applicant will work on Energy Transfer Studies using the tunable Spin Flip Raman Laser in collaboration with Drs R. T. Bailey and F. R. Cruickshank, and the laser physics group headed by Professor S. D. Smith at Heriot-Watt University. Most of the experimental work will be carried out at Heriot-Watt.

The post will be for a period of one year in the first instance commencing on September 1, 1974 or as soon as possible thereafter, with possibility of extension for one further year. Salary will be up to £2,058 per annum (under review) with placing according to age and experience and will attract one further increment if the appointment is extended for a second year.

Applications (quoting R25/74) should be sent to Dr. R. T. Bailey, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL.

(282)

UNIVERSITY OF SUSSEX

Research Studentships in Biochemistry

Applications are invited for:

- (1) An M.R.C. studentship to study either the control of protein synthesis in mammalian cells or some aspect of the growth control mechanisms operative in cultured human lymphocytes.
- (2) An S.R.C. studentship for studies on the mechanism of hormone action. Investigations into protein hormone-membrane receptor interaction and adenylate cyclase involvement in hormonal control processes in the adrenal cell are contemplated.

Applicants are expected to have a first or upper second class honours degree in Biochemistry or a related field, and should send a curriculum vitae and the names of two referees to Dr J. E. Kay (1) or Dr D. Schulster (2), School of Biological Sciences, University of Sussex, Brighton BN1 9QG.

(325)

COLAISTE NA HOLLSCOILE GAILLIMH

UNIVERSITY COLLEGE GALWAY

DEPARTMENT OF EXPERIMENTAL MEDICINE AND PRACTICAL PHARMACOLOGY

RESEARCH STUDENTSHIPS

Applications are invited from graduates in Biochemistry, Pharmacology, Physiology or related disciplines, who have a First or Upper Second class degree, for two research studentships under the direction of Professor B. E. Leonard. Students will be expected to register for a Ph.D.

Research will be concerned with the development of animal models of psychotic depressions and schizophrenia and will involve the use of biochemical, pharmacological and behavioural techniques; it is expected that the study of clinical material will form part of the investigations.

Stipendium will be equivalent to S.R.C./M.R.C. awards, plus small supplementary payments for departmental duties.

Applications, containing details of academic performance and the names of two referees, should be addressed to Professor B. E. Leonard, Professor of Pharmacology, University College, Galway, Ireland. Closing date—August 9, 1974.

(348)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL FELLOWSHIP IN ELECTROCHEMISTRY

Applications are invited from suitably qualified electrochemists, physical chemists, physical organic chemists or physicists for the two projects outlined below. The newly developed techniques of modulated specular reflectance spectroscopy will be used and supplemented by conventional electrochemical methods.

- (i) An industrially sponsored fellowship to study fundamental aspects of the mechanisms of cathodic hydrodimerisation processes with particular emphasis on the effects of the structure of the electrode/solution interface and the influence of surface active ions. The industrial importance of the work will be fostered by regular contact and the successful applicant will be expected to travel to the United States. For a suitably experienced person, the appointment can be made at a senior level with a salary up to £2,580 plus F.S.S.U.
- (ii) An S.R.C. fellowship for the application of modulated specular reflectance spectroscopy to studies in the area—adsorption on catalytic electrodes, the structure of the electrode/solution interface. Starting salary will be £2,118 plus F.S.S.U.

Applications with a curriculum vitae and the names of two referees should be sent to:

Dr A. Bewick, Department of Chemistry,
University of Southampton,
Southampton SO9 5NH.

(345)

UNIVERSITY OF OXFORD

Dyson Perrins Laboratory

Applications are invited from suitably qualified Organic Chemists for a Postdoctoral Research Assistantship to work on the synthesis and characterisation of sequential polypeptides. The appointment is available for the academic year 1974-5 at a salary according to age and experience up to a maximum of £2,247 with F.S.S.U. Previous experience in peptide chemistry would be advantageous but is not essential. Applications, giving a brief curriculum vitae etc., and naming two referees, should be sent as soon as possible to: Dr J. H. Jones, The Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY.

(344)

UNIVERSITY OF READING

Recent Graduate required in Department of Zoology, to work for three years on a S.R.C. C.A.S.E. Award Project on insecticide research leading to a Ph.D. degree. The project, which will be carried out together with I.C.I. Plant Protection, Jealotts Hill, is an investigation into the biochemical effects of a new insecticide upon insect cuticle. Apply to Professor Williams, Department of Zoology, University of Reading, Whiteknights, Reading. (Ref: MN35).

(366)

QUEEN ELIZABETH COLLEGE
(University of London)

DEPARTMENT OF MATHEMATICS

Applications are invited from suitably qualified graduates for a Science Research Council research Studentship. The successful candidate will work for a higher degree on one of the following: group theory, graph theory, mathematical biology, relativity and cosmology, topology.

Apply at once to Prof. W. B. Bonnor (N), Queen Elizabeth College, Campden Hill Road, London W8 7AH. (334)

KINGSTON POLYTECHNIC
SCHOOL OF CHEMICAL AND
PHYSICAL SCIENCES

RESEARCH ASSISTANT

Applications are invited for a postgraduate research assistantship to work on a project concerning synthesis and physico-chemical studies of Pyrimidines and related nitrogen heterocycles.

The assistant will work under the supervision of Dr D. T. Hurst, F.R.I.C., and will be expected to register for a higher degree. Salary range £1,427 to £1,537 (under review).

Further details and application forms (to be returned as soon as possible) from Appointments Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. 01-549 1366. (368)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF PHARMACOLOGY

Applications are invited for a Research Fellowship tenable in the Department of Pharmacology. The work will be mainly concerned with the pharmacokinetics of quaternary amines in man. Salary within the range £2,118 to £2,580 per annum and may be higher for a candidate with exceptional qualifications.

Applications, giving details of age, academic qualifications and experience, together with the names of two referees should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276136. (364)

UNIVERSITY OF
BIRMINGHAM

Applications are invited for an S.R.C. C.A.S.E. Studentship for research into factors influencing the allergenicity of various protein preparations in baboons and other experimental animals. The work will be carried out in collaboration with Unilever Research Laboratory, Colworth House.

Applicants should have a first or an upper second class honours degree in a biological science preferably with some experience in immunology. The successful candidate will be expected to register for a Ph.D.

Applications should be sent as soon as possible to Dr D. R. Stanworth, Dept of Experimental Pathology, The Medical School, Birmingham 15. (353)

USED COPIES required of all or any:- Chemical Abstracts 3rd, 6th, 7th, 8th collective indexes and Beilstein Handbuch 2nd suppl. vv 6-27, Index vv. 1-27 and general formula index.

Librarian,
Organon Laboratories Ltd.,
Crown House,
London Road,
Morden, Surrey.

(340)

GRANTS & SCHOLARSHIPS

LARGE TELESCOPE USERS

The Science Research Council invites applications to its Large Telescope Users' Panel for observing time as follows:

- (a) on the 98-inch Isaac Newton Telescope at Herstmonceux during the three months 1975 January to March inclusive; and
- (b) on the 20-inch and 40-inch reflectors at Sutherland during the three months 1975 April to June inclusive.

In considering the allocation of time, the Panel will especially support group applications, particularly where they result from collaboration between a university department and a Royal Observatory.

Applications must be made in respect of proposed programmes that cannot be accomplished on telescopes of smaller aperture, and will be considered by the Panel at its autumn meeting. They should be submitted by August 1 on a form obtainable from the Secretary of the Panel, Mr C. A. Murray, Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, Sussex. (289)

LECTURES AND COURSES

KINGSTON
POLYTECHNIC

Specialist one-year part-time courses leading to LRIC.

1. ADVANCED ANALYTICAL CHEMISTRY

This is a course in modern methods of analysis dealing with classical and instrumental methods of analysis in relation to a range of topics including environmental analysis, computing and analytical chemistry and laboratory automation.

2. ANALYSIS OF BIOMATERIALS

This course includes a consideration of the structure, function and analysis of various types of biomaterials. The principles of analytical procedures are covered (e.g. chromatographic, spectroscopic, enzymic, microbiological and radiochemical methods) and the application of these methods to the analysis of biomaterials is considered.

The courses start in September and are primarily offered to holders of HND/HNC in chemistry. They are also suitable as 'refresher' courses and applicants who do not hold the above qualifications will be considered on their merits. All candidates successfully completing the course will be awarded a Certificate of Supplementary Study (formerly known as an Endorsement Certificate) and subject to certain additional requirements, may qualify for admission as Licentiates of the Royal Institute of Chemistry.

Further information from the Secretary, School of Chemical and Physical Sciences, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366, ext 312. (341)

MISCELLANEOUS

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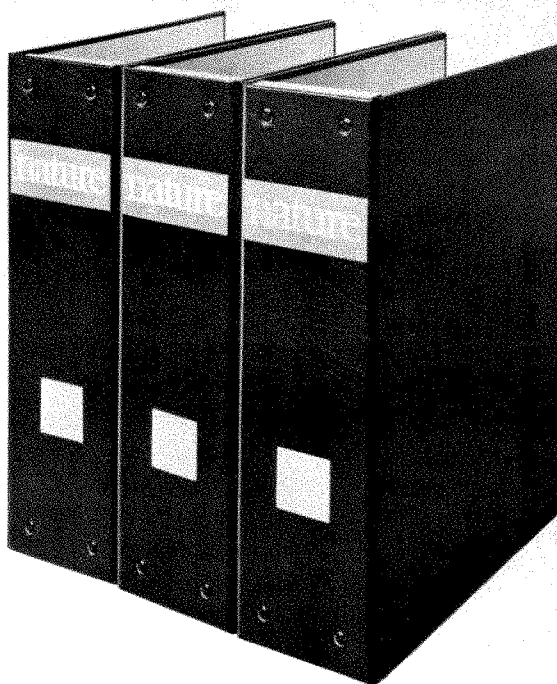
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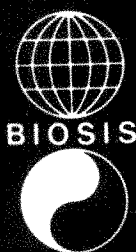
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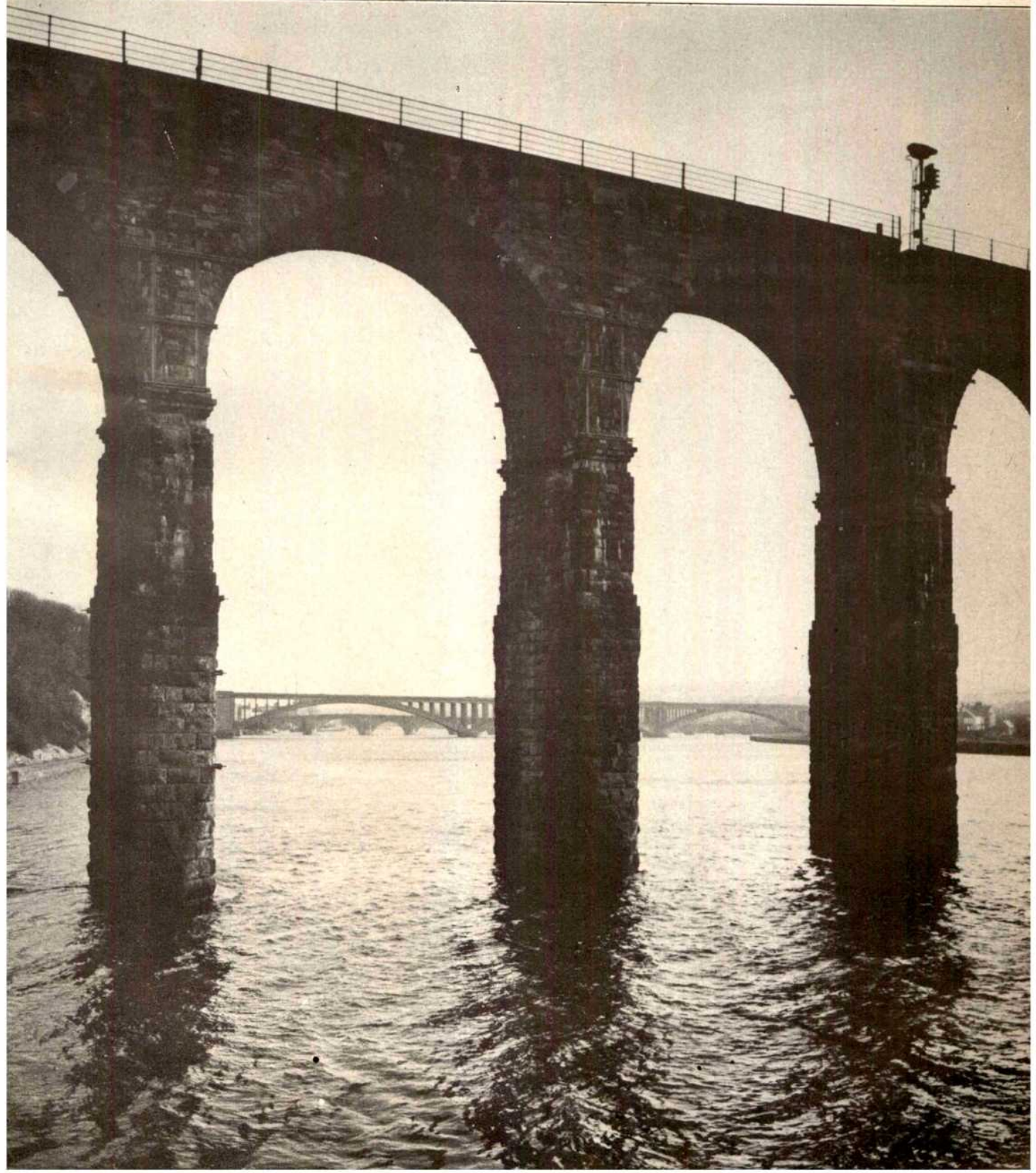


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Cover picture

The River Tweed at Berwick, where
10 million gallons of water flow
under the old railway bridge each
day. Britain's water resources and
plans for their development are dis-
cussed on p 276.

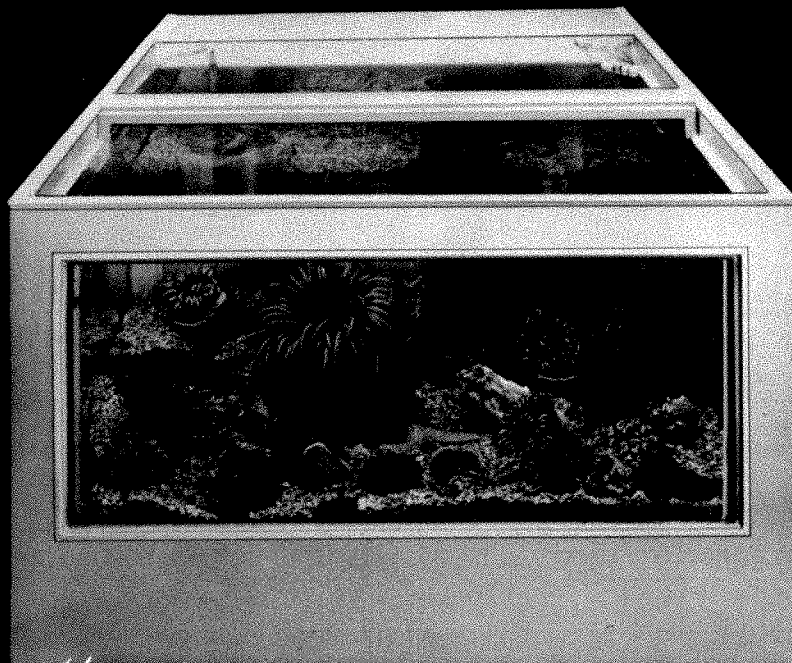


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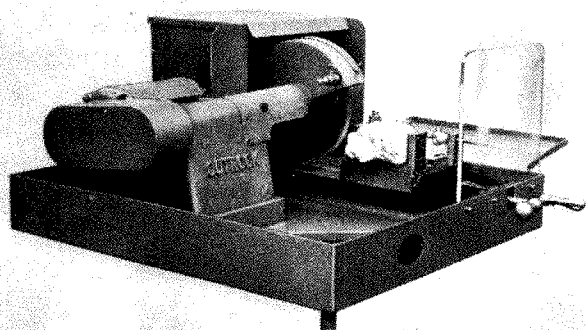
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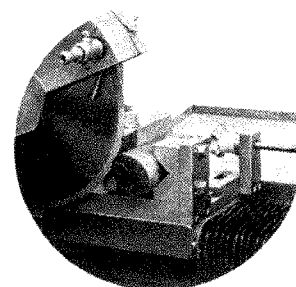
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Scientists don't move

DESCRIBING the British scientific system as positively arthritic, Sir Hermann Bondi introduced a report last week on "Interchange of Scientists" (available free from the Civil Service Department) which is a cautious first step towards easing the joints. It is small comfort that if anything Italy and Germany are even more fixed in their ways.

The report is necessary reading for anyone who worries that the British scientific scene is not so free-flowing and vigorous as its American counterpart, although most who feel that way (and clearly Sir Hermann's Task Force did) must hope that there is more to come and that the group will stay together even just as a continual stimulus to the body scientific and arthritic.

Mobility in the United States is much admired by those who live a trans-Atlantic existence, and with good cause. Scientists and engineers move regularly in pursuit either of greater job satisfaction or more dollars, and few would hazard a guess that they would be doing the same job in five years time. Movement into and out of the civil service, particularly to spend a time in Washington administering a programme they understand well, is something which many excellent scientists do and which gets them much credit in the community. But then 'the community' in the United States much more naturally includes industry and the civil service as well as the universities. Further there is a much greater freedom, it seems, to pay competitive salaries and in particular not to expect a bright young man to wait until he is forty before he begins to profit financially by being a scientist. Thus the problem with stimulating mobility in Britain is ultimately not so much one of improving administrative arrangements and making the physical move less disagreeable as of changing ways of thinking.

The central proposal of the task force (which the government has already promised to implement) is that within the civil service a small unit should be established to facilitate secondment between the civil service on the one hand and industry and universities on the other. Flow should be in both directions. The third side of the triangle—movement between industry and universities—could not be handled by the same unit, but there is an obvious need for an organisation to stimulate interchanges there too. The ever-cautious scientist, scared of venturing into the unknown, will generally be urged out of his shell by the existence of a return ticket to his first employer after a period of, say, two to four years.

The apparent obstacles to mobility which the task force report make stunning reading. The difficulty of moving house, problems with pensions (the university scheme is a particularly difficult one from which to transfer), inadequate allowances for transfer—all testify to feeble-mindedness among scientists and a lack of social responsiveness from employers. It is scarcely believable

that housing is sufficient of an obstacle that the task force has to recommend that "whenever possible, interchanges should be within the same geographical area to avoid moving house". Clearly a spirit of boldness in pushing back intellectual frontiers does not necessarily extend to much else. Not that employers are blameless—"to a large extent, those taking up permanent posts either in the civil service, or elsewhere, are not paid removal expenses". This medieval attitude must surely go.

One of the problems with stimulating mobility is that the potential mover must perceive other areas as attractive. This most certainly is not true at present, indeed the low regard that the three 'wings' of science often hold for each other must often dampen the enthusiasm of even the most willing mover. Furthermore the restraints on recruitment that the civil service has imposed on it ensured that in 1972, for instance, the Principal Scientific Officer grade, which numbers 2,000, comprised only ten who had been recruited from outside the service during the year. And all the signs are that mobility is on the decline. In 1958 half of all university chemists had outside experience. Now less than a third do, and one department of 33 has nobody with external experience!

There is no simple solution to this deplorable situation. The new unit will at least allow a few more temporary exchanges. But ultimately the whole question of the deployment of scientific manpower and the narrowness of institutional and individual attitudes needs penetrating examination. Maybe somewhere along that route the idea of allowing scientists to keep a year of their education in abeyance until their thirties would merit an airing. And it would be good to see more practising scientists in Whitehall.

100 years ago



THE tenacity of life of popular errors is well exhibited in the following extract from the *Californian Horticulturist* :—
 "The influence of forests in drawing moisture from the heavens may be seen from the experience of San Diego, California. Previous to 1863 there was yearly a rainy season, which made the soil nourishing and productive. In 1863 a destructive fire swept over the greater part of the country, destroying the forest and blackening the hills. Since then there has been no rainy season at San Diego." When will public writers learn that forests influence the climate by drawing water, not from the air, but from the soil?

As a result of the Water Act, 1973, river management, water supply and sewage disposal in Britain have now been integrated under the control of new regional water authorities. This is clearly a desirable move which will make planning and coordination easier within the regions. But of course there are still larger issues which must be settled on a national basis, at government level or by cooperation between regional authorities. To assist with these, a Central Water Planning Unit remains as a rump of the old Water Resources Board, under the auspices of the Department of the Environment (DoE), and a new Water Research Centre has been established by amalgamating the Water Research Association laboratories, the Water Pollution Research Laboratory, most of the Technology Division of the Water Resources Board and part of the Development Division of the Directorate General Water Engineering of the DoE.

As far as the study of long term trends is concerned, the CWPU is concerned directly with forecasting demand, and not with predicting how the supply of water (as rainfall) might vary. But the experience of the past three years, which have been very dry by established standards in Britain, has encouraged an awareness of the question of climatic change, and some steps are being taken to look into this problem.

This new weather pattern is seen by climatologists as part of a global trend which is causing droughts in parts of Africa and has also been responsible for bad harvests in Canada and the USSR. For Britain, the implications are that longer dry spells will be broken by occasional very heavy storms. This has serious repercussions for the farmer, but from the point of view of the CWPU reservoirs can be filled up just as conveniently in one or two great cloudbursts as by weeks of standard British drizzle.

The CWPU itself is situated on the banks of the River Thames at Reading, and members of the Unit (then part of the Water Resources Board) were treated to the sight of the river flooding its banks as recently as January 1974; the same high rainfall which produced that rise in the river helped to stock up the surface reservoirs. But the problems of replenishment of underground aquifers cannot be overcome so easily. Some 25 to 30% of the public water supply is derived from underground sources, and in periods of drought what precipitation there is tends to be absorbed in the surface layers of the ground, or runs off as floodwater, without having much effect on the aquifers; 10% less rainfall over a year can result in as much as a 50% drop in aquifer recharge.

Whither water?

Water services in Britain have recently been reorganised, with the Water Resources Board ceasing to exist on April 1, 1974. John Gribbin discusses future plans for water resources in the light of the last annual report of the Board and the growing awareness of the possible importance of climatic change.

This is where the possibility of longer term changes in rainfall becomes important.

The average rainfall of England and Wales has declined over the past five years; but is this a continuing trend, or is it likely to reverse in the immediate future? It is too early yet for panic measures on the strength of such evidence alone, although it is perhaps worth noting that Professor Hubert Lamb has commented that the first 50 years of this century, which are accepted as the climatic normal, were in

sources Board could, if it so wished, use any part of its research funds in support of climatic research. But now any proposal for extending the present investigation (or, indeed, starting any new project) must first go to the DoE before filtering back down through the CWPU or the Water Research Centre. A spokesman for the DoE confirmed recently that proposals for future research along these lines have been discussed with Professor Lamb, who heads the Climatic Research Unit, and that "the proposals are now under consideration by the Department". Meanwhile, the planners have plenty on their plate in working out how to distribute the rainfall we do get, without worrying too much about what might happen to that rainfall in future.

The way the CWPU would like to see things develop is towards what might loosely be termed a 'national water grid'. This would involve transfers from region to region—such as from the Severn to the Thames—and extending the present system of river regulation. Again, however, this depends on how things develop as the new regional authorities feel their way. There is nothing to stop a region opting for

Table 1 Rainfall over England and Wales, for year ended September 30, 1973

Month	Monthly rainfall (mm)	1916-50 Standard average (mm)	Percentage of average
1972			
October	32	92	35
November	99	95	104
December	104	88	118
1973			
January	44	92	48
February	40	66	61
March	24	57	42
Winter	343	490	70
April	67	60	112
May	84	63	133
June	63	55	115
July	91	79	115
August	63	81	78
September	86	76	113
Summer	454	414	110
Year	797	904	88

fact "perhaps the most abnormal period for the past thousand years" in terms of the British climate. What is important is that the CWPU, at least, takes the possibility of climatic change seriously. A spokesman said that they "would be very interested in seeing more work [of this kind] done, and are open to suggestions for programmes of research". The old Board has provided funds for research into the variability of rainfall in the English lowlands at the Climatic Research Unit of the University of East Anglia, but the present situation with regard to the continued funding of such work is not yet clear.

Under the situation which prevailed until April this year, the Water Re-

independence in water supply, and dotting the local countryside with reservoirs rather than 'importing' water from neighbours.

Indeed, the most nationalistic of the Welsh might press the case that England should be made to pay for 'Welsh' water—although the CWPU has a stock answer to that: build a dam across the border and see what happens if the Welsh do try to keep all their water!

In fact, of course, water from Wales is so important because of a coincidence of geography and climate. The water brought in from the Atlantic is dumped conveniently on the Welsh mountains, providing the possibility of storing it near the source of rivers and using it to regulate their flow. This makes schemes

to develop that potential still further more attractive than alternatives, such as barrages at the Wash or in Morecombe bay; indeed, one plan now being studied would involve expansion of the Craig Goch reservoir to produce 500 million gallons a day—more than twice the capacity of the Wash scheme in its latest form.

The Craig Goch scheme is representative of the change in philosophy of the water planners from the days when direct supply from reservoirs to consumers was fashionable. Now, the aim is to use rivers wherever possible, meeting the demand for abstraction by regulating the flow using reservoirs at the head of the river. This also improves the health of the river, as well as making it possible to take out more water than from a comparable 'wild' river.

Because of this philosophy of river regulation, the planners must now take increasing account of weather forecasting on a day-to-day basis. If water is released from a reservoir during a dry spell to maintain the flow of a river, it could be embarrassing if heavy rainfall a couple of days later were to occur lower down the river just as the surge from the reservoir arrived. This has provided one of the closest direct links between the old Board and the Meteorological Office in recent years, when both have collaborated with Plessey Radar Ltd and the Dee and Clwyd River Authority on a radar weather forecasting project which has formed part of the River Dee Regulation Research Programme.

Apart from the new outlook on surface water management—which depends very much on the cooperation of the new regional authorities—there are also plans to develop groundwater abstraction. In the tenth (and last) Annual Report of the Water Resources Board (HMSO, 79p) it is suggested that resources will be available to maintain the proportion of water derived from aquifer storage until the end of this century, even though total demand is expected to double by then. In the long term, there is the possibility of artificial recharge of aquifers from wells or by



Craig Goch reservoir

“infiltration” through lagoons or basins; pilot projects carried out in the London Basin, in Nottinghamshire, Sussex and other parts of England and Wales have shown the feasibility of refilling some strata with water and abstracting the water when required, and research into and development of such techniques must clearly have very high priority in the plans of the Water Research Centre and the CWPU.

But one superficially attractive proposal for the future seems to have been ruled out for some time to come. Desalination has been widely mooted as a practical system of obtaining pure water in Britain, because of the extensive coastline. But studies suggest that it will be simply too expensive in the fore-

seeable future (the next 25 years), especially when the rising costs of fuel are taken into account. In addition, the environment lobby, which by and large seems to favour the idea of desalination, has not, perhaps, taken full account of the impact on the coastal environment of unsightly desalination plants. Just about the only economically viable use of these plants in Britain at present would be to top up the supply of coastal resorts in the summer, when their population rockets and demand is highest (indeed, desalination is used in just this way in the Channel Islands). But on the other hand, the authorities of areas which depend on the income obtained from visitors attracted by the natural beauties of the coast are the least likely to authorise construction of new industry.

However the investigation of climatic change develops, the question of water supply is bound to occupy an increasingly important place in the thoughts of planners around the world as population increases. Desalination will obviously be important in some areas, if not in Britain. But the problems with desalination as a commercial proposition highlight the truth that although “two thirds of our planet is covered by water” we must think very carefully indeed about where we are going to get a drop to drink in the year 2001 □

Table 2 Water Resources Board expenditure on research

Financial Year	Research £	Section 90* £	Total £
1966-67	31,265	17,948	49,213
1967-68	41,260	41,553	82,813
1968-69	63,866	121,604	185,470
1969-70	120,878	107,998	228,876
1970-71	259,967	167,254	427,221
1971-72	226,679	194,027	420,706
1972-73	237,111	293,686	530,797
1973-74 (estimated)	436,300	347,700	784,000

* “Section 90” is the section of the 1963 Act which empowered the Water Resources Board to provide financial support for work and research carried out by the then river authorities.

international news

AN appeal to the scientific community by a committee of the National Academy of Sciences to refrain from conducting two types of genetic manipulation experiments because of potential hazards to society (see *Nature*, July 19) has drawn a swift and positive response from the National Institutes of Health (NIH). In a letter sent last week to Academy President Philip Handler, NIH Director Robert S. Stone indicated that he will establish a committee to define the possible hazards associated with such research and that NIH is willing to support an international meeting of scientists to discuss the matter.

The academy committee has urged that a moratorium should be placed on experiments which (I) involve the introduction into a bacterium of genes which either confer resistance to antibiotics or cause the formation of bacterial toxins and (II) those which involve the introduction of genes from viruses into bacteria. Furthermore, the committee suggested that experiments which introduce genes from animals into bacteria "should not be undertaken lightly".

The reason for concern is that the bacterium most commonly used for such studies is *Escherichia coli*, which

NIH backing for NAS ban

by Colin Norman, Washington

usually present in the human intestine. But the committee is quick to point out that the concern is "based on judgments of potential rather than demonstrated risk", and that the danger is yet to be precisely defined.

Each member of the committee, which was chaired by Dr Paul Berg of Stanford University, has agreed to eschew experiments of both sorts. And they have called on their colleagues throughout the world to join them in deferring such research until the hazards have been evaluated.

When the committee's statement was made public last week, Berg said that an international meeting is being planned for next February to "discuss whether there are in fact experiments that should or should not be done". The meeting, which will probably be attended by about 100-150 scientists, will not only attempt to define what types of research should be included in the embargo but also for how long it should be maintained.

Asked whether he expects that a

voluntary moratorium will be effective, Dr David Baltimore, a member of the committee, said that at present he knows of no laboratory which is planning to undertake experiments of type I or II, and that peer pressure on scientists now to eschew such studies will probably be sufficient to make the embargo stick. He pointed out, for example, that study groups at NIH—committees of scientists which provide initial peer review of grant applications—will be wary of approving funds for such studies and that the editorial boards of scientific journals will probably think twice about publishing research papers derived from experiments covered by the embargo. "To me", he said, "it is almost unthinkable that scientists would go out and do this type of work now".

The committee acknowledges, however, that its recommendations "will entail postponement or possible abandonment of certain types of scientifically worthwhile experiments". It therefore remains to be seen how long the scientific community will go along with this move toward self regulation, and much will depend on the outcome of the NIH committee's deliberations and next February's international meeting.

What British scientists say . . .

Molecular dirty tricks ban

THE critics of molecular biology are fond of pointing out the scarcity of practical benefits. It is a sad paradox that the very developments which could ultimately have immense value for production of useful, but rare, molecules, should result in an urgent cry of 'halt' from a committee of leading scientists involved, supported by no less than the United States National Academy of Sciences. The amplification of selected genes and their products by synthetic recombination with freely replicating DNA of bacterial plasmids, could, for example, revolutionise the commercial production of substances like insulin, or pituitary hormones, while the bulk synthesis of a transforming gene protein from an oncogenic virus might allow the design of specific antagonists. As more genes are identified on cleavage products of DNA from animal

cells and viruses, so the potential for practical application will increase, to say nothing of the basic knowledge gained.

Yet few will deny the wisdom of the appeal for a moratorium on certain classes of experiment until the implications are more clearly understood. It is not just the risk of unpredicted and explosive self replication of some sinister DNA sequences which causes concern, it is the possibility that by using *Escherichia coli*, the workhorse of molecular biology, the explosion might occur in someone's gut and then be transmitted like antibiotic resistance, throughout the world. No doubt a good many dirty tricks have been attempted and discarded by nature in the course of evolution, but the disquiet arises from the utterly novel associations of genetic material which are now possible. The potential benefits should, therefore,

be delayed, not for ever, but until consequences can be assessed, and preliminary experiments carried out under conditions of maximum security.

Most of the technology involved in all this has been developed in the United States and it is encouraging that the very leaders in the field have taken the initiative and have been supported by the academy. It is now to be hoped that academics and learned societies in other countries will add their weight, and that international organisations such as the European Molecular Biology Organisation will lend support. Granting agencies, and even editors of scientific journals, will also need to consider their policies in the face of wide support for a moratorium. For many it will be a test of self denial and social responsibility in the face of strong intellectual temptation.

Michael Stoker, ICRF

The indiscriminate use of antibiotics has exerted more pressure on the bacterial population than could be wielded by all research workers in the field put together.

from E. S. Anderson, Colindale

THE NAS statement on plasmid engineering evokes a mixed reaction from me. Whatever its justifiability, I wish it had been presented less pompously. We have been more aware since our first experiments in transferable resistance in 1964 that by introducing an R factor into a pathogen we reinforced this organism's pathogenicity with resistance to drugs that might be effective against the respective disease. In particular, when chloramphenicol resistance was introduced into the typhoid bacillus, we were manufacturing a virulent pathogen resistant to the drug of choice for typhoid fever. And we knew that if we presented such a strain with the opportunity of causing infection we would have been guilty of a grave antisocial offence. But R factors had become important in the enterobacteria and they demanded study. We could not do such work effectively if we shirked the step of introducing them into pathogens. So we transferred them to pathogenic enterobacteria, including *Salmonella typhi*, exercising our routine precautions for avoiding infection with the respective organisms, and for their safe storage. We have studied the typhoid bacillus and related organisms for many years, but no infections have resulted from our work, inside or outside the laboratory; so our protective measures must be reasonably efficient. Moreover, it is fair to say that these manipulations have contributed to the understanding of R factors and other plasmids, including the evolution of an easy method for establishing the plasmid nature of elements concerned with enterobacterial drug resistance, virulence or other properties.

The *in vitro* hybridisation of plasmid DNA with DNAs of widely different, even eukaryotic, origin, adds a new dimension to plasmid studies. Once introduced into the bacterial cell, the hybrid plasmids resume the relationships of their bacterial moiety and multiply with the cell. The eukaryotic regions, however, retain their original coding capacity and initiate the synthesis of the corresponding products.

What is the point of such experiments? First, they demonstrate that DNA-DNA hybridisation need not require extensive regions of base-pair homology. They therefore expose processes that may have important evolutionary significance or medical potential. Second, they may help to clarify the *modus operandi* and the nature of the products of the "Xeno-DNA", if one may coin such a term for the introduced foreign DNA. Normally, such

studies are hampered by the relatively slow cell growth and much greater complexity of the respective animals or

tissue cultures. In hybrid bacterial-animal plasmids, synthesis and replication are geared to the much higher kinetics of bacterial growth, so that the respective DNA and its products can be isolated in adequate quantities in a short time. This might accelerate the studies of these materials and could add

Alternative experiments?

SECTIONS of genetic material from any source can now be attached to a bacterial plasmid or bacteriophage and so introduced into the bacterial cell where they replicate quickly and can be recovered in relatively large amounts. By-passing the normal biological barriers between species in this way means that completely novel genetic combinations can be created and disseminated.

But it is at present impossible to predict all the properties and ecological consequences of these new genetic arrangements. Not unnaturally this has led to widespread concern over possible biohazards from such research, which has resulted in the National Academy of Sciences appeal. Concern is enhanced because the ubiquitous human enteric bacterium *Escherichia coli*, has been used as the organism in experiments carried out so far.

Anxieties arise on two main counts. (1) It is generally impossible to transpose selectively only those DNA fragments with known functions. Potentially harmful DNA fragments, such as those containing possible oncogenes, may unwittingly be transferred to a new host where their expression may not be properly controlled. (2) The vehicles used (in the work published so far) to transfer foreign DNA fragments to *E. coli* were plasmids that carry genes determining resistance to antibiotics.

The NAS embargo covers two types of experiment (which have already been accomplished by some of the signatories of the statement), namely the transfer of genes determining drug resistance between bacterial strains or species that do not normally carry such resistance, and the fusion of animal virus genes to transmissible systems.

The NAS request is both reasonable and responsible and deserves to be universally respected. It recognises both the difficulty in evaluating real or potential hazards that may be involved in such work, as well as the obvious criticism that these will remain obscure in the

absence of experimental study; urgent consideration of the latter is explicitly recommended.

Fears that the proposed limitations to experiments will seriously obstruct research in vital areas of biology seem unfounded. The NAS initiative, by focussing attention on the hazards involved, could well promote rather than hinder work on *in vitro* recombination in animal viral systems, an area believed by many to hold the key to gene therapy in its broadest terms. Similarly, the constraints on experiments with plasmids determining drug resistance need not preclude cautious use of the plasmids for cloning certain DNA fragments. Many experiments in this latter area can also be undertaken with bacteriophage λ which is a safer vector for *in vitro* recombination in that it has strict host specificity and carries no drug resistance determinants; it has the great additional attribute of bringing a wealth of genetic and biochemical experience to the service of studies on the cloned fragment of DNA. Use of phage λ does not, of course, eliminate hazards associated with the incorporation of unknown genes.

In view of the anxieties expressed, the wisdom of using a normal human enteric bacterium as host organism in these experiments might be questioned. Certainly there is no reason to believe that one could not select quite different microbial and viral systems that do not inhabit man or mammals, but the hazards, real or potential, would apply equally well to other ecosystems and thus eventually raise the same problem. To do this, however, would be to forsake the wealth of information and experience with *E. coli* and its viruses accumulated from decades of research. This would be prodigal to say the least.

While welcoming the NAS initiative, one is also appreciative of Anthony Tucker's perspective in his comment "Life Stylists" (*The Guardian*, July 19); equally, if we follow the moderate tone set by the NAS we shall be careful not to oversell the social benefits devolving from recent experiments.

Ken Murray, Edinburgh

substantially to the understanding of the respective systems. By hybridisation of oncogenic viral genomes with bacterial viruses or plasmids, cancer workers could perhaps selectively enrich the return of oncogenic material for study.

The signatories of the NAS letter define two types of experiment on which they recommend a moratorium. Type I comprises experiments in which plasmids coding for drug resistance or potential pathogenicity are introduced into pathogens or innocuous bacteria. The fear is of the risk to treatment if a newly resistant pathogen reached its host; or of the emergence of new pathogens that might become ecologically important. *In vitro* construction of plasmids for new drug resistance would also be suspended.

Type II experiments in the NAS letter are those involving hybridisation of plasmid with eukaryotic, or oncogenic or other viral DNA. Such hybridisation might create hazards of an unforeseen nature, since the potential of the hybrid genome cannot be accurately predicted. Hybrid plasmids may thus have oncogenic or other potential for man or other animals. And since the enterobacteria, most widely used for plasmid study, are ordinary inhabitants of the human or animal intestine, they may by accident or design enter the intestine of a suitable host, spread, and act as the vectors of cancer and related diseases, perhaps with augmented virulence or on an epidemic scale.

A sense of proportion must be retained in considering these matters. The widespread and indiscriminate use of

antibacterial drugs in man and animals has exerted immeasurably more pressure on the bacterial population than could be wielded by all the research workers in this field put together. These drugs are used prodigiously in human and animal medicine, and as animal feed additives. The long-predicted results, now realised, were the spread on a gigantic scale of transferable drug resistance plasmids in the ordinary intestinal bacteria such as *Escherichia coli*; and the emergence of epidemic drug-resistant and lethal lines of enterobacterial pathogens: *Shigella dysenteriae* I (Shiga's bacillus) in Central America, the typhoid bacillus in Mexico, India, South Vietnam and Thailand, and *S. typhimurium* and other salmonellae with massive drug resistance and apparently increased virulence in many countries. This has been the direct consequence, not of plasmid work, but of commercial pressures and administrative and professional irresponsibility.

For these reasons I feel little inclination to respond to the call for a moratorium on type I experiments in the NAS letter.

Hybrid plasmids formed by the introduction of normal, and oncogenic and other viral DNA, may present a potential threat, but it is difficult to see how this could be evaluated. Experiments on animals that are the natural hosts of particular viruses spring to mind. But these may not reveal potentialities of the hybrids that might be expressed in different animals. Naturally, tissue culture would be used and the range of experimental animals would include primates, but if negative results are obtained in these, how and

when can we be sure that the chimaeric plasmids are innocuous to man or other animals? The risks cannot be quantified as can energy release from nuclear reactions, because the processes are biological and complex, not physical and simple. And the techniques needed for reliable exposure of oncogenic potential may not yet have been devised.

There is therefore a need for caution in the type II experiments. Bacterial vectors of hybrid plasmids can, however, be adequately controlled by the ordinary sterile techniques of laboratories working with pathogens. This would call for technical re-education of the average microbial geneticist or molecular biologist, whose manipulation of bacteria chills the blood of anyone accustomed to handling pathogens. Cultures should be stored securely: methods for doing this already exist. Such precautions, which should be routine, minimise the risk of accidental spread of dangerous pathogens, and no technical distinction should be made between known pathogens and presumed non-pathogens.

Strict isolation techniques must be practised if animal experiments with bacteria carrying plasmids in the type II category are carried out.

The methods needed to maximise safety in such investigations are known, and plasmid researchers must learn them if work on these hybrid plasmids is to proceed. The only alternative is the abandonment of type II experiments, or at least a chosen proportion of them, which may mean the closure of potentially valuable avenues of research. There is a manifest need for expert international debate in this field.

THERE is a particularly irritating television commercial in the United States which contains the classic line that "all aspirin is not alike". Although that phrase was coined on Madison Avenue to make money rather than to make a scientific point, it is typical of the claims that are flying around in a bitter dispute between pharmaceutical manufacturers and the federal government over the quality and price of American drugs.

The dispute is simple enough. The government, backed by consumer groups, argues that a particular drug should produce the same effects no matter who manufactures it, and so government agencies should not throw money away by buying an expensive brand-name drug when the same product is available at a lower price.

But many drug manufacturers take a different view. They maintain that products containing the same ingredients but manufactured in different ways may produce different therapeutic effects. Therefore, they argue, if the

Differences between the same drugs

by Colin Norman, Washington

government insists on buying the lowest priced drugs it may also be getting the lowest quality products.

Last week, the Office of Technology Assessment (OTA), Congress's new scientific think tank, stepped into the debate. In its first report, prepared by a panel of ten eminent biomedical scientists, OTA provided at least partial backing for the arguments of the drug industry by stating that many drug products are not interchangeable, chiefly because shoddy manufacture and poor enforcement of quality standards by the federal government do not guarantee therapeutic equivalence.

The OTA panel, chaired by Dr Robert W. Berliner, Dean of the School of Medicine at Yale University and former Deputy Director of the National Institutes of Health, cited a number of

"well-documented and significant differences" between drug products that are supposed to be chemically equivalent. The differences, in fact, were not confined to drugs made by different manufacturers, but also cropped up among different batches of drugs made by the same company.

Perhaps the most clear-cut and alarming incidence of such disparities came to light during a study carried out at a New York City Hospital in 1971, which was singled out in the OTA report. That study turned up evidence that four different brands of a highly potent heart drug, digoxin, were so strikingly different in their effects that the peak concentration of the drug in the bloodstream differed by a factor of seven. Yet the margin of safety of digoxin is so narrow that lethal effects can occur if the dose given is only twice that needed to produce a therapeutic effect.

The OTA panel reckons that there is probably "roughly a score of drugs" for which there is evidence of dif-

ferences in so-called bioavailability among brands which are supposedly chemically equivalent. And it suggests that such differences have resulted in therapeutic failures or inadvertent poisonings when patients have been given doses containing too little or too much of the prescribed drug.

The importance of the OTA's findings is that they have a direct bearing on proposed new policies governing payment for drugs by the federal government. On December 19 last year, the Secretary of Health, Education and Welfare, Caspar Weinberger, sent a shiver of apprehension through the pharmaceutical industry by announcing that the federal government would limit the price paid for drugs under the state-run Medicaid and Medicare programmes to "the lowest cost at which the drug is generally available". About \$1,300 million is paid each year for prescription drugs under those programmes, and Weinberger estimated that the new policy would save between 5 and 8% of the costs.

But last week the Pharmaceutical Manufacturers' Association (PMA), the trade association which represents nearly all the big United States drug makers, claimed that the OTA report "completely undercuts the ill-advised proposal" for drug purchasing, because it challenges the basic principle that chemically equivalent drugs are interchangeable.

Berliner pointed out last week, however, that for most drug products biological equivalence is not critical because there is a wide margin of safety between the dose needed for therapeutic effects and the toxic dose. He reckoned that only "about 10 to 15%" of the drugs on the market may pose a problem because of therapeutic differences between brands.

Science studies in Amsterdam

by a Correspondent

At a time when the values of scientific activity are increasingly being questioned, so social studies of science are being included as optional or compulsory courses in universities round the world. British experience in this field—particularly that at the Universities of Edinburgh, Manchester and Sussex—has achieved considerable note during the past few years. So therefore it comes as no great surprise, even in these times of fluctuating interest in European collaboration, that the programme of teaching and research in science studies at Edinburgh will be the model for an unusual new unit at the Free University in Amsterdam. Moreover, British and Dutch collabora-

Confiscated manuscripts

by Vera Rich

THE scheduled date of the Moscow "seminar that never was" has passed, and the would-be organisers and participants have been, at least temporarily, released from custody—yet the history of this valiant venture in academic freedom seems far from complete. As might be expected, the attention of the authorities is still focused on Professor Aleksandr Voronel, whose Moscow apartment was to have been the venue of the seminar. On Friday July 19, 1974, his apartment was subjected to an 8-hour search by the

police who confiscated some 2,000 papers, mostly scientific manuscripts, including those submitted by intending participants in the international seminar. On the same day, the fourteen-year-old son of Dr Benor Gurfel, another of the seminar sponsors, was stopped in the street on his way to deliver certain manuscripts to Professor Voronel. These were also confiscated.

Professor Voronel and his wife are under constant and obvious surveillance, and are at present staying away from the apartment. This confiscation of manuscripts is seen by informed observers as an ominous sign—the intensive campaigns against major dissidents such as Solzhenitsyn began in precisely this way.

ration is further strengthened by the appointment from August of a British historian of science and former palaeontologist, Dr Martin J. S. Rudwick, from the Department of History and Philosophy of Science, Cambridge, as the unit's first director and also as the first professor of History and Social Studies of Science in the Netherlands.

The Free University has long had a liberal attitude to the education of its science students. A course in general philosophy has been compulsory since the university's foundation in 1880 and history of science was added to the curriculum of all students in the late 1940s on the appointment of the noted historian of science, Dr R. Hooykaas, as professor. When Professor Hooykaas retired—about the time of the student unrest in the Netherlands at the end of the 1960s—it was decided that it would be expedient to widen still further the scope of the curriculum by introducing courses in science and society. For the past two years staff from the History and Social Studies of Science Division at the Science Policy Research Unit, convened by Dr R. M. MacLeod, have helped Dr E. Boeker, Professor of Physics in the Natural Sciences Laboratory, to prepare the ground for the new unit which will be the cornerstone of the Centrum Algemene Vorming (Centre for General Education) in the Faculty of Mathematics and Natural Sciences.

All first and second year students in the five departments of the faculty must now attend 52 lectures a year on philosophy, and the history of 'science and society'. Since 1972/73, teaching has been divided between the staff of the science and philosophy departments and visiting scientists from industry as well as guest lecturers from Sussex. The Sussex staff have also assisted with the seminars for the science and society 'minor' option

which graduate students can take during their fourth and fifth years.

When Dr Rudwick assumes his appointment in August, the unit will then start acting as general co-ordinator of all the courses in general studies, including those at present taught by the different departments—the so called encyclopaedia. The first PhD student in science studies at the Free University (a physicist who is currently taking the MSc course at Sussex) will register next year and it is hoped that in time the unit will be able to carry out a regular programme of research. Dr Rudwick's present interest is the social and conceptual history of science; to complement this, it is expected that staff who specialise in the sociology of contemporary science will eventually join him in the Centrum.

Although science studies and science policy units are found in other universities on the continent—for example, at Heidelberg and Lund—they are usually separate from the central core of the science faculty. At the other five universities in the Netherlands, some science and society courses are available, but these are not as widely based as that offered at the Free University in Amsterdam. Chemistry students seem to be the most privileged in this respect; at the State University of Groningen, for example, the chemistry department offers a major in 'free chemistry' with courses in chemistry, physics, economics and sociology. There has been some success in introducing discussions on ethical problems into biology courses, but in physics and mathematics departments there has been more resistance, though in the department of physics at the Technical University, Eindhoven, students can do a minor in physics and society.

No takers

A WEALTHY businessman's plans to give grants to young scientists for basic biological research ended in disenchantment on both sides at a meeting in London recently, when it transpired that he would not consider any applicant who held any sort of religious belief.

Mr Ray Turner had hoped to set up a foundation to award grants to young biologists under 28 years old for "independent fundamental research into outstanding questions relating to the chemical organisation of life". The grants were to be at a rate 20% above the United Kingdom universities non-clinical academic scale with tenure for 5 years initially and the opportunity for repeated extension; in effect allowing the possibility of a lifetime's financial support.

Thirty-six applicants who, under the terms of the application, had been nominated by senior researchers, assembled at the Strand Palace Hotel at Mr Turner's expense for an informal meeting to present their research proposals for selection.

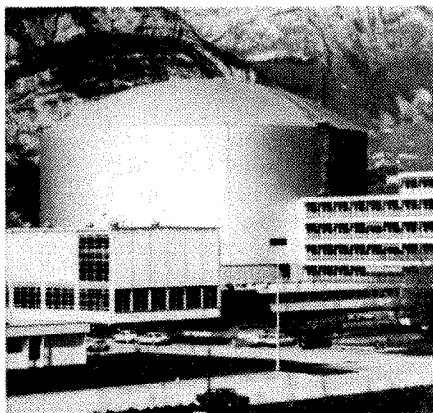
As most of them expected some sort of scientific panel, they were surprised to find only Mr Turner, his wife and Professor Bellamy of the Zoology Department at University College Cardiff, the 'scientific coordinator' who had signed the original circulars inviting applications which were sent to university heads of departments in early June.

Each applicant was asked for a brief resumé of his work and aims, and his ideas of "the single basic facet of the really great scientist". Mr Turner then said that he wished to ask a question which might surprise some people but that the motive would be explained later.

He then asked if anybody had religious beliefs. Four applicants put up their hands and their names were noted. The rest were then asked whether any of them held any belief in a "supernatural plan". More put up their hands and their names were also noted. The remainder resolved, after lively discussion, into what Turner classed as agnostics and atheists.

As it had become clear by now that selection was not going to be made on conventional scientific grounds, most of the participants thought that this might be some sort of 'management selection technique' but during lunch Mr Turner had a private discussion with one of the participants, Peter Leadley, who had put up his hand in the first group. Mr Turner told him that although he was considered among the most promising of those present, he could not be considered for a grant on account of his religious beliefs.

Now it's legal



Reactor in the Alps

It was a symbolic occasion at Grenoble last week as the ministers from Great Britain, France and West Germany signed an agreement bringing Britain into equal partnership with the other two countries in supporting the high flux neutron beam facility at the Institut von Laue-Langevin. Symbolic, because the partnership has existed *de facto* since January 1973. And yet, such is European collaboration on other things that since then the three countries have been trying hard to find a convenient date for ministers to participate in a ceremony giving tangible evidence that in some fields an international spirit still prevails.

The institute is hardly unfamiliar with symbolism; it exists in large part because France and Germany needed some collaborative scheme in the mid-1960's to patch up relations—indeed folklore goes so far as to say that Professor H. Maier-Leibnitz was asked to propose a project, suggested a high flux reactor and so it was. Not that it is in any way a white elephant. There was an urgent need for such a facility to further research in all fields from nuclear physics to biology, and it is

widely agreed that the institute is fulfilling its purpose admirably.

The 57-MW reactor was built by French and German firms and first went critical in August 1971. It delivers a maximum flux of 1.5×10^{15} neutrons $\text{cm}^{-2} \text{s}^{-1}$ and, in addition to supplying thermal neutrons to up to 50 separate experiments, has a cold source—25 litres of liquid deuterium as a low temperature (25 K) moderator—and a hot source—10 litres of graphite as a high temperature (2,000 K) moderator.

The main use of the neutrons so far has been in the study of condensed matter. Neutron diffraction can determine crystal structures, including the location of light atoms which are inaccessible to X-ray diffraction. Long wavelength neutrons are used to study defects and biological structures. The magnetic moment of the neutron is a valuable tool in understanding magnetic materials, and inelastic scattering is being used in studies of the dynamics of atoms and molecules.

As would be expected the scientific staff is international in character, although the British, who have been pioneers in neutron techniques, face problems in uprooting themselves for a period of several years in that return to a Britain with a depressed job market in high-flying science is bound to cause worries. On the other hand British technicians there speak with enthusiasm of their conditions and salary and some are prepared to stay indefinitely.

The running costs of the reactor and institute are £6 million this year but Professor Mössbauer, the director, is seriously concerned about inflation. Commitments in the newly signed document are inadequate for inflationary times and it has not been possible to hammer out tripartite agreement on including inflation automatically into each country's contribution. "As soon as the ink is dry", he said "I have to go back to the sponsors to negotiate more money for next year".

While the scientists were still debating whether even this might be some sort of psychological selection test, Mr Turner announced that he could not consider any of the applicants who had expressed any belief in a religious or supernatural power.

Mr Turner first said that he did not wish to give his reasons but during the three and a half hours discussion that ensued he eventually explained that he felt that if a scientist believed in any sort of supernatural power he would experience conflict in researching problems which enquired into the fundamental nature of life. He therefore thought that the very best scientists, the ones he wished to encourage, could not be religious.

At this point, Professor Bellamy who had taken little part in the proceedings so far, stated his opposition to the method of selection as did several of the 36 scientists, some of whom then left. The rest, according to a participant who stayed, tried to make Mr Turner realise that his sort of criteria could not be used as a basis for selecting scientific work and that it would be unacceptable to the scientific community at large. Mr Turner was asked whether he would consider handing over the money to scientific trustees to administer, but he refused.

Eventually it became obvious that deadlock had been reached and Mr Turner announced that he was withdrawing his offer.

news and views

Nutrition and immunity

As is demonstrated by the communication of Aschkenasy on page 325 of this issue of *Nature*, there is currently a broad focus of interest in the relations between nutrition and immunology. At first glance the association of these two disciplines may seem strange; their union grew from clinical and epidemiological experience in malnutrition and infectious diseases, experience that eventually led to the understanding that either of these conditions tended to augment the other. Many observations, such as that of an increased morbidity and mortality from measles among sub-optimally nourished children, suggested that nutritional deprivation tends to render persons more susceptible to certain disease, and suggested that some aspects of host resistance are functionally impaired. Meanwhile, laboratory workers were amassing evidence in support of the concept that nutritional manipulations could indeed affect the immune response. Much of the clinical and laboratory work concerning the nutrition-infection-immunity cycle was published in 1968 in a classic World Health Organisation monograph (*Monograph Ser. W.H.O.*, No. 57). As it became generally acceptable that the immune response accounts for much of host resistance, it also became more reasonable to ask if malnutrition affects the immune response. Answers to this type of question required the collaboration of nutritionalists and immunologists, and forged a new direction for both sciences.

Studies of nutritional-immunological relationships in man are difficult, for many reasons. In the first instance, pragmatists may assert, with some justification, that the answer to malnutrition is to provide more food. This is of course true, but until more food is provided the problem will exist, and famine and pestilence will continue. Another approach to the problem might be gained by asking what particular components of host resistance are affected by malnutrition, and by the subsequent use of modern immunology, nutrition or public health either to repair the lesion specifically or to circumvent the need for its function. Some persons have maintained that the problem is too complex to study because of the great number of uncontrollable variables. Logistics have been another impediment to the study of nutritional-immunological interactions in man. Malnutrition often exists in areas where medical and research facilities are limited, where weather and terrain are difficult and where many other social and medical problems require priority. In spite of these difficulties, several national and international centres and institutions have begun to construct a matrix of collaboration and communication with the purpose of defining and dissecting some of the effects of malnutrition on the immune response.

For the most part, these studies have sprung from individual efforts, but many of the investigators have elected to coordinate aspects of their studies through the World Health Organisation (*Bull. Wld Hlth Org.*, 46, 537; 1972). The WHO has responded in its usual efficient and effective way by formulating an international collaborative study (*Amer. J. clin. Nutr.*, 27, 638; 1974), the fabric of which has served to strengthen collaboration between national and international bodies, and to extend communications be-

tween field and laboratory workers. More information about this can be obtained by writing directly to the World Health Organisation in Geneva.

Although a great deal of work remains to be done, data from field and laboratory studies thus far do seem to be piecing together a preliminary but coherent picture of the immune response in malnutrition. For example, amounts of serum immunoglobulin (Ig) do not seem to be affected consistently by protein-calorie malnutrition (PCM), but elevated levels, especially of IgA, are not unusual (*J. Pediat.*, 81, 1194; 1972). Increased values for Ig in PCM have been attributed to intercurrent infections (*Archs. Dis. Childh.*, 45, 282; 1970). This seemingly increased synthesis of Ig needs to be reckoned with data that convincingly show serum albumin (*Lancet*, i, 63; 1973) and most components of the blood complement system to be depressed in PCM (*ibid.*, i, 1016; 1973). Amounts of antibody in malnourished persons are also inconstantly affected, but in these circumstances the effect seems to be antigen-dependent; for instance, vaccination against polio or measles produces measurable antibody (*Archos lat-am. Nut.*, 23, 345; 1973); but some viral and several bacterial antigens do not produce a high incidence of seroconversion (*Trop. geogr. Med.*, 18, 125; 1966). These observations would seem to be relevant to current vaccination programmes. They also suggest a clear need for more applied and basic research into problems such as the effectiveness of dietary supplementation before vaccination, the route of immunisation, the type of adjuvants, and the relative T- or B-cell dependance of antigens for human use.

Studies of cell-mediated immunity (CMI) in human malnutrition have suggested that the thymus-derived components of the immunological system are damaged. Indeed, histopathological studies have reported a virtual absence of thymus tissue (*Envir. Child Hlth.*, 217; September 1972). These morphological alterations seem to be mirrored in tests of T-cell function; for instance, results of skin testing for delayed hypersensitivity reactions to many antigens, particularly to tuberculin following BGG vaccination (*Lancet*, ii, 719; 1965), have been reported to be significantly depressed (*ibid.*, i, 506; 1973). Insofar as positive tuberculin reactions are thought to be associated with protection following BCG, the absence of such reactions is likely to be relevant to the incidence of tuberculosis among sub-optimally nourished persons. Other measures of CMI have also been reported to be depressed in malnutrition. The response of human peripheral blood lymphocytes to phytohaemagglutinin *in vitro* is generally regarded as a measure of T-cell function, and this reaction is reportedly suppressed in PCM (*Lancet*, ii, 939; 1971). Numbers of T cells can also be measured *in vitro* by allowing them to form rosettes with other cells, and results using this test have again suggested that malnourished persons have diminished numbers of T cells. The message of these several lines of evidence seems to point to a type of T-cell immunodeficiency in malnutrition. If this is true, it does not, of course, alter the dictum that the answer to the cycle of malnutrition and infection is to provide more food, but it does suggest that perhaps the cycle can be prophylactically or therapeutically approached from another bias.

Several biochemical and cellular events important in the nutrition-immunity-infection cycle have also been studied. Biochemical studies of phagocytic cells from malnourished

persons have reported a depressed glycolytic pathway (*Amer. J. clin. Nutr.*, **24**, 272; 1971), and these defects are reversed when patients are given a diet rich in calories and proteins (*Biochem. J.* **127**, 255; 1972). Functional studies of similar cells have reported a delay in the killing of phagocytosed bacteria, and this defect is also reversed by adequate diet (*Clin. Exp. Immunol.*, **17**, 121; 1974). Another defect in the killing of phagocytosed bacteria is corrected simply by the addition of iron (*Archs. Dis. Childh.*, **48**, 863; 1973). If these various defects represent different mechanisms which are corrected by different types of dietary supplementation, it would seem to be wise to know more about the mechanisms involved. The bacterial-killing defect, which is presumably lysosomal, may be related to the immunodeficiency discussed earlier; for instance, one mechanism for activating macrophages to kill phagocytosed bacteria is by way of products from antigen-activated T cells. This type of basic information is being integrated into modern nutrition and public health programmes, the practical effects of which are yet to be determined.

A great deal of work remains to be done. Almost nothing is known about the effects of intrauterine malnutrition on the development of the immunological system, the long-term effects of early malnutrition on the immune response, or the effectiveness of vaccination programmes in marginally nourished populations (*Lancet*, **ii**, 675; 1972). Answers to these questions would seem to be relevant to public health programmes in both developing and developed countries. Many of these questions can be addressed to animal models as has been done by Aschkenasy (*Immunology*, **24**, 617; 1973) and many others. This brief comment has not covered experimental models because of their obvious importance. Neither immunologists nor nutritionists pretend to have an answer to the malnutrition-infection cycle, but their combined approach has broadened understanding of an area of human biology that concerns us all.

W. PAGE FAULK

Evolution of nucleotide-binding proteins

ONE of the most fascinating questions about the formation of life and its immediate development is how the enzymes needed to sustain the basic life processes were derived. It seems reasonable that the relatively large number of enzymes required by even a primitive bacterium were derived from a much smaller number of basic, relatively non-specific enzymes—probably by gene duplication and subsequent divergence leading to a diversification of function and a narrowing of specificity. On this basis it would be expected that the large number of present-day enzymes could be grouped into a much smaller number of families, each of which was derived from a common ancestor. But if the common ancestor is very remote, there may be insufficient indication from the amino acid sequence to establish relatedness. On the other hand, since it is known from the evolutionary studies of the globins, the serine proteases and the cytochromes that tertiary structures change very slowly, X-ray diffraction studies of protein structure seem to be the most promising method for identifying family relationships. During the past year or so X-ray structure determination of a number of intracellular metabolic enzymes have produced some exciting results that seem to provide the basis for characterising an extensive family of nucleotide-binding enzymes.

The structural relationship was first seen clearly when the structures of liver alcohol dehydrogenase (LADH) (Brändén, *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2439;

1973) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Buehner *et al.*, *ibid.*, 3052) were compared with the homologous lactate and malate dehydrogenase (LDH and MDH) molecules. The polypeptide chains of all four dehydrogenases, composed of between 327 and 374 amino acid residues, are folded into two distinct units or domains. One of these domains is responsible for binding the common NAD cofactor and the other domain carries the binding site for the specific substrate of each enzyme and its catalytic site. In all four molecules the NAD-binding unit has a common fold that is characterised by a β -sheet core composed of six parallel strands (A-F), with a strand order CBADEF, and by a similar disposition of the mainly α -helical intrastrand loops above or below the sheet. The NAD cofactor is bound to the domain at an equivalent site in all four molecules. Earlier, Rao and Rossman (*J. molec. Biol.*, **76**, 241; 1973) had found that this domain seemed to be composed of two similar three-stranded units each associated with the binding of a mononucleotide, and they proposed that this unit was utilised to bind flavin mononucleotide in flavodoxin and to form the aromatic specificity site in subtilisin. In sharp distinction to the similarity of the NAD-binding domain, the catalytic domains, except in the case of LDH and MDH, are structurally quite distinct.

This pattern of relationship was subsequently observed in phosphoglycerate kinase (PGK) (Blake and Evans, *J. molec. Biol.*, **84**, 585; 1974; and Bryant, Watson and Wendell, *Nature*, **247**, 14; 1974), where the suggestion that the ATP binding domain of PGK has the characteristic fold and binding site of the NAD binding domain has been confirmed by the latest results. As with the dehydrogenases the catalytic domain has a distinct structure. The nucleotide-binding domain corresponds to the N-terminal part of the chain in LDH, MDH and GAPDH and the C-terminal part of the chain in LADH and PGK.

This pattern of structural relationships points strongly towards the evolution of the NAD-binding protein by gene duplication of the mononucleotide binding unit and its subsequent incorporation in the dehydrogenases, and probably in a modified form in the kinases, by a genetic fusion process involving protein fragments carrying the specific binding and catalytic properties of each particular enzyme. This hypothesis can only be established by amino acid sequence comparisons. Those comparisons of dehydrogenase sequences that have been made failed, however, to reveal this striking relationship, probably because the remoteness of the common ancestors makes it very difficult to align the sequences.

Rossman, Moras and Olsen in last week's issue of *Nature* (**250**, 194; 1974) reported that these problems could be overcome by using the structural and sequence data together to trace the evolutionary history of the nucleotide-binding unit. By superposing the hydrogen bond arrangement in the β -sheet core, they have been able to determine homologous amino acids in the sequences of the various dehydrogenases, flavodoxin and subtilisin. After aligning the sequences in this way, the calculation of the minimum base change per codon was used as a measure of the elapsed time since the occurrence of a common ancestor. The evolutionary tree produced in this way is consistent with the divergent evolution of the nucleotide-binding unit of the dehydrogenases and flavodoxin from a common ancestral binding protein. This common ancestor seems to be exceedingly remote and Rossman and his colleagues suggest that it is older than 3.2×10^9 yr, indicating that it may have been present during precellular evolution. Though evolutionary relationships need many data to establish them with any certainty, this analysis seems to indicate a line of protein development that is as old as life itself, which may link together two of the largest groups of enzymes, the dehydrogenases and kinases. At the same time it shows that

gene fusion may be an important process in molecular evolution, particularly as it gives the resultant molecule a binucleic structure that is an almost universal feature of enzyme molecules.

The structures of two other enzymes that may also be members of this family have been reported recently in *Nature*. In this week's edition, Campbell, Watson and Hodgson (*Nature*, **250**, 301; 1974) describe the structure of phosphoglycerate mutase (PGM) determined by a 3.5 Å resolution electron density map. The molecule is a tetramer of four identical subunits, whose polypeptide chains are about 220 residues long. The core of each subunit is formed by a 6-stranded β sheet, in which four parallel strands are followed by an antiparallel pair, flanked by five of the six α helices in the subunit. Though the order of the β strands is not the same as that in the nucleotide-binding unit, the linear order and spatial arrangement of the five flanking helices is remarkably similar to that of LDH, although PGM has no known nucleotide-binding capacity. The authors show how the strands in the β sheet may have been rearranged if PGM has evolved from an ancestral protein with LDH folding, but at the same time point out that the general helix-sheet structure observed in these molecules may represent a particularly stable fold and imply, therefore, that it could be the property of more than one enzyme family.

The other enzyme, adenylate kinase (AK) was the subject of three papers in *Nature* for July 12. In the first, Schulz, Elzinga, Marx and Schirmer (*Nature*, **250**, 120; 1974) reported the structure of AK obtained from a 3 Å resolution Fourier map. The enzyme's single polypeptide chain has 194 amino acid residues and it is folded into two domains, the larger of which has a five-stranded parallel β -sheet core with a BCADE strand order. Although X-ray analysis of substrate binding has not yet been carried out, the active site histidine is found near two hydrophobic pockets in a position equivalent to that of the adenine and nicotinamide binding sites in the NAD-binding unit, that may represent the AMP and ATP binding sites. In the second paper Schulz and Schirmer (*Nature*, **250**, 142; 1974) report the use of a method for comparing AK with other potentially related molecules that is based on the topologies of their

polypeptide chain folds. They define a "figure of topological relatedness" as the triple product of the number of ways of ordering the strands in a β sheet, the number of ways the connecting loops can be disposed on either side of the sheet and the number of possible locations for binding sites. The figure of relatedness is highest when AK is compared with subtilisin and lower, but still significant when AK is compared with flavodoxin or the dehydrogenase nucleotide-binding unit.

Schulz and Schirmer point out that topological comparisons cannot be used to distinguish convergent and divergent evolutionary relationships. But a more fundamental problem in using topologies or defining structural pathways to relate molecules with somewhat different chain folds is that such methods are not supported by any firm knowledge of the chain folding process. The best that can be done in this direction is demonstrated in the third article on AK, by Schulz and the "Who's Who" of the structure prediction world (*Nature*, **250**, 140; 1974). Using the joint probability of up to ten different prediction methods, they show that α helices, β -sheet strands and reverse turns can be predicted from the amino acid sequence with a fairly high degree of success, for this particular molecule at least. Although this is very promising, there is still a long way to go before, for example, the strand order in a β sheet can be predicted. Until this can be done structural comparisons must be treated with caution. Unlike its amino acid sequence, the tertiary structure of a protein reflects changes in the genetic material in an essentially unknown way and is likely to undergo discontinuous changes, such as the rearrangement of the order of strands in a β sheet, that have no intermediates. In these circumstances it would be unwise to use structural comparisons as more than indicators of possible evolutionary relationships. Though such relationships can only be established by sequence comparisons they are best carried out in the light of the structural information. It is by no means impossible that within this large group of apparently closely and more distantly related enzymes there are both homologous and analogous relationships, and it is vitally important to distinguish them if the path of molecular evolution is to be accurately traced.

C. C. F. BLAKE

State of play in the neural hypothesis of muscular dystrophy

THE most striking clinical and pathological change in the muscular dystrophies in man is the severe and progressive degeneration of the skeletal muscles in the absence of clinically or pathologically significant peripheral nerve abnormality. The motor nerve, however, is known to have a very marked effect on the function and metabolism of the skeletal muscle. The finding by McComas and Mrozek¹ that 27% of skeletal muscle fibres in mice with muscular dystrophy were denervated suggested that the motor nerve may have an even more important part to play in regeneration. Further studies showed a marked decrease in the number of motor units in the extensor digitorum brevis muscle in Duchenne and other human muscular dystrophies²⁻⁴, and in murine muscular dystrophy⁵. As a result of these studies, McComas and colleagues propounded the hypothesis that muscular dystrophy is caused by the "sick" motor neurone, the abnormal influence of which on the skeletal muscle fibre caused it to break down.

This hypothesis received support from the experiment of Salafsky⁶ in which muscle transplanted from dystrophic mice into normal hosts regenerated with the physiological characteristics of normal muscle. Further support came from

Gallup and Dubowitz⁷ who found that dystrophic mouse spinal cord would not support the regeneration of muscle in tissue culture, whereas normal spinal cord supported full regeneration both of dystrophic and normal muscle.

Much of this original evidence in support of the neural hypothesis has now been called into question. Harris and Marshall⁸ have failed to confirm the finding of denervated fibres in murine muscular dystrophy and have suggested that the original findings by McComas and Mrozek could have resulted from hypoxia of the preparations leading to artefactual presynaptic failure. Panayiotopoulos and colleagues^{9,10} and Ballantyne and Hansen^{11,12} have both criticised the technique used for motor unit recording by McComas and colleagues, and by more refined techniques have found that the number of motor units in the extensor digitorum brevis muscle of patients with various muscular dystrophies is normal. Several groups of workers have obtained results entirely contradictory to those reported by Gallup and Dubowitz^{13,14}. Preliminary results in my laboratory suggest that the contraction believed by Salafsky⁶ to arise from the transplant may in fact have been transmitted from the host's underlying normal muscles to which the transplant

is intimately fibrosed (unpublished work of A. Montgomery, W. G. Bradley and M. Jenkinson). Abolition of the contraction of these host muscles virtually abolishes the tension produced by the transplant.

Despite the contradiction of many of the basic observations leading to the neural hypothesis, experiments which bear on the nerve in muscular dystrophy are still being performed. In reviewing these it is important to remember that there are several different diseases both in man and in animals which have been classed under the title of the muscular dystrophies, and that the aetiology of each may be different. Desmedt and Borenstein¹⁵ found that motor nerves in patients with Duchenne muscular dystrophy are quite capable of sprouting to reinnervate denervated muscle fibres, and thus show no sign of being 'sick'. The total number and structure of the anterior horn cells in human Duchenne¹⁶ and murine muscular dystrophy¹⁷ are normal. Focal peripheral nerve abnormalities are found in murine muscular dystrophy but not in Duchenne dystrophy or in dystrophic hamsters¹⁸. The peripheral nerve abnormality in mice both of the 'severe' (dydy)¹⁹ and 'benign' (dy²³dy²³) (my and M. Jenkinson's unpublished work) forms of muscular dystrophy consists of a decrease in the number of Schwann cells and apparent arrest of myelination at the foetal stage of axons in the anterior and posterior roots, and the proximal parts of certain peripheral nerves. Axons run through these areas to become myelinated distally. My group has found that this abnormality of Schwann cell investment and myelination is present in the lumbo-sacral and cervical anterior and posterior roots, disappears with normal myelination in the region of the dorsal root ganglia, but recurs several millimetres distally in patches in the upper part of the lumbo-sacral plexus. We have also found it in the facial and trigeminal nerves.

These dystrophic mice also show an abnormality of axoplasmic flow in the peripheral nerves, with a decrease in the amount of slow flowing protein¹⁹ and of choline acetyl transferase²⁰, and an increase in the amounts of faster flowing proteins, phospholipids and cholesterol^{19,21,22}, with a probable decrease in the amounts of the fastest ('superfast') flowing proteins²¹. An abnormality of the peripheral nerve proteins probably derived from myelin has been reported in dystrophic mice²³.

Peterson's technique²⁴ of chimaera formation from normal and dystrophic mouse morulae is of considerable interest. He used isozymes to follow dystrophic and normal nuclei in the mouse which developed from these morulae. Some skeletal muscles had up to 94% of dystrophic nuclei, and many individual muscle fibres had only dystrophic nuclei. Despite this there was little or no pathological degeneration of these skeletal muscles. Conversely in some of

these mixed chimaeras, muscle containing no dystrophic nuclei sometimes showed pathological changes. The data suggest that in these mice the muscle degeneration is caused by some extramuscular abnormality. Peterson suggested that this might lie in the motor neurones, but it could equally be some abnormality conveyed in the blood stream.

This study is very important, and it must be extended to other enzyme markers, and to other animal models of muscular dystrophy. It is important to remember that the study does not necessarily apply to man and that the short history of the neural hypothesis is scattered with apparently conclusive studies which fail to be confirmed by other workers. Thompson and colleagues have pointed out²⁵ that the neural hypothesis will not explain what is known about female carriers of the gene for Duchenne muscular dystrophy. According to the Lyon hypothesis, half of the neurones of these carriers will contain a single active X chromosome carrying the dystrophic gene, and half the normal gene. The creatine kinase level in the blood of a carrier might therefore be expected to be half of the level in a male with Duchenne muscular dystrophy of the same age if the neurone caused the skeletal muscle breakdown. This prediction is not substantiated, the values of creatine kinase being much lower.

The state of play may be summarised as follows. Much of the original basis of the neural theory has been contradicted, but neural abnormalities both of structure and function have been found in murine muscular dystrophy. The muscle degeneration in the latter may be of extramuscular origin though the chimaera experiment needs further study. On the whole I consider that there is little incontrovertible direct evidence for the neural hypothesis and that it remains likely that a primary muscle degeneration occurs in many, if not all, of the muscular dystrophies.

There are reasons to turn attention to membrane abnormalities in the muscular dystrophies. There is some suggestion that the sarcolemma leaks creatine kinase at the earliest stages of Duchenne muscular dystrophy, some months or years before muscle fibre necrosis begins²⁶. Red cell membrane abnormalities have been seen with the scanning electron microscope both in murine²⁷ and human Duchenne²⁸ muscular dystrophy, though preliminary studies in my laboratory have failed to confirm acanthocytosis in murine muscular dystrophy, nor demonstrate abnormalities of plasma lipoproteins. It would, however, clearly be interesting to study the other muscular dystrophies with the techniques which have enabled Roses, Appel and colleagues to identify biochemical abnormalities in the membranes of patients with dystrophia myotonica^{29,30}.

W. G. BRADLEY

¹ McComas, A. J., and Mrožek, K., *J. Neurol. Neurosurg. Psychiat.*, **30**, 526 (1967).

² McComas, A. J., Sica, R. E. P., and Currie, S., *J. Neurol. Neurosurg. Psychiat.*, **34**, 461 (1971).

³ Sica, R. E. P., and McComas, A. J., *J. Neurol. Neurosurg. Psychiat.*, **34**, 469 (1971).

⁴ McComas, A. J., Campbell, M. J., and Sica, R. E. P., *J. Neurol. Neurosurg. Psychiat.*, **34**, 132 (1971).

⁵ Harris, J. B., and Wilson, P., *J. Neurol. Neurosurg. Psychiat.*, **34**, 512 (1971).

⁶ Salafsky, B., *Nature*, **229**, 270 (1971).

⁷ Gallup, B., and Dubowitz, V., *Nature*, **243**, 287 (1973).

⁸ Harris, J. B., and Marshall, M. W., *Exp. Neurol.*, **41**, 331 (1973).

⁹ Scarpalezos, S., and Panayiotopoulos, C. P., *Lancet*, **ii**, 458 (1973).

¹⁰ Panayiotopoulos, C. P., Scarpalezos, S., Papapetropoulos, T. A., and Caronis, A., *J. Neurol. Sci.* (in the press, 1974).

¹¹ Ballantyne, J. P., and Hansen, S., *Lancet*, **i**, 1060 (1974).

¹² Ballantyne, J. P., and Hansen, S., *J. Neurol. Neurosurg. Psychiat.* (in the press, 1974).

¹³ Hamburgh, M., Bornstein, M. B., Peterson, E. R., Crain, S. M., Masurovsky, E. B., and Kirk, C., *Brain Res.*, **40**, 497 (1973).

¹⁴ Paul, C. V., and Powell, J. A., *J. Neurol. Sci.*, **21**, 365 (1974).

¹⁵ Desmedt, J. E., and Borenstein, S., *Nature*, **246**, 500 (1973).

¹⁶ Tomlinson, B. E., Walton, J. N., and Irving, D., *J. Neurol. Sci.*, **22**, 305 (1974).

¹⁷ Papapetropoulos, T. A., and Bradley, W. G., *J. Neurol. Neurosurg. Psychiat.*, **35**, 60 (1972).

¹⁸ Bradley, W. G., and Jenkinson, M., *J. Neurol. Sci.*, **18**, 227 (1973).

¹⁹ Bradley, W. G., and Jaros, E., *Brain*, **96**, 247 (1973).

²⁰ Jablecki, C., and Brimijoin, S., *Nature*, **250**, 151 (1974).

²¹ Komiya, Y., and Austin, L., *Exptl Neurol.*, **43**, 1 (1974).

²² Tang, B. Y., Komiya, Y., and Austin, L., *Exptl Neurol.*, **43**, 13 (1974).

²³ Salafsky, B., and Stirling, C. A., *Nature new Biol.*, **246**, 126 (1973).

²⁴ Peterson, A. C., *Nature*, **248**, 561 (1974).

²⁵ Thompson, W. H. S., Sweetin, J. C., and Elton, R. A., *Nature*, **249**, 151 (1974).

²⁶ Bradley, W. G., Hodgson, P., Larson, P. F., Papapetropoulos, T. A., and Jenkinson, M., *J. Neurol. Neurosurg. Psychiat.*, **35**, 451 (1972).

²⁷ Morse, P. F., and Howland, J. L., *Nature*, **245**, 156 (1973).

²⁸ Matheson, D. W., and Howland, J. L., *Science*, **184**, 165 (1974).

²⁹ Butterfield, D. A., Chesnut, D. B., Roses, A. D., and Appel, S., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 909 (1974).

³⁰ Roses, A. D., and Appel, S. H., *Nature*, **250**, 245 (1974).

'Hot' hydrogen initiates amino acid production

from D. O. Hall

It is just 21 years since Miller reported his now classic experiments in a paper entitled "A Production of Amino Acids under Possible Primitive Earth Conditions". This non-biological synthesis of amino acids by sparking for a week a gaseous mixture of methane, ammonia, hydrogen and water vapour initiated a new phase of research on the origin of life. Since then an impressive list of many different amino acids, sugars, aromatic hydrocarbons and nucleotides and nucleosides have been synthesised using various energy sources such as sparking, heat, ultraviolet (UV) light, α , β and γ rays, and shock waves. These experiments are well described in Miller and Orgel's recent book *The Origins of Life on the Earth* (Prentice-Hall, New Jersey; 1974).

The sulphur-containing amino acids which are so essential components of proteins have been conspicuous by their absence among the products of all these types of experiments. In 1971, however, Sagan and Khare (*Science*, 173; 417) solved this problem by including H_2S in a UV-irradiated gas mixture which included methane, ammonia, ethane and water; cysteine was produced as one of the six amino acids detected. The H_2S provided the source of sulphur and importantly also provided the means by which UV light at wavelengths below 266 nm could be absorbed as an energy source to catalyse the prebiotic synthesis of organic compounds. These authors calculated that about 200 kg of amino acids could have been produced by UV light absorbed by H_2S per square centimetre on the primitive Earth in 10^9 years. This large amount of amino acids must have allowed a high concentration to accumulate even allowing for considerable destruction by other processes.

Hong, Hong and Becker (*Science*, 184, 984; 1974) have now confirmed and extended this earlier work by showing that H_2S or CH_3SH (methyl mercaptan) can act as UV photon absorbers and can initiate by releasing 'hot' hydrogen atoms the production of up to nine amino acids, including the important cysteine. Whereas Sagan and Khare reported that a two-carbon substrate such as ethane is required to produce amino acids, Hong *et al.*'s experiments worked well with just the one-carbon methane. A simple mixture of H_2S , NH_3 , CH_4 and H_2O will produce nine amino acids but interestingly

cysteine may require a two-carbon substrate in order to be synthesised. These authors state that the amino acids produced were in the free form and were not breakdown products of polymers which would have formed as polypeptides—as was suggested by Sagan and Khare. These differences in experimental procedure and products may be important in future 'prebiotic experiments' where the aim is to synthesise polypeptides.

The 'hot' hydrogen atoms produced by the action of ultraviolet light on H_2S or CH_3SH have kinetic energies of about 17 to 32 kcal mol⁻¹, which is sufficient to provide the activation energy for the synthesis of organic compounds in interstellar space or on the primitive Earth. That these types of syntheses may occur in interstellar space has become a real possibility with the recent discoveries using radio astronomy, of compounds such as ammonia, hydrogen, hydrogen cyanide, acetaldehyde, methyl alcohol and hydrogen sulphide in interstellar clouds. In 1971 Buhl reviewed this rapidly developing field (*Nature*, 234, 332; 1971) and concluded with this fine paragraph: "The important parallels appear to be between the formation of interstellar molecules and the synthesis of amino acids in prebiological chemistry experiments. It is here that the key to the evolution of interstellar molecular clouds lies".

Limited progress at GR7

from P. C. W. Davies

ALTHOUGH the location of the Seventh International Conference on Gravitation and General Relativity at Tel Aviv University from June 24–28, was somewhat controversial—few Eastern European countries were represented and some eminent Western relativists were also absent—nevertheless, there was a generous sprinkling of familiar names among the participants and the usual atmosphere of conviviality seemed unimpaired.

On the theoretical side, only modest progress seems to have been made since the sixth conference in Copenhagen. Both S. Deser (Brandeis University) and J. Goldberg (Syracuse University) made discouraging remarks about quantum gravity, reinforcing the prevailing opinion that traditional covariant quantisation techniques are going round in circles, while only hinting at the possible direction of more radical approaches. At a less sophisticated level, the recent claim by Hawking that quantum field theory in a classical gravitational field predicts the disinte-

gration of microscopic black holes seemed to have been readily accepted in spite of the rather unusual nature of the result. (Hawking himself was not present.) The effect was discussed at some length by W. Press (California Institute of Technology) in a summary of astrophysical processes occurring near black holes. Helpful review talks on cosmology and exact solutions were delivered by R. Matzner (University of Texas) and W. Kinnersley (University of Montana) respectively, and a sparkling account of exotic mathematical developments connected with the BMS group was given with much humour by E. Newman (University of Pittsburgh).

The experimental results were rather more dramatic. M. Rees (University of Cambridge) gave a spirited and carefully balanced review of the theoretical and observational aspects of the detection of black holes, concentrating particularly on the situation regarding the X-ray source Cygnus X-1. Having presented some evidence to suggest that this source has a mass of at least $6M_\odot$, he commented that explanations of this object involving models of differentially rotating white dwarfs or three-body systems seemed "contrived and *ad hoc*", and that in his opinion Cygnus X-1 was instead a "strong candidate" for a black hole. Characteristically, his optimism was tempered by the remark that this was "not a firm conclusion", but that the balance of evidence did seem to favour the black hole explanation. If this was so, then there was a new opportunity to test general relativity by examining the behaviour of the accretion disk which would surround such an object and produce the X rays, possibly by relating this behaviour to curious fluctuations in intensity which have been observed from this source.

By far the most controversial issue of the conference was left to the last day, with a panel discussion on gravitational wave detection. During the past few months the interpretation of J. Weber's epic experimental data in terms of gravitational pulses from the galactic centre has been increasingly open to doubt. The subject reached a new peak of controversy with the presentation of results from other similar experiments being carried out in Munich/Frascati, Glasgow and the Bell Telephone Laboratories/University of Rochester. First, Weber (University of Maryland) reasserted his existing position in confident and ebullient style, claiming a further high event rate for the period May 21–June 15, 1974 using improved equipment. He did admit, however, that the use of a new algorithm favoured by other groups led to a greatly diminished event rate, but asserted that his original algorithm had a very much better detection efficiency.

P. Kafka (Max Planck Institute,

Munich) described the Munich/Frascati data since August 1973, claiming a substantial improvement on sensitivity over Weber, but with no significant events detected. R. Drever described data from his wide bandwidth detector in Glasgow during the period September 1972–April 1973 with the same result—no effect. He described the situation as “saddening” and went as far as to express the opinion that Weber was probably mistaken. Finally, J. Tyson (Bell Telephone), claiming a sensitivity for the Bell Laboratories/Rochester experiment very much greater than other experiments, also reported no events.

In view of the importance of the issue at stake, the atmosphere remained remarkably restrained. Weber countered the negative results of his colleagues by rejecting their claims of higher sensitivity. When challenged on the all-important issues of the sidereal anisotropy (which seems to indicate a correlation of his events with the centre of the galaxy), Weber seemed to be saying that he had not yet checked recent data for this effect because of other pressures. The overall impression was that the issues involved hinge on complicated technical details, but that Weber's position among the experimentalists is now growing increasingly isolated as more and more experiments fail to confirm his data.

Stranded whales in Britain

from our
Marine Vertebrate Correspondent

THE gathering of data on stranded cetaceans on the coast of the British Isles has now been in progress for well over half a century. Because of the special historical claim that the Crown had over ‘royal fishes’ on much of the English, Welsh and Scottish coasts, the collection of reports of strandings was already in progress and their publication on a regular basis was a logical step which was initiated by the late Sir Sidney Harmer in 1913.

The latest *Report on Cetacea stranded on the British Coasts from 1948 to 1966* compiled by Fraser (No. 14, 1–65, 9 maps; British Museum (Natural History) 1974, £3.00) contains a full listing of the reported stranded cetaceans (with notes on a few sightings) on the British and Irish coasts for the eighteen year period to 1966. It is unfortunate that even the most recent records in the report are now eight years old, and that the opportunity has not been taken to bring the list as nearly up to date as possible. Nevertheless, there is much of interest in the accumulated records despite their age, and there is good reason

in bringing them all together in the hope that common patterns will emerge from the data.

Among the records of especial interest is the full documentation of two strandings of narwhal (*Monodon monoceros*), both in 1949 (February and July), a species known to have been found only three (or possibly four) times before. A pair of narwhals was also reported as sighted off the Orkneys in June 1949. The white whale (*Delphinapterus leucas*), only stranded once before since 1913, was sighted on three occasions (1948, 1950 and 1965) off the British Isles, and, as Fraser points out, was also reported off the French coast in December 1948, and in Dutch waters in June 1965 and 1966. The coincidence in some of these dates of sighting and later stranding suggests that the same specimens may have been involved in some cases, but this is not possible to confirm. What may also be significant (although Fraser does not comment on this) is that these two species are both Arctic cetaceans which occurred in unprecedented numbers in 1948–1949 in European waters following a winter of unusual severity in 1947–1948.

Other records of particular interest in Fraser's report are the first recorded occurrence of the pigmy sperm whale (*Kogia breviceps*), off Co. Clare, Ireland, in April 1966, and two unrecorded strandings of the euphrosyne dolphin (*Stenella coeruleoalba*). This species had hitherto only been reported twice (1937 and 1939) in British waters, but the two additional records predate these, one based on an old photograph of 1923, and the other on a skull in the British Museum collection, previously identified as a common dolphin, which was stranded in 1934.

The total number of identified stranding since record keeping began in 1913 now amounts to 1,550. Of these 631 were of the common porpoise (*Phocoena phocoena*), 185 of the bottle-nosed dolphin (*Tursiops truncatus*) and 135 of the common dolphin (*Delphinus delphis*). By analysis of the occurrences of the common porpoise, Fraser has shown that strandings occur most frequently during the period July to October, with small peaks in January to March, and on the central and southern North Sea coast. Fraser cites earlier literature which suggests that there is an annual migration out of the Baltic between November and February, and conjectures that this may be correlated with the summer-time peak of strandings on the East Coast of Britain. The pattern of records which Fraser presents is certainly in keeping with the suggestion that porpoises enter the North Sea mainly in the northern sector and then follow the main current systems southwards

in an anti-clockwise direction. On the other hand, the incidence of strandings seems to be high in each of the major bights round the English and Welsh coasts, and the southern North Sea and the eastern English Channel can be seen as particularly large bights. The explanation for the distribution of porpoise strandings may thus be due to the configuration of the coast line and possibly the presence of shoal water, rather than to purely biological phenomena such as annual migrations of the animals.

Lead accretion in alluvial deposits

from Peter D. Moore
Plant Ecology Correspondent

THE release of undesirable but easily recognised substances into the environment, during such processes as mining and industrial smelting or by the explosion of nuclear devices, does have certain compensations. It can provide datum levels in recently deposited sediments which allow the calculation of accretion rates. For example, Bradbury and Waddington (in *Quaternary Plant Ecology*, edited by Birks and West, Blackwell, Oxford, 289; 1973) have used the abundance of haematite granules in the recent sediments of Shagawa Lake, Minnesota, to determine ediment ages. Haematite grains first appear in the sediments when iron ore mining began in the area in 1889 and reached a peak in 1902 when production was maximal; subsequently they declined gradually until 1951 when there was a sharp decrease corresponding to a decline in mining.

The isotope caesium-137 has been used as a datum in both peats and limnic sediments (for example, Pennington, Cambray and Fisher, *Nature*, **242**, 324; 1973). Fallout reached a maximum in 1963 following the widespread proliferation of nuclear testing above ground. Lee and Tallis (*Nature*, **245** 216; 1973) have used aerial lead fallout to provide datings of stratified peat deposits in the Southern Pennines.

Davies and Lewin (*Envir. Pollut.*, **6** 49; 1974) have also studied lead, but this time in alluvial deposits of the River Rheidol. The build-up of alluvium in the meander loops of the river has been mapped or photographed on eight occasions during the past 130 years; this has allowed the successive zones of alluvial soil to be dated fairly accurately. The upper catchment of the Rheidol has been mined for lead since Roman times, but peak activity occurred during the nineteenth century. Davies and Lewin have analysed the zones of alluvial soils in an attempt to determine the effects of these activities

upon the floodplain materials of the river.

Deposits laid down in the period 1845–1886 had the highest concentrations of lead (1,500 p.p.m. dry soil). Following this, in the zone 1886–1904, values dropped to 1,011 p.p.m.; the fall could reflect the 1876 Act of Parliament requiring the cleansing of mine effluents. Mining ceased at about 1900 and the subsequent zones have lower concentrations of lead: 1904–1951, 785 p.p.m.; 1951–1971, 503 p.p.m.; 1972, 368 p.p.m.

These data show that, despite the reworking of older deposits during the formation of meanders, the lead contents of alluvial soils do reflect the contemporary conditions of lead pollution in the river. This technique could be used for the historical studies of alluvial pedogenesis in similar situations but where precise mapping is unavailable. The accumulation of heavy metals in alluvium is also a salutary reminder that the precise drainage conditions which make these soils the recipients of leached and eroded plant nutrients, producing high fertility, also result in the concentration of the toxic materials eroded from the catchment. The advisability of using such alluvial soils for arable agriculture or grazing will depend on conditions of river pollution during the period in which accumulation of the substrate took place.

Evaporation and gas exchange in nature

from P. S. Liss

THE evaporation of water from natural water bodies is largely controlled by aerodynamic processes in the air above the water surface. By contrast, exchange of sparingly soluble gases, such as oxygen, carbon dioxide, nitrogen and the inert gases, across air–water interfaces in the environment is almost entirely dependent on the degree of turbulence in the liquid phase. This fundamental difference leads to very different approaches in the study of evaporation and gas exchange in nature. For instance in order to describe evaporation, sophisticated mathematical treatments of atmospheric turbulence are necessary, whereas it seems that simple laminar models can go a long way towards elucidating air–water exchange rates for dissolved gases.

In order for water molecules to evaporate from a water surface they must have sufficient energy to escape from the liquid. Furthermore, the aerodynamic regime in the air above the interface must be such that the water molecule is prevented from con-

densing back onto the surface. At the 22nd Bat-Sheva seminar, which this year took for its subject "Mechanism of Evaporation and Gas Exchange in Nature" and was held from June 16–30 at the Weizmann Institute of Science, Rehovot, W. H. Brutsaert (Cornell University) presented a detailed mathematical treatment of evaporation and showed how semi-empirical theories of atmospheric turbulence can be used to help solve the differential equations involved. These lectures were complemented by those of C. B. Tanner (University of Wisconsin) who reviewed the practical methods so far used to measure evaporation rates over land surfaces. He illustrated the importance of such studies by pointing out that evaporation represents on average 70% of the total outflow from catchments in the United States. In arid regions the figure may rise as high as 95%. The best estimates of evaporation seem to be those based on measurements of the energetics of the process, especially when these methods are locally calibrated. G. Stanhill (Volcani Center, Bet-Dagan) led a discussion on the various methods of preventing evaporation from water bodies and plants. For open waters, monolayers have been tried, but it is hard to maintain a continuous film at the surface when there is any appreciable wind. Pumping cold water from the bottom of the lake to decrease the temperature of the surface and so reduce evaporation is a novel technique which deserves attention. There has been some success in laboratory experiments in which plant leaves are sprayed with a chemical in order to increase their stomatal resistance and so reduce evapo-transpiration, but such techniques do not seem to work well when applied to crops in the field.

A laminar layer model has been used by W. S. Broecker (Lamont Geological Observatory) to characterise exchange of sparingly soluble gases across the air–sea interface. From measurements of Rn-222 and its parent Ra-226 near the water surface, it is possible to calculate the thickness of the laminar layer at the sea surface. From such measurements, made as part of the GEOSECS programme in the Atlantic and the Pacific, there are now reasonable average values for gas exchange rates in the major oceans. These values can be used to calculate the fluxes of many atmospheric trace constituents (both natural and anthropogenic) across the sea surface. S. Emerson (Swiss Federal Institute for Water Protection) showed how similar techniques have been applied in lakes to obtain rates of CO₂ transfer across the interface. His results indicate that, despite reports to the contrary, dissolved carbon is unlikely to be a limit-

ing factor in algal growth in lakes, because CO₂ can transfer from the atmosphere to the water at a rate fast enough to supply the carbon required for phytoplankton growth.

The molecular structure of liquid water was considered by R. A. Horne (Arthur D. Little Inc.). Of especial importance in the context of the seminar was his analysis of how the properties of the very surface layers of water molecules (vicinal water) differ from those of the bulk liquid. Vicinal water seems to differ from the bulk fluid over a wide range of physical (freezing point, viscosity, thermal expansion) and chemical (solubilities of ionic and non-polar solutes) properties, but its thickness is still a subject of much debate.

Thermal disruption in the asthenosphere

from Peter J. Smith
Geomagnetism Correspondent

THE possibility that nonlinear viscous heating could produce thermal runaways in the Earth's interior was first recognised by Gruntfest (*Trans. Soc. Rheol.*, **7**, 195; 1963) who used the heat equations to show that, for one-dimensional plane viscous flow under constant stress in a slab, the temperature and strain rate may become arbitrarily large in finite time under adiabatic or near-adiabatic conditions. Many of the ideas initiated by Gruntfest were then later applied by Shaw (see, for example, *J. Petrol.*, **10**, 510; 1969) and Shaw *et al.* (*Bull. geol. Soc. Am.*, **82**, 869; 1971) to specific geological situations such as the rheology of basalt, the relation between Earth tides and magmatism in the Sierra Nevada, and Hawaiian volcanism. More recently, Fujii and Uyeda have been quoted as having used the ideas of both Gruntfest and Shaw to explain the size of intrusive dykes and the eruptive history of volcanoes.

But talk of magmatism, volcanism and viscous flow leads naturally to deeper thoughts about the asthenosphere in mind that O. L. Anderson and Perkins (*J. geophys. Res.*, **79**, 2136; 1974) have been looking again, but in a rather different way, at the possibility and implications of thermal runaways in the Earth. They begin by drawing attention to the fact that the heat flow equation applicable to one-dimensional viscous flow at constant stress is the same one-dimensional equation as that used to describe thermal explosions in solids, and then go on to explore the consequences of this connection between two apparently quite different situations. The fact is that the common equation implies fea-

tures common to both processes; and this, in turn, suggests that the known characteristics of one process may be used to predict the properties of the other; for example, solid explosives possess instabilities which, when triggered, produce the explosion. The fact that viscous flow is governed by the same basic heat flow equation then implies that regions of viscous flow in the Earth (for example, the asthenosphere) should also possess inherent instabilities which, if triggered, should produce thermal runaways.

Insofar as thermal runaways in the Earth were proposed by Grunfest more than a decade ago, this prediction by analogy may not seem particularly useful. Fortunately, however, there is more to be gained, for, as Anderson and Perkins point out, thermal explosions have been studied in some detail, and the knowledge obtained thereby may be used to extend the analogy with viscous flow. Pursuing this line of attack and making reasonable assumptions about the properties of the heat flow equation, Anderson and Perkins conclude that the potential for thermal runaways in the asthenosphere does indeed exist and that the time taken for the temperature to rise to the very high values necessary would probably be a few tens of million years after the initiating event.

Once such a thermal event has been triggered, the hotter material will rise towards the lithosphere in what Anderson and Perkins choose to call a surge. Some of the stronger surges would actually reach the bottom of the lithosphere but would probably not penetrate it. Instead, the surging material would spread out horizontally and then sink as it cooled, although completely circular paths would not be produced thereby because any given surge would die out in about the same time as it took to grow. On the other hand, the time scale involved here is tens of million of years, so that regions of high temperature (hot spots) would be expected to persist near the lithosphere–asthenosphere boundary for periods significant even in geological terms.

The geological implications of such a situation are clearly important. For example, although the hot asthenospheric material itself may not penetrate the lithosphere, its presence immediately beneath would facilitate partial melting in the upper mantle and lower crust, and thus crustal igneous activity. The significant point to note here is that zones of partial melting produced in this way would be distributed irregularly and would thus not necessarily bear any regular temporal or spatial relationship to steady-state subduction processes. Acceptance

of surges would therefore mean that it would be no longer necessary to force complex arrangements of igneous activity into patterns dictated by plate tectonic models. The case that Anderson and Perkins have in mind here is the wide extent and complex patterns of Cainozoic igneous activity in the south-western United States. The problems involved in attempting to explain this activity in plate tectonic terms have already been emphasised by several workers; Noble *et al.* (*Bull. geol. Soc. Amer.*, **84**, 1393; 1973), for example, noted that the late Tertiary volcanic field of south central Nevada contains materials generated from different sources or different levels or both.

Finally, if thermal runaways produce surges in the asthenosphere, what triggers them? Thermal explosions in solids are triggered by thermal impulses and, by analogy, asthenospheric 'explosions' could be initiated by friction at the lithosphere–asthenosphere boundary, mantle plumes or changes in activation energy (for example, by dilution with a suitable volatile). Anderson and Perkins, however, also draw attention to other possible triggers which are less directly thermal and more concerned with changing asthenospheric flow patterns. Examples are the break-up of continents and the replacement of subduction zones by transform faults. It is clear, for instance, that the encroachment of the Pacific ridge on Atlantic plate must have profoundly affected flow patterns in the region and thus severely disrupted the asthenospheric thermal gradients.

Models of the Earth

from P. G. Richards

THE classical subjects of theoretical geophysics dominated a symposium held from June 25 to July 5 at Cambridge: no neat and easily solved novelties here, but the staples of mantle convection, dynamo motions in the core, scattering theory, weather prediction and earthquake source mechanisms.

At a session honouring Sir Edward Bullard, who is retiring from the Cambridge chair of Geodesy and Geophysics, F. Gilbert and A. Dzeiwonski (University of California, San Diego and Harvard) described notable new levels of sophistication in their study of the Earth's free oscillations. Several hundred new modes have been identified, and the inversion of 1066 data yields an improved Earth model in which the source mechanisms of two deep focus earthquakes can be retrieved accurately. Their results back

up a controversial claim, made earlier, that the source region experiences compression, beginning about 80 s before the origin time as determined from body wave observations.

Since 1972, when the International Union of Geodesy and Geophysics sponsored its first symposium on mathematical geophysics, there has clearly been progress in the understanding of the Earth's core. M. Chinnery (Lincoln Laboratory, MIT) gave a new formulation for finding static deformations of an Earth model with a compressible fluid core, showing that current debate and major disagreements in the literature on this subject can be resolved by working with bulk properties in the fluid (density, pressure) rather than with particle displacements. D. Crossley (Memorial University, Newfoundland) presented results for the gravitational undertones in a subadiabatic core of a rotating Earth, finding that eigenperiods are confined to the range 0–12 h. The written versions of these two papers are awaited with interest, for the basic problem has challenged Love, Jeffreys, Pekeris, and many another of the sharpest theoreticians in geophysics.

In weather prediction and seismic wave propagation, it is now possible to recognise statistical elements which limit the deterministic results naively hoped for a few years ago. However well an initial state of the atmosphere is known, C. Leith (National Center for Atmospheric Research, Boulder) reported, there is no basis for predicting atmospheric pressures beyond about two weeks. Likewise, the seismologist should not expect to interpret every detail on a seismogram, although K. Aki (MIT) has used a standard back-scattering theory to determine some interesting differences in the heterogeneity of California, Japan and Norway. Subsequent corridor discussion showed that scattering theories are being widely investigated, as, for different length scales of heterogeneity, completely different scattering modes may be appropriate. Even the standard formulations (based on acoustic waves) may give different asymptotic results for P–S scattering in the elastic case.

The effect of specified inhomogeneity on sufficiently long period waves should, of course, be deterministic, and quite realistic problems are now often attacked with the finite element method. A major computational advance here was reported by W. D. Smith (University of California, Berkeley), who has found a subtle way to eliminate unwanted reflections from the grid boundaries. He uses two sets of boundary conditions for which the sum of solutions sees the boundary as transparent, even for elastic (P–SV) waves.

Fossil marginal basin in the southern Andes

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In the southern part of the South American continent a marginal basin opened behind an active andesitic island arc in the earliest Cretaceous. The basin closed again in the middle Cretaceous, during the period of fast seafloor spreading, causing the penetrative deformation of the southern Andean Cordillera.

THE Andes are widely regarded as the 'type' continental margin orogenic belt¹⁻⁴. The regular Cordillera-bounded, western continental margin of South America is frequently contrasted with the island arc festooned eastern margin of Asia^{5,6}. Our recent field work demonstrates that a marginal basin with a mafic floor, like those behind the island arcs of the western Pacific, opened up in the southern Andes during the Cretaceous as previously suggested by Katz⁷. This has important consequences not only for the history of the Pacific Ocean basin but also for concepts of mountain building at continental margins.

A narrow discontinuous belt of mafic igneous rocks extends along the spine of the Andean Cordillera from Cape Horn at 56°S at least as far north as 51°S, a distance of 350 km (Fig. 1). These rocks, commonly termed the *rocas verdes* (green rocks) by Chilean geologists, were considered to be mainly lavas and have been variously referred to as 'eugeosynclinal' or 'ophiolite' assemblages⁷⁻¹¹.

The belt of mafic rocks is flanked on the Pacific side by the Patagonian batholith, a vast continuous granodioritic body extending from Cape Horn to 50°S and beyond, averaging more than 60 km in width and reaching almost to the continental margin. The continental rocks on the Atlantic side consist of Palaeozoic (? and older) metamorphics unconformably overlain by an extensive silicic volcanic layer 0-2 km thick of middle-late Jurassic age, with a cover of Cretaceous and Cainozoic sediments. The silicic volcanic layer extends in the subsurface as far as the eastern continental margin.

The Patagonian batholith is interpreted as the root of an andesitic volcanic chain initiated in the Late Jurassic and comparable with the Central Andes of today. The *rocas verdes* are thought to represent the mafic floor of a marginal basin that formed in the latest Jurassic-earliest Cretaceous, separating the active andesitic volcanic chain from stable continental South America.

Volcanic chain development

A thick sequence of sediments was deposited in the southern Andes from Kimmeridgian to Aptian time. Along the Atlantic side of the High Cordillera uniform black mudstones of shelf facies were deposited, thickening markedly from 400 m in the east to more than 2 km towards the west. Within the Cordillera a marked facies and a further change in thickness occurs. Abundant, thick greywacke beds and thin radiolarian cherts appear interbedded with the black mudstones. Towards the Pacific where the sequence exceeds 3 km, the greywackes thicken and become more abundant¹². They are rich in immature

andesitic volcanic detritus, which suggests the presence of an active volcanic source during sedimentation. Variations in thickness and facies indicate that the source lay on the Pacific side of the sedimentary basin. The presence of andesitic lavas and volcanic breccias in the southern part of the basin, interbedded with the sediments but geographically restricted to the Pacific margin, supports this interpretation¹³ (see Fig. 2b).

The Patagonian batholith now lies directly along the Pacific margin of the sedimentary basin. It is a composite,

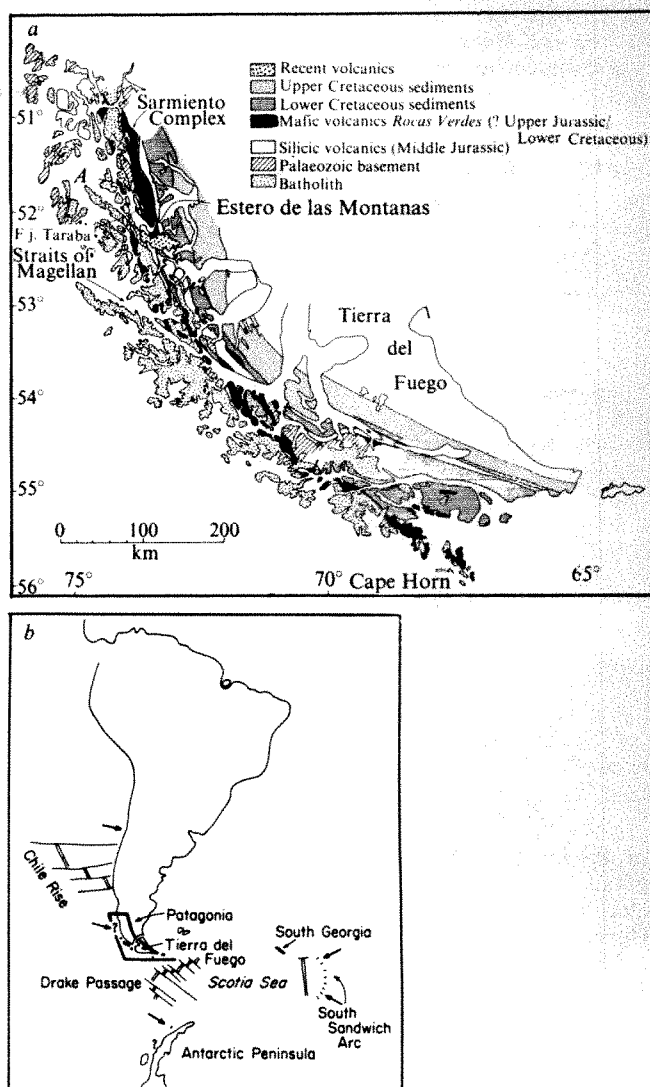


Fig. 1 a, Simplified geological map of the southern Andes, A = Isla Young. b, Location map, arrows show general subduction directions.

and as yet poorly mapped unit consisting of numerous small plutons of coarse grained 'granitic' rocks ranging from tonalite to adamellite. The batholith is intruded into and contains large xenoliths of Palaeozoic metasediments and Late Jurassic silicic volcanics, both readily recognisable as

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parts of the South American continental 'basement' that underlies the Cretaceous shelf sediments to the east (Figs 1 and 2). The Patagonian batholith is strikingly similar to the Peruvian batholith recently described by Cobbing and Pitcher¹⁴.

Hamilton¹⁵ has proposed that large batholiths of intermediate composition, such as those that are more or less continuous along the western North and South American continental margins, are the plutonic equivalents of overlying contemporaneous calc-alkaline volcanic chains. In the southern Andes there is strong support for such a comagmatic origin. Radiometric dating, largely by Rb-Sr whole-rock-mineral isochrons¹⁶, indicates that the batholith was initiated by the Late Jurassic (150 Myr) and that plutonic activity continued intermittently until at least the end of the Cretaceous. Thus it was coeval with the andesitic volcanic activity on the same (Pacific) side of the sedimentary basin.

Thus we conclude that the Patagonian batholith was overlain by an andesitic complex emplaced into South American continental crust, as in the present central Andes, and supplying detritus to the basin on its eastern flank. This then suggests that subduction of the floor of the Pacific Ocean beneath this part of South America was initiated by the late Jurassic.

Marginal basin development

The floor of the basin consists of large lenses (up to at least 120 km long and 20 km wide and more than 2,000

overlap on to these blocks, usually being separated from them by a variable sequence of clastic (often volcanoclastic) sediments including conglomerates, mafic and silicic tuffs, and lavas. The extrusives dip moderately inwards (Fig. 2a). Thus the mafic lenses are funnel shaped in cross section, have normal igneous contacts and are autochthonous.

The sheeted dyke complexes are entirely composed of dykes with chilled margins against each other and basaltic-doleritic textures. They are as much as tens of metres thick but average 1–2 m. Where studied in detail (north of the Straits of Magellan where the Andies trend north-south) they strike consistently subparallel to the Cordillera. Swarms of mafic dykes and gabbro sills are present even outside the main mafic complexes, cutting the rocks of the continental blocks. Where large volumes of these intrusives are present in the continental blocks, especially near the margins of the mafic lenses, remelting and assimilation of the older silicic material is frequently evident. This process has almost certainly resulted in the generation of some of the more silicic phases associated with the gabbros.

Although the original igneous textures are generally preserved in the mafic rocks, the latter generally display zeolite facies mineral assemblages increasing with depth to lower amphibolite facies. At present these assemblages seem to represent an 'ocean floor' type of metamorphism.

The mafic lens we have studied in most detail forms Cordillera Sarmiento north of the Straits of Magellan (Fig. 1). Here the extrusive unit is conformably overlain by fossiliferous sediments dated as Lower Cretaceous (A. Cañon, personal communication). The absence of extensive

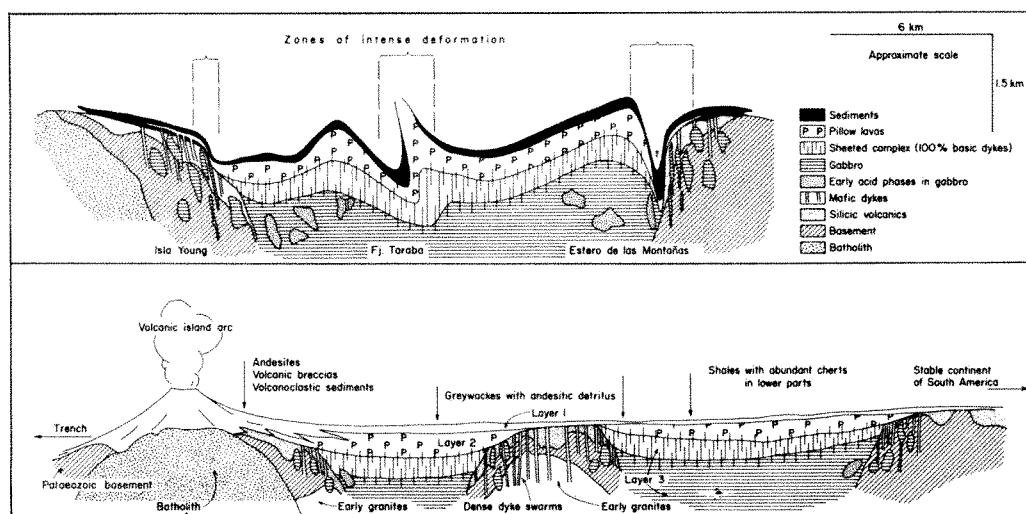


Fig. 2 a, Composite east-west section across the Sarmiento complex (for location see Fig. 1) b, Restored diagrammatic section across the southern Andes in the early Cretaceous.

m thick) of mafic rocks (Fig. 1). The lenses are subparallel to the Cordillera. They intrude and separate blocks of Palaeozoic schist and the overlying Middle–Upper Jurassic silicic volcanics (Fig. 2a).

The mafic rocks represent the upper parts of ophiolite assemblages. An intrusive unit, consisting of gabbro that may show cumulative layering, is cut by, and passes upwards into, a sheeted dyke complex. At still higher levels the sheeted complex is overlain by an extrusive unit of considerable thickness consisting of near horizontal pillow lavas, pillow breccias and tuffs. The intrusive–extrusive transition is complex, the dykes thinning and becoming irregular and more widely separated by screens of pillow lavas. Individual dykes frequently feed pillows. The extrusives are in turn overlain by, and intercalated with, minor amounts of chert and jasper.

The intrusive unit has steeply dipping igneous contacts with the continental blocks. In places the extrusive units

flyschlike sediment layers within and below the mafic extrusives suggests that the latter were extruded very rapidly during a major pulse of magmatism in the early Cretaceous and that marginal basin formation was initiated during the evolution of the andesitic volcanic complex along the Pacific continental margin.

The floor of the marginal basin consisted of huge strips of 'oceanic' crust separated by elongate blocks of older continental crust and must have formed by extension and intrusion of basaltic magma behind an active andesitic volcanic chain. Extension moved the volcanic chain towards the Pacific, relative to South America, producing a volcanic island arc based on continental crust and separated from the stable continent by a marginal basin whose floor had geophysical characteristics partly 'oceanic' and partly 'continental'. The geotectonic setting must have been analogous to the present Japan Sea area in these respects. The apparent absence of ultramafic complexes

from the otherwise complete ophiolite suites of the southern Andes may be the result of structural level, because in Cordillera Sarmiento the gabbros are at sea level. The question of whether or not the crust of marginal basins is identical to that of large ocean basins remains, however, to be settled.

Closure of marginal basin

The youngest dated rocks of the basin infilling are Aptian. The whole sedimentary pile, together with the underlying basin floor, shows signs of strong deformation. These tectonic structures are cut by undeformed granodioritic plutons that have been dated as 80–90 Myr old^{16,17}. It is clear, therefore, that the marginal basin rocks were deformed after the Aptian and before the end of Coniacian¹⁸. We consider this deformation to have occurred when the volcanic arc was translated back towards the continental margin. The resultant shortening between the arc and continent caused the deformation not only of the rocks of the marginal basin but also of its margins, particularly the eastern continental margin.

The very varied basin floor assemblages were very inhomogeneously deformed. Zones of intense and locally polyphase deformation alternate with large areas of virtually undeformed rocks across the width of the basin, the areas of high strain often being concentrated along major lithology contrasts. North of the Straits of Magellan the floor rocks of the basin display essentially vertical planar structural elements (shear zones, slaty cleavage and major fold axial surfaces) that parallel the strike of the Cordillera.

By contrast the sedimentary fill of the basin appears to be more uniformly deformed. A persistent, well developed, slaty cleavage that is axial planar to related asymmetric folds occurs throughout the cover sediments, striking parallel to the Cordillera. South of the Straits of Magellan the cleavage and fold axial surfaces dip towards the Pacific. The vergence of the folds towards the Atlantic^{19,20} is accentuated by small thrusts in the anticlinal hinges that translate the rocks in the same direction. This fold vergence is also predominant in southern Tierra del Fuego where the Cordillera trends west-east^{12,19}. Moreover, if recent reconstructions of the North Scotia Ridge are correct^{21,22}, the flyschlike Cumberland Bay and Sandebugten sequences of South Georgia^{23,24} also represent basin fill, and the Cumberland Bay has the same Pacific to Atlantic vergence^{23,24} and is thrust towards the Atlantic over the Sandebugten sequence that has predominantly opposed vergence²⁵.

The rocks to the east of the marginal basin on the South American continental margin of the early Cretaceous show varying intensity of deformation. Along the whole length of the Cordillera the silicic volcanic layer beneath the Cretaceous and Cainozoic sedimentary cover has been structurally uplifted and crops out in a narrow belt along the Atlantic margin of the High Cordillera. In the north deformation of the Palaeozoic basement schists, like that of the mafic lenses, is confined to narrow zones²⁰. In the east–west trending Cordillera in Tierra del Fuego, by contrast, widespread reactivation of the Palaeozoic basement has occurred^{20,26}. Everywhere the intensity of deformation dies away rapidly towards the Atlantic.

The rocks of the volcanic arc itself were also probably deformed at this time. Some narrow subvertical shear zones in the batholith may be related to basin closure. To the north of the Straits of Magellan the Palaeozoic schists into which the batholith was emplaced are deformed in zones as on the eastern margin of the basin.

The basin must have closed by obduction, subduction or internal deformation of the basin floor and its infill, or by a combination of these processes. As the rocks of the basin

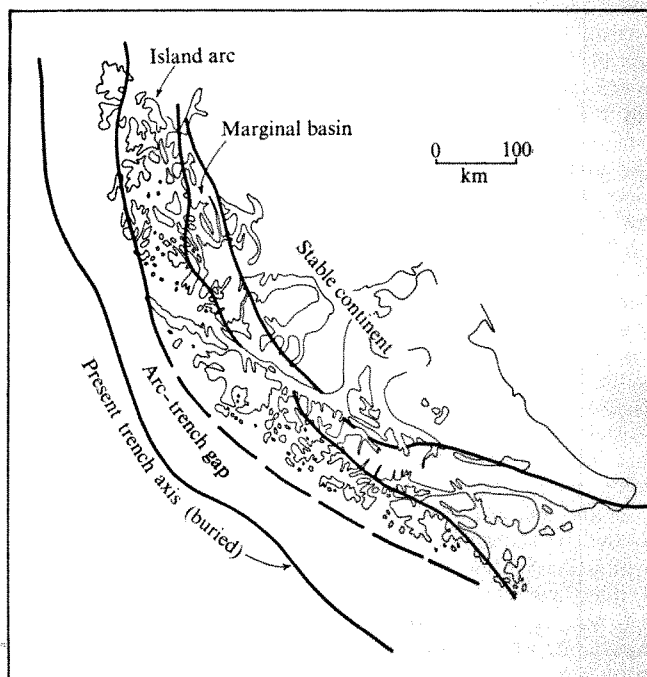


Fig. 3 Present location of the main tectonic divisions of the southern Andes, as interpreted in this paper.

floor appear to be entirely autochthonous, obduction can be rejected. The marginal basin rocks we have studied were certainly horizontally shortened by internal strain. It has not yet been determined whether this resulted from subduction of the basin floor. If subduction occurred the mafic lenses now preserved merely represent relics of the easternmost part of an originally more extensive marginal basin.

Geotectonic implications

Several significant conclusions can be drawn:

(1) There is positive evidence in the southern Andes that the Mesozoic batholith along the Pacific continental margin represents the root of an andesitic volcanic chain that was initiated on South American continental crust by the late Jurassic. We relate the formation of the volcanic chain and its continued activity into the Cretaceous to subduction of the Pacific Ocean floor beneath the South American continent.

(2) In the latest Jurassic–early Cretaceous, basaltic magmas were intruded into the continental crust behind the volcanic complex that was consequently translated towards the Pacific relative to South America, creating a marginal basin between a volcanic island arc and the South American continent. The marginal basin seems to have formed during a single major pulse of rapid extension, and was infilled during the early Cretaceous by andesitic detritus from the active arc. The original width of the basin is not known.

(3) During the middle Cretaceous the arc moved back towards the continent accompanied by the deformation of the basin rocks, the South American continental margin and probably the arc itself. Recognition of the inhomogeneous deformation of the mafic lenses leads us to emphasise that the dating of ocean floor remnants solely on the basis of the presence or absence of deformation fabrics such as has been attempted in the Appalachian/Caledonian orogen^{27,28} must be undertaken with the greatest caution (J. R. Burnsall and M. J. de Wit, in preparation).

(4) Based on fossils in the sediments overlying the mafic

lenses, it can be interpreted that the marginal basin opened before 118 Myr (base of the Barremian). From the age of the plutons cutting the deformed basin infill, it closed before 88 Myr (base of the Coniacian). Thus whereas the opening took place during a period of 'normal' seafloor spreading rates in the Pacific, closure and accompanying deformation coincided with the period of particularly high spreading rates between 110 and 85 Myr ago²⁹.

The opening of the marginal basin took place by extension and intrusion behind the arc, apparently in the manner suggested by Karig³⁰ and Uyeda³¹. Field observations clearly demonstrate that the 'ophiolitic' rocks do not represent old oceanic crust fortuitously trapped between South America and an island arc migrating across the Pacific^{2,6}. Closure of the basin did not take place by obduction of the basin floor. Horizontal shortening was certainly involved and Pacific-ward subduction may have taken place.

In the Central Andes and in West Antarctica ophiolitic rocks are unknown^{10,14,22}, although there is substantial evidence of Mesozoic subduction^{32,33}. Hence it seems that a marginal basin with a mafic or partially mafic floor did not develop in these areas. At least in the central Andes, however, an early Cretaceous sedimentary basin infilled with flysch-type sediments full of andesitic detritus and mafic extrusives, did develop behind the Mesozoic volcanic chain, perhaps indicating more limited extension. Significantly, the mid-Cretaceous deformation there was far less intense and of a different style, consisting of block faulting and gentle warping. Penetrative fabrics are wholly absent. Thus it seems that large penetrative strains at high crustal levels are not generated behind a volcanic arc by subduction alone but may be related to 'collision' between volcanic arcs and continents during closing of marginal basins.

The data show that marginal basins are not characteristic solely of the western side of the Pacific Ocean. They have existed in the past along the eastern margin. Hence the present western South American continental margin is not representative of all Mesozoic-Cainozoic Andean geological history. Recognition of this fact leads us to consider the factors involved in the opening and closing of marginal basins.

Wilson and Burke⁵ have suggested that marginal basin formation is related to the relative motion of lithospheric plates and the mantle underlying the asthenosphere. According to their hypothesis the consuming plate at a subduction zone, such as the American plate at the Peru-Chile trench, if advancing over the lower mantle, will develop no marginal basin. If, however, the over-riding plate is stationary over the mantle or retreating from the plate being consumed, then marginal basins will develop as in the western Pacific.

Scholz *et al.*³⁴ have suggested a more general explanation that marginal basin formation is related to the level of the horizontal component of compressive stress across the consuming plate boundary. An increase in this component could arise from an increase in spreading rate and it may not be a coincidence that the age of closure of the marginal basin in the southern Andes coincides with the world-wide pulse of high spreading rates²⁹. In addition to changes in relative motion of plates over the mantle, increase in spreading rate and certain changes in plate configuration, it has been suggested that marginal basin closure could also result from weakening of the mafic floor of the basin because of high heat flow below an insulating 'blanket' of volcanoclastic sediments. The metamorphic assemblages of the southern Andean mafic lenses could be taken to support this view but the fact that a uniform penetrative fabric is lacking seems to argue against it.

The question of why the basins open and close is at present unresolved, and it is increasingly apparent how crucial the answer is for understanding the evolution of continental margins and mountain belts. Recognition not only that a marginal basin developed in the Andes, but that it opened and closed before those of the present western Pacific were initiated, may be of great significance for understanding of the evolution of continental margin orogenic belts and the history of the Pacific Ocean basin.

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- ¹ Dewey, J. F., *Nature*, **222**, 124 (1969).
- ² Dewey, J. F., and Bird, J. M., *J. geophys. Res.*, **75**, 2625 (1970).
- ³ James, D. E., *Bull. geol. Soc. Am.*, **82**, 3325 (1971).
- ⁴ James, D. E., *Scient. Am.*, **229**, 60 (1973).
- ⁵ Wilson, J. R., and Burke, K., *Nature*, **239**, 448 (1972).
- ⁶ Moores, E., *Nature*, **228**, 837 (1970).
- ⁷ Katz, H. R., *Nature*, **237**, 331 (1972).
- ⁸ Katz, H. R., *Geol. Rdsch.*, **54**, 195 (1964).
- ⁹ Borrello, A. V., *Dirección Nacional de Geología y Minería (Argentina) Museo, Anales XIV*, (1969).
- ¹⁰ Gansser, Q., *Jl geol. Soc. Lond.*, **129**, 93 (1973).
- ¹¹ Butterlin, J., *Trans. sixth Caribbean geol. Conference* (edit. by Perzall, Cecily), 265 (Caracas, Venezuela, 1973).
- ¹² Katz, H. R., and Watters, W. A., *N. Z. Jl Geol. Geophys.*, **9**, 323 (1966).
- ¹³ Kranck, E. H., *Acta geogr., Helsingf.*, **4**, 1 (1932).
- ¹⁴ Cobbing, E. J., and Pitcher, W. S., *Q. Jl geol. Soc. Lond.*, **128**, 421 (1972).
- ¹⁵ Hamilton, W., *Ore. Dept Geol. Min. Industries Bull.*, **65**, 175 (1969).
- ¹⁶ Halpern, M., *Bull. geol. Soc. Am.*, **84**, 2407 (1973).
- ¹⁷ Halpern, M., and Rex, D. C., *Bull. geol. Soc. Am.*, **83**, 1881 (1972).
- ¹⁸ Anonymous, *Q. Jl geol. Soc. Lond.*, **120**, 200 (1964).
- ¹⁹ Dalziel, I. W. D., in *Antarctic Geology and Geophysics* (edit. by Adie, R. J.), 47 (Universitetsforlaget, Oslo, 1972).
- ²⁰ Dalziel, I. W. D., and Cortes, R., *26th Int. geol. Congr.*, **3**, 316 (1972).
- ²¹ Dalziel, I. W. D., and Elliot, D. H., *Nature*, **233**, 246 (1971).
- ²² Dalziel, I. W. D., and Elliot, D. H., in *The Ocean Basins and their Margins: I. The South Atlantic* (edit. by Stehli, F. G., and Nairn, A. E. M.) (Plenum, New York, 1973).
- ²³ Trendall, A. F., *Falkland Islands Dependencies Surv. scient. Rep.*, **7** (1953).
- ²⁴ Trendall, A. F., *Falkland Islands Dependencies Surv. scient. Rep.*, **19** (1959).
- ²⁵ Dalziel, I. W. D., Dott, R. H., jun., Winn, R. M., and Bruhn, R. L., *Geol. Soc. Am. Abstracts with Programs*, **590** (1973).
- ²⁶ Palmer, K. F., and Dalziel, I. W. D., *Antarctic J. U.S.*, **VIII**, 11 (1973).
- ²⁷ Dewey, J. F., Rickards, R. B., and Skevington, D., *Norsk. geol. Tidsskr.*, **50**, 19 (1970).
- ²⁸ Bird, J. M., Dewey, J. F., and Kidd, W. S. F., *Nature phys. Sci.*, **231**, 28 (1971).
- ²⁹ Larson, R. L., and Pitman, W. C., *Bull. geol. Soc. Am.*, **83**, 3645 (1972).
- ³⁰ Karig, D. E., *J. geophys. Res.*, **76**, 2542 (1971).
- ³¹ Uyeda, S., *Tectonophysics*, **12**, 269 (1971).
- ³² Pitcher, W. S., *24th Int. geol. Congr.*, **2**, 156 (1972).
- ³³ Vergara, M. M., *24th Int. geol. Congr.*, **2**, 222 (1972).
- ³⁴ Scholz, M. L., Barazangi, M., and Sbar, M. L., *Bull. geol. Soc. Am.*, **82**, 2979 (1971).

Crystallographic study of the interaction of urea with lysozyme

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High-quality crystals suitable for an X-ray crystallographic study have been obtained for urea-denatured lysozyme

As more protein structures become known through the use of crystallographic methods, knowledge of the basic structure, and also of perturbations of the structure will be required to understand the forces involved in the architecture of the proteins and the catalytic power of enzymes. One of the perturbations which may prove useful, is the unfolding of proteins caused by high concentrations of urea. Although many solution probes such as nuclear magnetic resonance (NMR), electron spin resonance (ESR) and fluorescence, have been used in denaturation studies, these tools, powerful as they are, can probe only small portions of the protein at a time. Such a partial picture makes it difficult to relate structure change with activity since only some of the changes are known. A comprehensive recording of all changes could be obtained by crystallography but unfortunately crystallisation of proteins in urea has seemed difficult theoretically or recalcitrant experimentally. It was therefore decided to explore conditions for obtaining enzyme crystals from urea by the manipulation of urea and salt concentrations. This has now been achieved for lysozyme and excellent X-ray diffraction patterns have been obtained from crystals in equilibrium with high urea concentrations.

Preparation of lysozyme crystals

Native, tetragonal crystals of hen egg white lysozyme chloride were grown following the method of Alderton and Fevold¹: 400 mg of lysozyme were dissolved with thorough stirring in 5 ml of 0.04 M acetate buffer pH 4.7 and the stirring was continued for a further hour to dissolve nuclei. Sodium chloride solution (5 ml, 10% w/v) was added dropwise over several minutes, the final solution was filtered into a polythene bottle and left undisturbed for up to two weeks. Crystal nucleation generally occurred within a few hours but growth was regarded as complete only after two weeks had elapsed.

Tetragonal crystals of lysozyme were crystallised with, for example, 6 M urea by a simple variation of the above procedure: 300 mg of lysozyme were dissolved in 2 ml of a solution which was 4 M in acetate buffer pH 4.7, and 9 M in urea. After equilibrium (assumed to be up to 1 h), 1 ml of saturated sodium chloride solution was added and after further gentle stirring the solution was filtered and left. Nucleation and growth of the crystals was generally as rapid as for the native crystals.

Equilibration of native lysozyme crystals with urea solutions was achieved by first stabilising the crystals with a solution of 0.2 M acetate buffer, 12.3% w/v NaCl and subsequently with solutions of the same salt content but

with the appropriate urea concentration. Equilibration was achieved by adding the final stabilising solution dropwise over several days, with gentle agitation at each addition, to the crystals in their original mother liquor.

Hen egg white lysozyme (3X-crystallised, dialysed and lyophilised), Lot 70C 8110, and N-acetyl glucosamine (NAG) (Sigma (London) Chemical Co.), Tri-N-acetylchitotriose (tri-NAG) and analytical grades of urea, glacial acetic acid, sodium acetate trihydrate and sodium chloride

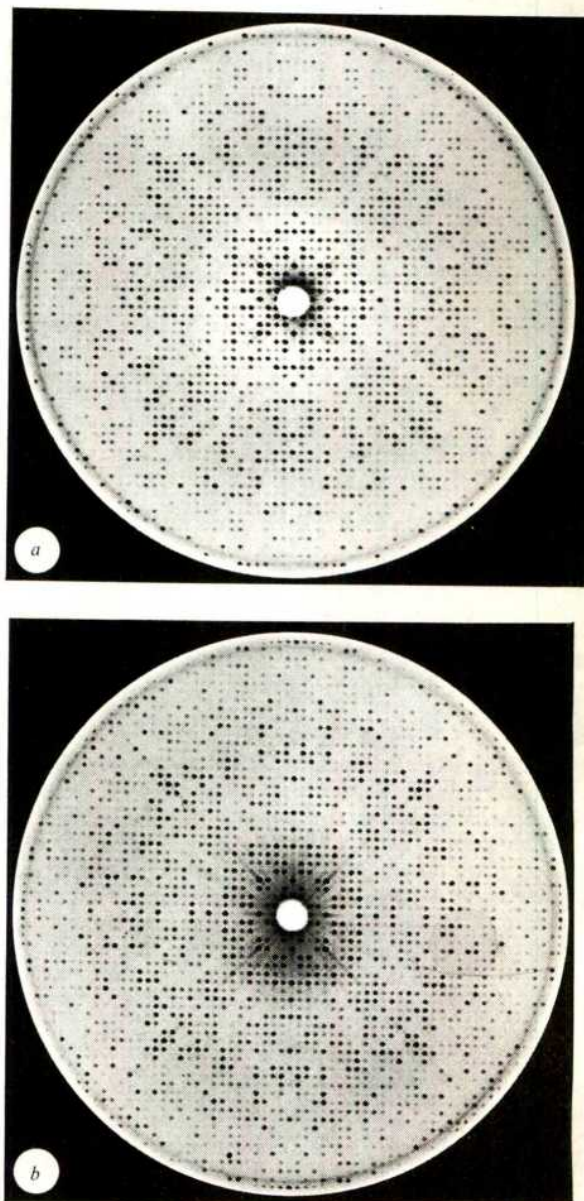


Fig. 1 18° HKO (2.5 Å) precession photographs of tetragonal crystals of hen egg white lysozyme chloride: *a*, native crystals; *b*, crystals equilibrated with 9 M urea.

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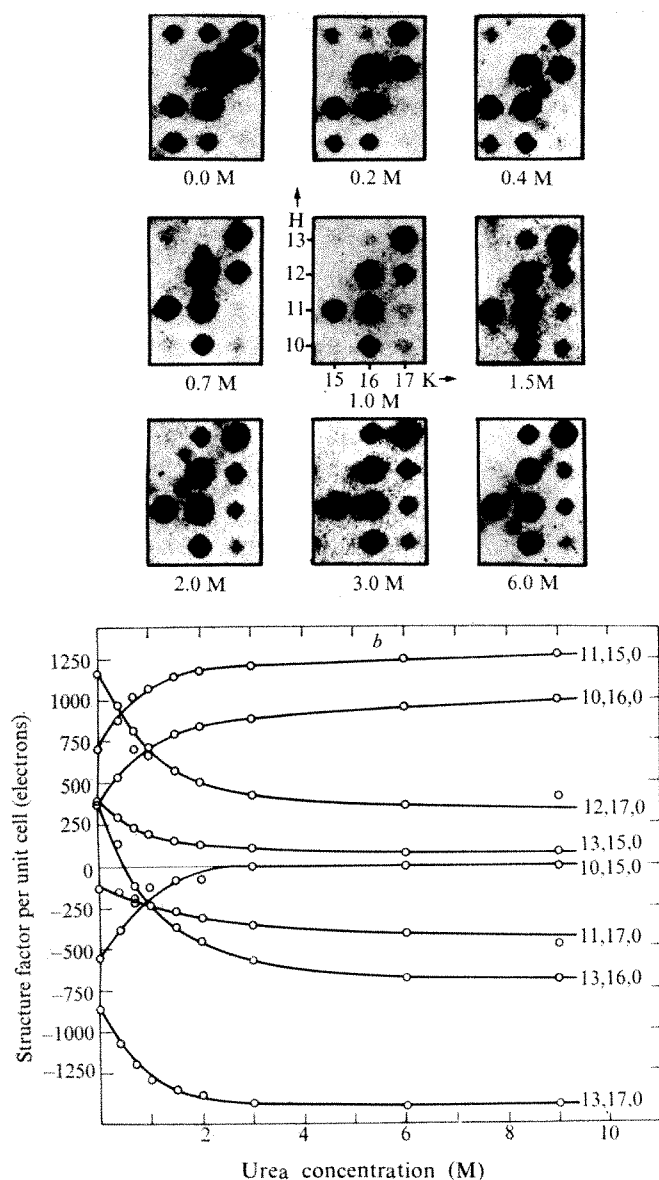


Fig. 2 *a*, Enlargement of a representative group of medium resolution HKO reflections showing the variation of intensities with urea molarity. *b*, A plot of the variations with urea concentration of the structure factors of some of the best-phased reflections of *a*.

(British Drug Houses, Ltd) were used. The urea was freshly recrystallised from glass distilled water immediately before use and only freshly prepared urea solutions were used. Screened precession photographs (18°) of the (centric) HKO and HOL zones were obtained using Ni-filtered copper radiation from an Elliott Automation rotating anode, Type GX3. Photographic intensities were estimated using a Joyce-Loebl double-beam recording microdensitometer Mark III CS. Crystals were conventionally mounted and sealed in contact with the vapours of their respective equilibrium soaking solutions.

Isomorphism of crystals

Crystals of hen egg white lysozyme in equilibrium with solutions of urea show diffraction patterns indicating isomorphism with native crystals². Significant alterations in the intensities of reflections were observed without any loss of order in the crystals or any large change in cell dimensions (compare Fig. 1). The detailed X-ray crystallography therefore confirmed the qualitative observation that good crystals existed in the urea plus salt mixture and that the

crystals give X-ray patterns of the same quality as do the native crystals.

Examination of the photographs (of which Figs 1 and 3 include two representative examples) shows large intensity changes in many of the reflections. These intensity changes indicate that urea causes extensive structural alterations without any resultant loss of crystallinity. This clearly allows a structural investigation of the lysozyme molecule in high concentrations of urea to be carried out using X-ray techniques. Extensive studies of lysozyme structure and activity in urea solutions have been made³⁻¹¹, which have indicated that the lysozyme structure is generally rather resistant to denaturation by urea but that changes do occur. X-ray crystallography confirms these findings in general but allows more specific conclusions to be made. Edelhoch and Steiner⁵, for example, found that the molecule retained some structure, that is, was not a random coil, even in 9 M urea. The X-ray results are in agreement with this conclusion and indicate a surprising degree of order for a solvent of such denaturing capacity.

The changes in intensity are unfortunately much too great (compare Fig. 1) for a conventional difference synthesis against the known native structure to be useful in defining the precise nature of the changes induced by urea. In these circumstances there is no alternative to a complete *de novo* structure determination of the denatured molecule, and a search for useful heavy-atom derivatives has been started. Some useful information of a general nature can, however, be deduced from the intensity changes. The alterations in intensity are particularly pronounced at high resolution, indicating that changes occur largely to the detailed structure of the molecule. A three-dimensional difference map between the 3 M urea and native forms, using terms to 3 Å resolution, while confirming our judgement that the intensity changes were too extensive to be useful in detail, showed that the major difference density features were confined within the molecular envelope. This indicates that while some contribution to the intensity changes can probably be expected from perturbation of the solvent structure, it is clear that the major contribution arises from conformational changes distributed throughout the molecule itself.

Variation with urea concentration

Preliminary phase information from the heavy-atom search together with an analysis of the variation of X-ray intensities as a function of urea concentration allow some broad but definite inferences to be made about the lysozyme-urea system under study. An examination of precession photographs extending to 2.5 Å resolution shows that the intensities of a majority of the centric HKO and HOL reflections vary in a monotonic manner. The detailed intensity variations of a small number of representative medium-resolution reflections are shown in Fig. 2*a*, and a plot of the structure factors of the best phased reflections in this group in Fig. 2*b*. Figure 2 shows that the major changes in intensity have occurred by the time the concentration of urea has reached 3 M, and that the further changes up to 9 M are small. This suggests that the major conformational changes induced by the urea have occurred at these low concentrations, and that thereafter further alterations are minor.

A small number of reflections seem to behave in a different way. Their intensities first decrease to zero as the urea concentration increases and then subsequently increase at higher concentrations. The preliminary phase information indicates, however, that the reflections behaving in this way have all undergone a change of sign (see, for example, 13, 16, 0 in Fig. 2*a* and *b*) and when this factor is taken into account, all the reflections examined are found to vary monotonically. The finding of such

monotonic changes in the diffraction pattern might indicate a two-state denaturation model, or at least be consistent with such a model. But, the knowledge of protein structure which already exists, and the failure of two-state models to account for the kinetics of either denaturation^{12,13} or refolding^{14,15} suggests that such a model is unlikely, and indeed a plot of fractional structure factor changes as a function of urea concentration for individual reflections gave differing curves indicating deviations from a mutually linear relationship. Additional small intensity changes due to the minor concentration-dependent changes in cell dimensions cannot be corrected for, however, and these data *per se* cannot exclude a two-state model. It is, of course, conceivable that, because we are dealing with the structure in a crystal, there are certain constraining relationships which favour an apparent two-state model. A definitive answer to this problem must await the complete three-dimensional crystallographic study which is now in progress.

Stability of the protein

A particularly interesting feature of this X-ray study is the thermodynamic stability both of the protein and of the tetragonal crystal form. Changes in the diffraction patterns seemed to be complete within 24 h of the introduction of urea, suggesting a rapid attainment of equilibrium, but there were no further changes when the crystals were subsequently exposed to the urea solutions for a period of weeks. Crystals equilibrated with high urea concentrations could, in addition, be leached with urea-free high salt solutions and were found within 24 h to regain in full their original native diffraction patterns. That this stability represents a true thermodynamic minimum was demonstrated by the crystallisation of lysozyme directly from urea solutions. Crystals obtained from 1 M urea had diffraction patterns which were identical to those of soaked crystals, whereas those obtained from 6 M urea showed only minor high-angle differences when compared with their corresponding soaked crystals (compare Fig. 3). This retention of isomorphism is especially interesting in view of the appreciable conformational changes indicated both by physical studies and by the present work, and confirms that the basic form and packing of the molecules is not altered as much as might be expected.

No glutaraldehyde or other cross-linking material has been used to cross-link the crystals. The main feature that has changed the urea from a medium which dissolves protein to one in which crystals are stable is the use of high salt concentrations.

While this work was in progress, we learned of the study of Berthou and Jolles¹⁶ who have also crystallised lysozyme from urea. Their studies on temperature effects and polymorphism should offer additional valuable insights into lysozyme properties.

Further uses

In summary, these studies indicate that crystallisation of proteins from urea is possible and that the X-ray crystallography of these structures will be valuable in interpreting the role of conformation in the activity and folding of proteins. The use of urea for structural changes has considerable advantages in studying protein structure since it is the differences rather than the creation of a total structure that is being dealt with. Furthermore, the use of lower concentrations of urea can in some cases produce smaller perturbations of structure. We hope that further work will relate conformational changes to the activity of the lysozyme. Preliminary (unpublished) results indicate that activity is regained and conformational changes are induced when polysaccharides are added.

X-ray crystallography is particularly valuable for such structure-activity correlations. The examination of any

one group, such as by using reporter groups is useful, but the role of a residue may be ambiguous if the perturbations of other groups are not known. Crystallography gives an overall three-dimensional picture which would be difficult or impossible to achieve with individual probes, but is also more time consuming. Eventually parallel relationships in which the probing of an individual group by a reporter group can be correlated with key X-ray structures may offer the complementary advantages of completeness and economy of effort. Our present X-ray results with lysozyme make possible such interrelated studies.

The generality of the method is supported by unpublished studies on chymotrypsin which have shown that it can be crystallised from 6 M urea and remain isomorphous with the native enzyme. Thus, the results found here in relation to lysozyme can be applied to a variety, possibly a majority, of protein molecules.

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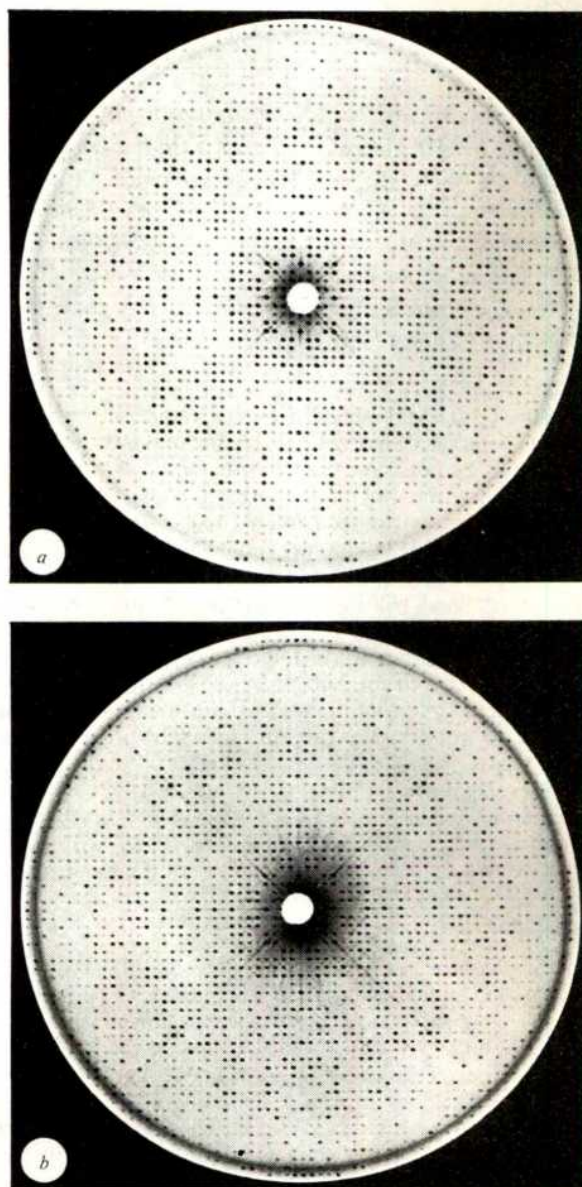


Fig. 3 HKO precession photographs of crystals of hen egg white lysozyme: *a*, soaked in 6 M urea; *b*, cocrystallised from 6 M urea.

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¹ Alderton, G., and Fevold, J., *J. biol. Chem.*, **164**, 1 (1946).

² Blake, C. C. F., Johnson, L. N., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarman, V. R., *Proc. R. Soc. Lond.*, **B167**, 348 (1967).

³ Hamaguchi, K., and Rokkaku, K., *J. Biochem.*, **48**, 358 (1960).

⁴ Donovan, J. W., Laskowski, M., jun., and Scheraga, H., *J. Am. chem. Soc.*, **83**, 2686 (1961).

⁵ Edelhoch, H., and Steiner, D., *Biochim. biophys. Acta.*, **60**, 365 (1962).

⁶ Leonis, J., *Archs Biochem. biophys.*, **65**, 182 (1956).

⁷ Hamaguchi, K., and Kurono, A., *J. Biochem.*, **54**, 111 (1963).

⁸ Shimaki, N., Ikeda, K., and Hamaguchi, K., *J. Biochem.*, **70**, 497 (1971).

⁹ Wein, R. W., Morrisett, J. D., and McConnell, H., *Biochemistry*, **11**, 3707 (1972).

¹⁰ Meadows, D. H., Markley, J. L., Cohen, J. S., and Jardetzky, O., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1307 (1967).

¹¹ Allerhand, A., Childers, R. G., and Oldfield, E., *Biochemistry*, **12**, 1335 (1973).

¹² Tsong, T. Y., and Baldwin, R. S., *J. molec. Biol.*, **69**, 145-149 (1972).

¹³ Anfinsen, C. B., *Science*, **181**, 223 (1973).

¹⁴ Teipel, J., and Koshland, D. E., Jun., *Biochemistry*, **10**, 792 (1971).

¹⁵ Sachs, H. A., Schechter, A. N., Eastlake, A., Anfinsen, C. B., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3790 (1972).

¹⁶ Berthou, J., and Jolles, P., *FEBS Lett.*, **31**, 189 (1973).

Plate movement relative to rigid lower mantle

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The vertical extent of the flow in the upper mantle may be governed by the layering of the Earth, with the lower mantle rigid enough to act as a frame of reference. A theorem deduced from basic mechanics gives the movement of the plates relative to this frame. The fit with velocities deduced from the migration of deep volcanism is excellent. Absolute velocities of trenches may be related to the slope of the sinking slabs of lithosphere.

THERE have been several successive steps in the hot spot theory¹. It was first suggested that volcanic chains of the Hawaiian type are produced by hot spots deep in the mantle. Then came the suggestion that the hot spots form a consistent frame of reference. If this were so, then at a particular depth, the bulk of the mantle outside the hot plumes should be subject to shrinking at a negligible rate only. Furthermore, horizontal movements should be confined within the upper mantle and near to the mantle-core interface. The third suggestion was that hot spots could also provide the motive force for continental drift, with horizontal currents in the asthenosphere flowing radially away from each of the plumes. I here deal with the first two suggestions, but do not consider the third idea which is much more controversial.

The movement of the lithospheric plates relative to the lower mantle can be deduced directly, using basic mechanics, as long as the model fits some general conditions. The fit between the relevant calculations supports the validity of the model used.

Necessary assumptions

I assume that the lower mantle, below the 700 km discontinuity, is rigid enough away from hot plumes, to be taken as a frame of reference. Velocities relative to this frame will be termed 'absolute velocities'.

This assumption conflicts with some questionable estimations of the viscosity at depth. McConnell's method of calculating velocities from the profiles of raised beaches² gives no pertinent information about regions below 800 km. Although Goldreich and Toomre³ have explained the nonhydrostatic bulge of the Earth by assuming a mean viscosity $< 10^{24}$ poise, an earlier explanation⁴, which suggested a mean viscosity $\approx 10^{26}$ poise has not been disproved. A mean viscosity of 10^{23} poise at the

mantle-core interface, with a shear strain rate of 10^{-16} s^{-1} has been calculated⁵. It is, however, the shear stress, not the shear strain rate, which is spread over the whole mantle with the same order of magnitude (for instance 5-10 bar), with convection, or without it. With such a shear stress in the asthenosphere, where the melting point is reached, Weertman's law⁶ would give a shear strain rate of 10^{-8} to 10^{-9} s^{-1} , a value 10^6 times too high. By modifying the numerical factor accordingly, the viscosity at the mantle-core interface rises to 10^{27} poise.

My second assumption is that, with the exception of hot plumes and descending slabs, there are no lateral variations in the viscosity of the upper mantle. A three-layer model can be adopted. The top layer is composed of rigid lithospheric plates, which may be limited downwards at an isobaric surface p_0 without substantially changing the dynamical problem. Under this is a low viscosity layer (for instance 4×10^{20} poise). The bottom layer is more viscous (for instance 10^{22} poise), and is of uniform thickness and horizontal extent. The viscosities of layers 2 and 3 are assumed to be Newtonian for the very slow motions involved.

My third assumption is that the viscous layers have densities ρ_1 and ρ_2 , respectively, with horizontal variations which are much larger than the vertical variations within a single layer. The horizontal variations are a function of temperature changes which are governed by the convection processes, and so this assumption implies that any reversal of the current should occur near an interface between layers.

Absolute velocities

Departures from the hydrostatic values are here termed 'perturbations'. The equations of quasistatic equilibrium and of viscosity can be written in a linear form with respect to the perturbations. This is not so, however, with the heat equation, because the convective term in $\mathbf{u} \cdot \nabla T$ is the main one; thus it is not introduced, and the horizontal density gradients $\nabla \rho_1$ and $\nabla \rho_2$ are considered as unknown functions.

I have shown⁶ that, in the two-dimensional case, any perturbation may be expressed as a linear function of four perturbations. With the x -axis horizontal, the velocity $u_{0,x}$ at any point on a plate may be written:

$$u_{0,x} = C_1 \partial \zeta / \partial x + C_2 \partial \rho_1 / \partial x + C_3 \partial \rho_2 / \partial x + C_4 \tau_{2,x} \quad (1)$$

where ζ denotes the vertical deflection of the isobaric surface p_0 and $\tau_{2,x}$ is the shear stress, in the x direction, between the

third layer and the motionless lower mantle. C_i is constant (for a given model) independent of the perturbations. Equation (1) holds to the second order only, but because with reduced variables C_i is of the order of unity and the perturbations are of the order of 10^{-3} – 10^{-4} , this inaccuracy is of little concern.

In the three-dimensional case, equation (1) holds in the x direction, and in a transverse horizontal direction (substituting y for x) because, to the second order, the perturbations in the x and y directions do not interfere with one another. Therefore:

$$\mathbf{u}\partial = C_1\nabla\zeta + C_2\nabla\rho_1 + C_3\nabla\rho_2 + C_4\tau_2 \quad (2)$$

According to my third assumption, equation (2) is valid over the whole Earth with the same constants C_i , with the exception of some small areas (oceanic ridges, subduction zones, and so on). Of course, for points on one plate the values of \mathbf{u}_0 are linked and thus the four vectors on the right hand side of the equation are not independent. When crossing a ridge or a subduction zone, ζ does not undergo a discontinuity. The same is true, to the second order, for ρ_1 and ρ_2 , as the conditions are very close to hydrostatic.

(It should be noted that the nullity of the total mass transport through the three layers is not introduced, as was the case in previous articles^{6,7}. Even if there were no hot plumes rising from the lower mantle, this nullity would hold only in the two-dimensional case, that is for a 'cylindrical Earth'. This point will be developed elsewhere.)

There can be a horizontal vector, \mathbf{a} , defined at any point on the Earth's surface. The resulting moment of $\mathbf{a}dS$ over the whole Earth, with respect to an arbitrary diameter DD' is

$$\iint (\mathbf{OD} \times \mathbf{OM}) \cdot \mathbf{a} dS = \mathbf{OD} \cdot \iint (\mathbf{OM} \times \mathbf{a}) dS \quad (3)$$

(taking the radius of the Earth, OM , as unity of length). This quantity vanishes when, and only when:

$$\mathcal{M}_0(\mathbf{a}) \equiv \iint (\mathbf{OM} \times \mathbf{a}) dS = 0 \quad (4)$$

It can easily be shown that, when \mathbf{a} is the gradient of a function continuous over the whole Earth, $\mathcal{M}_0(\mathbf{a}) = 0$

Such is the case for ζ , ρ_1 , ρ_2 , and thus:

$$\mathcal{M}_0(\nabla\zeta) = \mathcal{M}_0(\nabla\rho_1) = \mathcal{M}_0(\nabla\rho_2) = 0 \quad (5)$$

Moreover, as the rigid lower mantle is assumed to be spherical, the quasistatic equilibrium of the whole system means that $\mathcal{M}_0(\tau_2) = 0$. Thus, according to equation (2)

$$\mathcal{M}_0(\mathbf{u}_0) = \iint (\mathbf{OM} \times \mathbf{u}_0) dS = 0 \quad (6)$$

For any closed path drawn on a single plate, the conservation of mass requires,

$$\oint (\mathbf{OM} \times \mathbf{u}_0) ds = 0 \quad (7)$$

where ds is an infinitesimal arc.

As this relationship no longer holds when the path crosses a ridge or a trench, equation (6) is important.

Ω is the instantaneous rotation vector of the plate at point M , and, relative to the lower mantle, $\mathbf{u}_0 = \Omega \times \mathbf{OM}$. Equation (6) can thus be written

$$\iint (\mathbf{OM} \times \Omega \times \mathbf{OM}) dS = \iint \Omega dS - \iint \mathbf{OM} \cdot (\Omega \cdot \mathbf{OM}) dS = 0 \quad (8)$$

Ω is different from plate to plate.

In Cartesian coordinates, with the origin at the Earth's centre O , the components of \mathbf{OM} are x , y , z , and of Ω : Ω_x , Ω_y , Ω_z . Equation (8) becomes:

$$\iint \Omega_x dS - \iint x(x\Omega_x + y\Omega_y + z\Omega_z) dS = \iint (y^2 + z^2) \Omega_z dS - \iint x(y\Omega_y + z\Omega_z) dS = 0 \quad (9)$$

with two other similar relationships obtained with permutations of x , y , and z .

ω of components ω_x , ω_y , ω_z , can be the rotation vector of any plate relative to a reference plate (the Antarctic plate in these computations), and Ω_0 , of components a , b , c can be the 'absolute' rotation vector of the reference plate. Then:

$$\Omega_x = \omega_x + a \quad \Omega_y = \omega_y + b \quad \Omega_z = \omega_z + c \quad (10)$$

As:

$$\iint xy dS = \iint xz dS = 0 \\ \iint (y^2 + z^2) dS = 8\pi/3 \quad (11)$$

it follows that:

$$a = -3/8\pi \iint (y^2 + z^2) \omega_x dS \\ + 3/8\pi \iint x(y\omega_y + z\omega_z) dS \quad (12)$$

with two similar relationships for b and c , obtained by permuting x , y , and z .

Comparison with former estimations and results

In an earlier publication⁷ the two-dimensional relationship of equation (1) has been extended to cover the whole Earth. Only the plates of the equatorial zone were considered, and the effect of other plates was neglected. That is a very crude model of a 'cylindrical Earth'. This procedure was equivalent to neglecting the second integral on the right hand side of equation (12). If there were a very large number of plates, with random rotation vectors, this second integral should in fact vanish.

McKenzie⁸ has suggested that the term 'polar wandering', which described the motion of the pole relative to a frame of reference, should be defined such that

$$\iint \Omega dS = 0 \quad (13)$$

In other words, he defines 'absolute' velocities of continental drift by:

$$a = -1/S \iint \omega_x dS \quad (14)$$

To study of the causes of polar wandering it seems preferable to use equation (12) instead. 'Polar wandering' would then be the motion of the pole relative to the bulk of the mantle, excluding its outer viscous shell, that is, relative to the rigid body which carries most of the Earth's momentum. It follows from equation (7) that both definitions would be identical if $\mathbf{OM} \cdot \Omega \approx 0$ for all of the plates. This, however, is not the case.

The computation has been done using Chase's determination of the relative rotation vectors⁹, which takes into account the velocities at the ridges (deduced from the magnetic lineations of the ocean floor) and the direction of the transform faults, but which does not use data on the migration of volcanism (which may then be used as an independent check). To render the integrals discrete, the Earth's surface has been divided into bands of 5° of latitude, and for each band the boundaries between the plates have been located to within 1° of longitude.

Table 1 gives the 'absolute' rotation vector of the Antarctic plate according to McKenzie's definition (equation 14), to the two-dimensional approximation, and to my present theory (equation 12). The differences are not negligible. Table 2 gives the relative rotation vectors calculated from Chase's data, and the absolute rotation vectors according to my present theory.

Table 1 Absolute rotation vector of the Antarctic Plate (10^{-7} degree $^{-1}$ yr $^{-1}$)

	McKenzie's definition	Two-dimensional model	Present theory
a	-0.622	-0.549	-0.207
b	-0.079	-0.223	-0.394
c	2.381	2.539	2.658

Table 2 Relative and absolute* rotation vectors (10^{-7} degree $^{-1}$ yr $^{-1}$)

Plate and area (Earth's surface = 4π)	ω relative to the Antarctic Plate	Ψ , relative to the lower mantle Cartesian coordinates†	Spherical coordinates
Antarctic 1.431	$\omega_x = 0$ $\omega_y = 0$ $\omega_z = 0$	$\Omega_x = -0.21$ $\Omega_y = -0.39$ $\Omega_z = 2.66$	$\lambda = 81^\circ$ $\phi = -18^\circ$ $\Omega = 2.70$
American 2.627	0.42 -1.39 -2.96	0.22 -1.78 -0.31	-10° -83° 1.82
Eurasian 1.689	-1.01 -0.72 -1.21	-1.21 -1.11 1.45	41° -138° 2.19
Pacific 2.743	-0.98 3.20 -9.18	-1.18 2.80 -6.52	-65° 113° 7.19
African 1.944	1.64 -2.03 0.18	1.43 -2.43 2.84	45° -59° 4.00
Indian 1.637	5.70 2.54 1.79	5.49 2.15 4.45	37° 21° 7.39
Cocos 0.095	-6.90 -15.02 4.74	-7.10 -15.42 7.40	24° -115° 18.52
Nazca 0.400	-1.90 -4.26 3.38	-2.11 -4.65 6.04	50° -114° 7.91

* According to present theory.

† x—axis points towards 0° N, 0° E; y—axis towards 0° N, 90° E; z—axis towards 90° N, 0° E.

It seems that the South American plate, because it has a pole of rotation on itself, and the Antarctic plate, because it has a pole of rotation very near itself, are almost motionless. (Some symmetrical, eastward motions are occurring in Patagonia and the Antarctic Peninsula. These increase towards the Drake Passage.) Africa and the Indian Plate are moving towards the north-east, Europe towards the east, Indochina and Indonesia towards the south-east, and Alaska towards the south. This asymmetry of absolute motions on both sides of the Pacific Ocean may be related to the asymmetry in the slope of the descending slab¹⁰. Any descending slab should have a tendency to sink vertically downwards under its own weight. Nevertheless, if the trench is moving towards the ocean, the horizontal velocity of the trench must be added to the vertical velocity, to give the slope of the slab. This should be the case on the western side of the Pacific Ocean. There, the absolute velocity of the Eurasian plate, and the extension of the marginal seas (which has its own causes), add together to give a large eastward absolute movement of the Japanese–Kuril Trench. Thus, the sinking slab under the Japanese sea has a uniform slope of 30° until its very end, at a depth of 600 km whereas the lower end of the sinking slab under South America is almost vertical.

Comparison with migration of volcanism

If λ_p and ϕ_p are the latitude and longitude, respectively, of a pole of absolute rotation, then its distance, γ , from a point, λ , ϕ , in the corresponding plate is given by the relationship:

$$\cos \gamma = \cos \lambda \cos \lambda_p \cos (\phi - \phi_p) + \sin \lambda \sin \lambda_p \quad (15)$$

The absolute velocity, u_0 , at (λ, ϕ) is then

$$u_0 = (10/9) \Omega \sin \gamma \text{ cm yr}^{-1} \quad (16)$$

where Ω is expressed in 10^{-7} degree yr $^{-1}$

The azimuth (angle with the meridian) A is given by:

$$\cos A = (\Omega_x \sin \phi - \Omega_y \cos \phi) / \Omega \sin \gamma \quad (17)$$

For the Hawaiian chain, $u_0 = 7.6 \text{ cm yr}^{-1}$, $A = 63^\circ 1/2$, and for the Toubouai Islands (Austral chain), $u_0 = 7.7 \text{ cm yr}^{-1}$, $A = 65^\circ 1/4$. These values may be compared with those deduced from the hot spot theory¹¹. The azimuths are correct to less than 1° , and the velocities are within the limits of confidence.

According to our theory, the absolute motion of the Cocos Plate at the Galapagos Islands is 11.4 cm yr^{-1} ($A = 48^\circ 3/4$), and the absolute motion of the Nazca Plate at the same point is 7.1 cm yr^{-1} ($A = 71^\circ 3/4$). According to Johnson and Lowrie¹², the Galapagos hot spot is generating the Cocos and Carnegie Ridges. Our azimuths coincide exactly with the Cocos and the Malpelo Ridges. This is consistent with the idea that the Malpelo Ridge was formed in former times by the Galapagos hot spot on the Nazca Plate, to become extinct when the boundary between both plates, migrating northwards, crossed the ridge. The Carnegie Ridge would be a distinct feature, probably an extinct ridge like the Alpha Cordillera of the Arctic Basin.

The Walvis Ridge is composite, and does not present a clearcut migration of volcanism¹³. Nevertheless, my theory provides a correct azimuth for its southern part (55° ; $u_z = 4.4 \text{ cm yr}^{-1}$).

Lastly, Duncan *et al.*¹⁴ have discussed the migration of volcanism in central Europe. Using my theory the absolute motion at its western extremity (the Eifel), is 2.4 cm yr^{-1} ($A = 65^\circ$). This velocity is exactly the same as that given by the migration of volcanism, but the direction is consistent with that at the western tip of the volcanic range only. But the central European volcanics do not lie on a straight line, and the opening of the Atlantic may have seriously changed the plate motion in the past, and so the fit is satisfactory.

Thus, for five plates out of eight, in the instances in which, a check is possible, the absolute movement deduced from our theorem is consistent with that deduced from the migration of volcanism. This cannot be mere coincidence, and strengthens the idea that our assumptions are sound: there are no lateral variations in the viscosity (except in a few anomalous areas), and the lower mantle is rigid enough to act as a frame of reference for plate motions. This means that in the future, the determination of absolute velocities could have two important uses. First, they can be used to distinguish recently active volcanic chains which are created by hot spots, in the lower mantle, from other volcanic chains. Second, they could be used to calculate movements in the asthenosphere near sinking slabs and marginal basins, taking into account the absolute velocity of the trench relative to the rigid lower mantle. Such a calculation has never been done so far.

I thank Miss Discrezenzo for computing the integrals giving Ω_0 . Discussions with colleagues have helped to improve the presentation.

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¹ Morgan, W. J., *Nature*, **230**, 42 (1971).

² McConnell, R. K. jun., *J. geophys. Res.*, **70**, 5171 (1965).

³ Goldreich, P., and Toomre, A., *J. geophys. Res.*, **74**, 2555 (1969).

⁴ Munk, W. H., and McDonald, G. J. F., *The rotation of the Earth* (Cambridge University Press, Cambridge, 1960).

⁵ Weertman, J., *Rev. geophys. Space Phys.*, **8**, 145 (1970).

⁶ Lliboutry, L., *J. geophys. Res.*, **77**, 3759 (1972).

⁷ Lliboutry, L., *J. geophys. Res.*, **74**, 6525 (1969).

⁸ McKenzie, D., in *The Nature of the Solid Earth* (edit. by Robertson, E. C.), 323 (McGraw Hill, New York, 1972).

⁹ Chase, C. G., *Geophys. J.*, **29**, 117 (1972).

¹⁰ Isacks, B., and Molnar, P., *Rev. Geophys. Space Phys.*, **9**, 103 (1971).

¹¹ Johnson, R. H., and Malahoff, A., *J. geophys. Res.*, **76**, 3282 (1971).

¹² Johnson, G. L., and Lowrie, A., *Earth planet. Sci. Lett.*, **14**, 279 (1971).

¹³ Burke, K., and Wilson, J. T., *Nature*, **239**, 387 (1972).

¹⁴ Duncan, R. A., Petersen, N., and Hargraves, R. B., *Nature*, **239**, 82 (1972).

Structure of yeast phosphoglycerate mutase

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A 3.5 Å resolution electron density map of yeast phosphoglycerate mutase has been calculated which shows that much of the tertiary structure of this enzyme resembles that found in a number of nucleotide binding enzymes although the mutase itself has no known nucleotide binding requirement.

THE extension of the three-dimensional study of the structure of yeast phosphoglycerate mutase (PGM) by X-ray diffraction to 3.5 Å resolution gives us the first opportunity to report some of the structural details of a 'mutase' enzyme. Rather unexpectedly we find that the most striking structural feature of the enzyme resembles that found in certain enzymes which bind nucleotides¹⁻⁷. The phosphoglycerate mutase reaction,

3-phosphoglycerate \rightleftharpoons 2-phosphoglycerate, uses the cofactor 2,3-diphosphoglycerate and has no apparent nucleotide requirement. The results of the present study could therefore provide an important clue in the puzzle of enzyme evolution or alternatively strike a note of caution against over interpretation of results where structural similarities are involved.

Structure determination

The reflection data were measured, in four shells of increasing resolution, on a computer-controlled four-circle diffractometer. The potassium mercuriiodide derivative used in our earlier studies⁸ has now been replaced by a potassium chloroplatinite (5 mM) complex. This new derivative showed better isomorphism than the mercuriiodide derivative though in two experiments it was observed that, after a crystal had been exposed to X-radiation for about 60 h, there seemed to be a rapid and substantial change in the cell parameters. The relevant parameters for the new

derivative are included in Table 1 which summarises the 3.5 Å resolution refinement results. Anomalous dispersion data were measured for both the $K_3UO_2F_6$ and K_3PtCl_4 derivatives. Since our earlier paper we have redefined the origin of the unit cell to coincide with the molecular centre of the tetramer. Independent phase refinement was carried out for each of the four resolution shells of reflections, cycles of maximum probability phase refinement being alternated with the least squares refinement of the heavy atom parameters⁹. The four sets of phased reflections were scaled together using reflections, measured from a single crystal, covering the complete resolution range to 3.5 Å. An electron density map was calculated using centroid phases for nearly 8,000 reflections (average figure of merit 0.80) and plotted on perspex sheets, stacked perpendicular to the crystallographic two-fold axis, on a scale of 0.5 cm per Å.

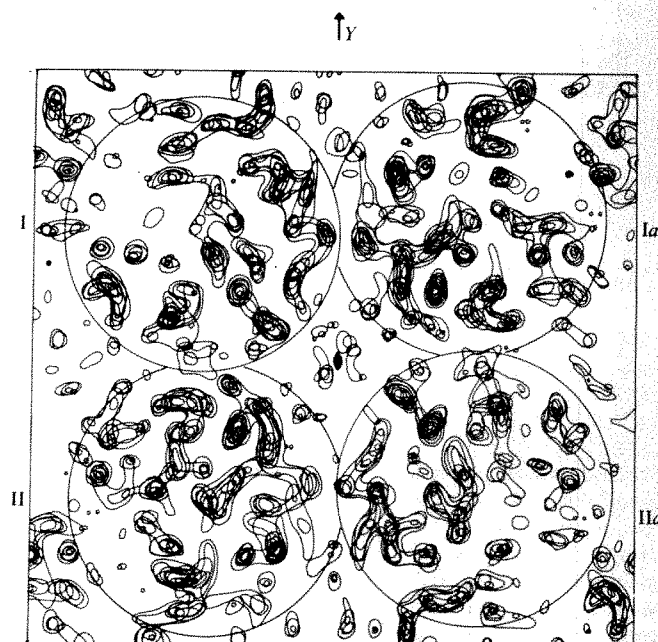


Fig. 1 Part of the yeast phosphoglycerate mutase 3.5 Å electron density map sectioned normal to one of the local molecular two-fold axes. The other local two-fold axis relates subunits I and II, and Ia and IIa respectively. The direction of the crystallographic two-fold axis, Y, is also indicated.

Table 1 Summary of phase refinement

Shell number	1	2	3	4
Resolution range	6.0–15.0 Å	4.4–6.0 Å	3.8–4.4 Å	3.4–3.8 Å
No. of reflections	1,438	2,242	2,134	2,039
R factors				
K_3HgI_4	0.50	—	—	—
$K_3UO_2F_6$	0.41	0.47	0.52	0.49
K_3PtCl_4	—	0.45	0.58	0.65
E/f				
K_3HgI_4	0.45	—	—	—
$K_3UO_2F_6$	0.32	0.43	0.46	0.43
K_3PtCl_4	—	0.40	0.51	0.61
Average figure of merit	0.82	0.83	0.78	0.77
Averaged heavy atom parameters				
Derivative	Site number	Relative occupancy	x	y
$K_3UO_2F_6$	I	1.00	0.151	–0.220
	II	0.94	0.164	0.220
	I	0.57	0.020	–0.120
	II	0.75	0.020	0.130
K_3HgI_4	III	1.36	0.183	–0.001
	IV	0.76	0.310	0.771
K_3PtCl_4	I	0.73	0.060	–0.085
	II	0.79	0.237	0.088
	III	0.15	0.429	0.795
	IV	0.15	0.142	0.704

Space group C2; $a = 96.6$ Å, $b = 86.0$ Å, $c = 81.8$ Å, $\beta = 120.6^\circ$. Local 2 fold axes at $y = 0.0$, E r.m.s. lack of closure error, f r.m.s. heavy atom structure factor.

Electron density map

Detailed visual inspection of the averaged and normal electron density maps showed no significant differences between the two subunits within one asymmetric unit, thus confirming the 222 symmetry of the PGM tetramer indicated in the previous structural work on the enzyme^{8,10}. Part of the electron density map showing the local two-fold symmetry is reproduced in Fig. 1. The fact that the two non-crystallographically related but chemically equivalent¹¹ subunits have apparently identical conformations gives us confidence in the accuracy of the structure determination and also indicates that the conformation of the subunit is stable and not unduly influenced by intermolecular forces within the crystal.

The subunits were named as shown in Fig. 1 with I and II being a pair of subunits in an asymmetric unit and with

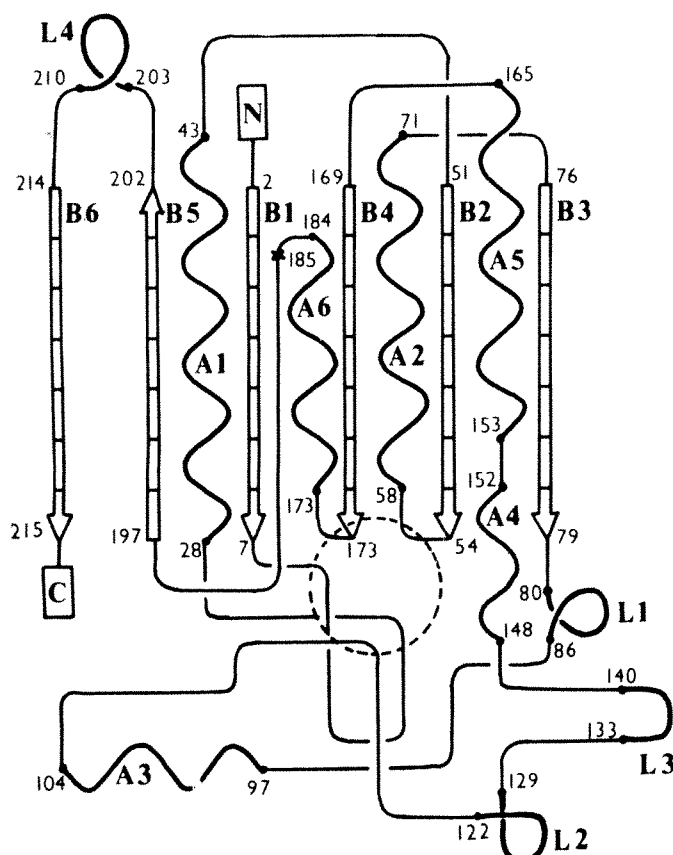


Fig. 2 Schematic representation of the structure of yeast phosphoglycerate mutase showing its regions of secondary structure. These regions are numbered from the N-terminal end of the polypeptide chain with A1-A6 being α -helices, B1-B6 being strands of the β -pleated sheet and L1-L4 being surface loops. The extent of each element of secondary structure can be calculated from the residue numbers marked on the figure. The area enclosed by the broken circle indicates the proposed region for the active site of the enzyme.

Ia and IIa being related to them by the crystallographic two-fold axis. A reasonable course was followed for the polypeptide chain throughout subunit II. In the few places where the interpretation of the map required that the polypeptide chain had to bridge a small gap in the electron density, it was usually found that the density in the map associated with the corresponding point in subunit I was continuous. The interpretation of the electron density map for subunit II fitted equally well the density associated with subunit I apart from small regions near the chain termini. These are at the surface of the molecule where, in general, the electron density is slightly less well defined than for the interior of the molecule. There is no suggestion, however, that the conformations of the subunits are fundamentally different.

The positions of many side chains were clearly visible in the electron density map and this information was used to determine the probable chain direction in all major helical regions. In each case the direction was consistent with the interpretation of the map based merely on the continuity of the electron density. Throughout the map, coloured markers were placed on the probable α -carbon atom positions indicating a total of 220 residues, some 30 less than has been predicted for this enzyme subunit based on molecular weight and amino acid composition data¹². Although a number of forms of the enzyme have been reported^{13,14} in which up to nine residues have been removed from the subunit by autolysis, the present crystallographic result would suggest that the molecular weight may be

slightly lower than 110,700.

In yeast PGM there are six strands forming a β -pleated sheet. The first four strands encountered when tracing the chain from the amino terminus are all parallel and the remaining two strands form an antiparallel pair (Fig. 2). There are five α -helical regions flanking the pleated sheet region (Fig. 3a, b) and, in addition, a further α -helical region making six in all. These α -helices together with helical segments in the regions 11-15 and 108-113 (Fig. 2) account for some 80 residues which, when taken with the 27 or so residues contained in the β -pleated sheet region, indicate that almost half the residues in the molecule are involved in some form of major secondary structure.

The contact between the mutase subunits related by the crystallographic two-fold axis is not very extensive involving mostly the loop L3 (for nomenclature see Fig. 2) from each subunit and the helix A5. The contact between the subunits I and II is much more extensive and involves the side chains of helix A2, the main chain leading into helix A2 and the main chain coming out from this helix which also forms one edge of the β -pleated sheet. The quaternary structure of yeast PGM is illustrated in Fig. 4.

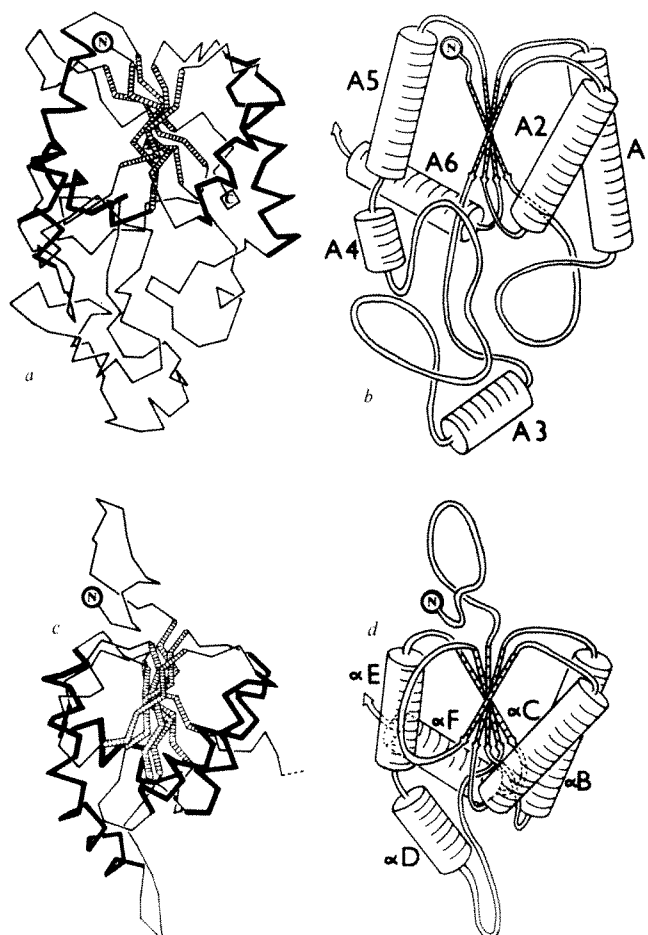


Fig. 3 a, Complete mutase subunit in which strands of the β -pleated sheet have been emphasised as thick shaded lines and in which the α -helices on either side of the β -pleated sheet have been drawn with thick black lines. b, First 185 residues of the mutase subunit drawn in a simplified manner to illustrate the chain folding within this region of the subunit. The helices are labelled using the nomenclature defined in Fig. 2. c, First 165 residues of the lactate dehydrogenase structure drawn for comparison with the mutase subunit in a. The coordinates used in preparing this drawing were from Adams *et al.*¹⁷. d, First 150 residues of LDH drawn diagrammatically for comparison with the part of PGM illustrated in b. The helices are labelled using the nomenclature of Rossmann *et al.*^{1,2}.



Fig. 4 A stereographic drawing of the yeast phosphoglycerate mutase tetramer. All four subunits are drawn as being identical. The view is chosen to give as clear a picture as possible of the inter-subunit contacts.

Mutase structure

In the absence of primary sequence data and conclusive substrate binding work the most interesting result of the current study is the striking resemblance that part of the mutase structure seems to bear to a structural feature found in the dehydrogenases¹⁻³ and the kinases^{6,7} in which a region of β -pleated sheet flanked by α -helices seems to have a common folding pattern. Detailed comparison (Fig. 3) of part of the mutase structure (the first 185 residues involving the first four parallel strands of the β -sheet and helices A1-A6) and part of LDH (the first 150 residues involving the first five parallel strands of the β -sheet and helices α B- α F) suggests that the similarity is more than superficial as evidenced by the fact that the helices A1, A2, A4, A5 and A6 of PGM are connected in the same order along the polypeptide chain as the corresponding helices in LDH. There are however significant differences, namely that, in the region described, PGM has one less strand in the β -sheet and the order of the strands in the sheet is not the same. We cannot see how the present electron density map can be reinterpreted to give the exact LDH folding but it is nevertheless quite possible that the mutase folding, as reported here, has evolved from a protein with LDH folding. The change would have resulted from the insertion of a loop of about 35 residues in a position near strand BD causing this strand to be disrupted from the β -sheet with the consequent rearrangement of two of the remaining strands as shown in Fig. 5.

It is also interesting to note that the substrate binding region tentatively proposed for the mutase in our earlier

paper⁸ is in a closely similar position relative to the α - β structure as that found for the nucleotide substrates of both the kinases and dehydrogenases¹⁻⁷.

Evolutionary significance

It has been suggested^{15,16} that the α - β structure, observed first in the dehydrogenases and more recently in the kinases, is a common feature of these enzymes relating their nucleotide binding function. The presence of a similar feature in the mutase, where no nucleotide binding is required, suggests that this particular structural feature is an easily formed stable conformation which could serve as a suitable core for building intracellular enzymes but may also reflect an ancestral gene from which enzymes in one or a group of related biochemical pathways have evolved.

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- ¹ Adams, M. J., Ford, G. C., Koekoek, R., Lentz, P. J., McPherson, A., Rossmann, M. G., Smiley, I. E., Schevitz, R. W., and Wonacott, A. J., *Nature*, **227**, 1098 (1970).
- ² Rossmann, M. G., Adams, M. J., Buehner, M., Ford, G. C., Hackert, M. L., Lentz, P. J., jun., McPherson, A., jun., Schevitz, R. W., and Smiley, I. E., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 179 (1971).
- ³ Hill, E., Tsernoglou, D., Webb, L., and Banaszak, L. J., *J. molec. Biol.*, **72**, 577 (1972).
- ⁴ Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossmann, M. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3052 (1973).
- ⁵ Brändén, C.-I., Eklund, H., Nordström, B., Boiwe, T., Söderlund, G., Zeppezauer, E., Ohlsson, I., and Åkeson, A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2439 (1973).
- ⁶ Bryant, T. N., Watson, H. C., and Wendell, P. J., *Nature*, **247**, 14 (1974).
- ⁷ Blake, C. C. F., and Evans, P. R., *J. molec. Biol.*, **84**, 585 (1974).
- ⁸ Campbell, J. W., Hodgson, G. I., and Watson, H. C., *Nature new Biol.*, **240**, 137 (1972).
- ⁹ Lipscomb, W. N., Coppola, J. C., Hartsuck, J. A., Ludwig, M. L., Muirhead, H., Searl, J., and Steitz, T. A., *J. molec. Biol.*, **19**, 423 (1966).
- ¹⁰ Campbell, J. W., Hodgson, G. I., Watson, H. C., and Scopes, R. K., *J. molec. Biol.*, **61**, 257 (1971).
- ¹¹ Sasaki, R., Sugimoto, E., and Chiba, H., *Agric. Biol. Chem.*, **34**, 135 (1970).
- ¹² Sugimoto, E., Sasaki, R., and Chiba, H., *Agric. Biol. Chem.*, **27**, 222 (1963).
- ¹³ Chiba, H., Sugimoto, E., and Kito, M., *Bull. Agric. Chem. Soc. Japan*, **24**, 428 (1960).
- ¹⁴ Sasaki, R., Sugimoto, E., and Chiba, H., *Archs Biochem. Biophys.*, **115**, 53 (1966).
- ¹⁵ Rao, S. T., and Rossmann, M. G., *J. molec. Biol.*, **76**, 241 (1973).
- ¹⁶ Rossmann, M. G., Moras, D., and Olsen, W., *Nature*, **250**, 194-199 (1974).
- ¹⁷ Adams, M. J., Ford, G. C., Liljas, A., and Rossmann, M. G., *Biochim. biophys. Res. Commun.*, **53**, 46 (1973).

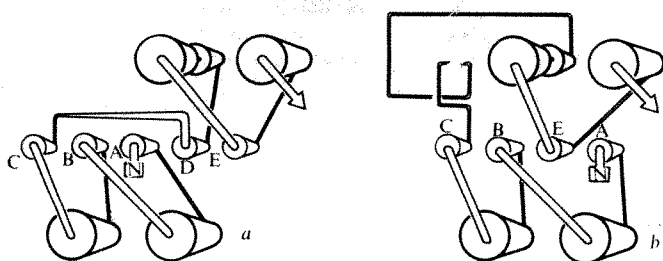


Fig. 5 Diagrammatic representations of the first 150 residues of LDH (a) and of the first 185 residues of PGM (b) showing that the two structures have the same linear connectivity even though the order of strands in their β -pleated sheets is slightly different. Strands of the β -pleated sheet are lettered according to the LDH numbering scheme^{1,2} although the prefix β has been omitted. The flanking helices (see text) are shown above and below their respective pleated sheets.

Effects of succinyl-con A on the growth of normal and transformed cells

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Covering con A binding sites on virally transformed mouse fibroblasts with succinyl con A has no significant effect on their growth pattern.

CULTURED mouse 3T3 fibroblasts and their viral transformants, SV40-transformed 3T3 (SV3T3) and polyoma-transformed 3T3 (Py3T3) cells, serve as models to demonstrate the differences in growth control between normal and malignant cells^{1,2}. Under normal culture conditions the growth of 3T3 cells and SV3T3 cells is controlled by serum growth factors^{3,4}. As these factors become exhausted, 3T3 cells stop growing and arrest in the G₁ phase of the cell cycle without loss of viability⁵. Under identical conditions SV3T3 cells grow to a much higher cell density than 3T3 cells⁶ as a result of a reduced serum requirement⁷. SV3T3 cells do not arrest in the G₁ phase as serum factors are depleted. Instead they continue to grow, moving slowly through all stages of the cell cycle before they eventually die^{8,9}.

Table 1 Minimum concentration of lectin required for agglutination of cells

Cells	Lectin concentrations ($\mu\text{g ml}^{-1}$)	
	Succinyl con A	Con A
SV3T3	250	10
3T3	>1,000	50

Cells were removed from the dishes by successive washes with 0.15 M NaCl-0.01 M sodium phosphate pH 7.2 (PBS) and with PBS containing 5×10^{-6} M EDTA, followed by a 10 min incubation at 37° C in PBS containing 5×10^{-5} M EDTA²⁶. The cell suspension was washed once with PBS and the cells resuspended at 2×10^6 cells ml⁻¹. Agglutination assays were performed in Linbro 16 mm multi-well tissue culture plates²⁷. Cells (0.2 ml) were mixed with lectin (0.2 ml) and the trays placed on a rotary table²⁸ (1-2 Hz) for 20 min at room temperature. Agglutination was estimated using an inverted light microscope.

It is possible that changes in the cell membrane are responsible for malignant growth¹⁰⁻¹². Recently it was reported that Py3T3 cells grown in the presence of trypsinised concanavalin A (con A) revert to normal growth¹³. Native con A is a plant lectin which binds terminal α -D-glucosyl and α -D-mannosyl sugar residues^{14,15}, and it was suggested that the binding of trypsinised con A to glycoproteins on the surfaces of Py3T3 cells caused the reversion to normal growth.

Although originally described as monovalent, subsequent characterisation has shown that protease-treated con A is a complex mixture which includes unmodified protein and totally inactive digest products¹⁶. The complexity of trypsinised con A makes analysis of its biological effects difficult. Extensive succinylation of con A converts the native tetrameric molecule to a dimer with the same binding affinity as the native molecule for methyl α -D-glucoside¹⁷. Succinyl con A is less toxic than the native lectin and does not readily agglutinate cells^{17,18}. Because succinyl con A has a low toxicity but has a similar binding specificity to that of the native lectin, we tested its effects on the growth of normal and transformed cells.

Agglutination and lectin binding

Agglutination studies and lectin binding assays were performed with succinyl con A to find conditions under which the con A binding sites on the cell surface of SV3T3 cells could be covered without inducing cell agglutination. Twice crystallised con A (Miles-Yeda, Kankakee, Illinois) was used without further purification; succinyl con A was prepared and characterised as described¹⁷. Cells from confluent cultures of 3T3 and SV3T3 cells were used in the agglutination assays and the specificity of the lectin-induced agglutination was demonstrated by the inhibition of agglutination by 100 mM methyl α -D-mannoside. Though con A agglutinated SV3T3 cells at a concentration of about $10 \mu\text{g ml}^{-1}$, the concentration of succinyl con A required to give detectable agglutination was about $250 \mu\text{g ml}^{-1}$ (Table 1). Succinyl con A at 1 mg ml^{-1} did not agglutinate 3T3 cells.

The binding of ¹⁴C-succinyl con A and ¹²⁵I-con A to almost confluent 3T3 and SV3T3 cells attached to plastic tissue culture wells was measured in PBS at 4° C. Low temperature was chosen to minimise endocytosis of lectin molecules bound to the cell surface¹⁹. Since the binding of succinyl con A and con A was complete after 30-40 min, cells were incubated with various concentrations of lectin for 50 min. Although succinyl con A did not agglutinate cells as efficiently as the native lectin, succinyl con A bound almost as well as con A to the surface of SV3T3 cells (Fig. 1), and in other experiments bound to the surface of 3T3 cells as well as the native lectin.

Binding experiments were also carried out in PBS at 37° C in the presence of 10% calf serum to measure binding under the conditions of the growth experiments described later. Under these conditions succinyl con A bound more efficiently than the native lectin although the maximal number of molecules of either lectin bound under these conditions was slightly reduced: at a concentration of $100 \mu\text{g ml}^{-1}$ succinyl con A, 4.2×10^6 molecules per cell were bound and more than 90% of the available binding sites were saturated.

Competition binding studies were carried out to confirm that succinyl con A bound to the same receptor sites as the native lectin. Succinyl con A could inhibit the binding of at least 60% of the ¹²⁵I-con A molecules which bound to SV3T3 cells (Fig. 2). Succinyl con A did not, however, inhibit binding as efficiently as the native lectin. Taken together the results of the agglutination studies and binding experiments suggest that succinyl con A can be used to cover con A binding sites on SV3T3 and 3T3 cells without inducing cell agglutination.

Growth studies

The effects of succinyl con A on the growth of SV3T3, Py3T3 and 3T3 cells have been observed over a wide range of succinyl con A concentrations and under a variety of different growth conditions. For most experiments the effects of succinyl con A at a concentration of $100 \mu\text{g ml}^{-1}$ were tested since the binding studies showed at this concentration more than 90% of the maximal number of succinyl con A molecules were bound.

3T3 cells and SV3T3 cells were obtained from Dr R. Dulbecco, and Py3T3 cells from Dr W. Eckhart. Cells were maintained in Dulbecco's and Vogt's modification of Eagle's medium supplemented with 10% calf serum, 250 units ml^{-1} penicillin and 50 $\mu\text{g ml}^{-1}$ streptomycin at 37°C in a water-saturated atmosphere at 10% CO_2 and 90% air. No mycoplasma were detected by autoradiography. Cells ($3\text{--}5 \times 10^4$) were seeded in 30 mm Nunc plastic tissue culture dishes in 1 ml of culture medium containing 10% calf serum. The next day (day 0) the medium was replaced with fresh medium (2 ml) containing 10% calf serum and the required concentration of succinyl con A. Cell number in duplicate cultures was determined daily by removing the cells from the dishes with 0.05% trypsin and counting the cells in a Coulter counter. Similar results were obtained if replicate cell counts were made on cells removed from the dish with trypsin containing

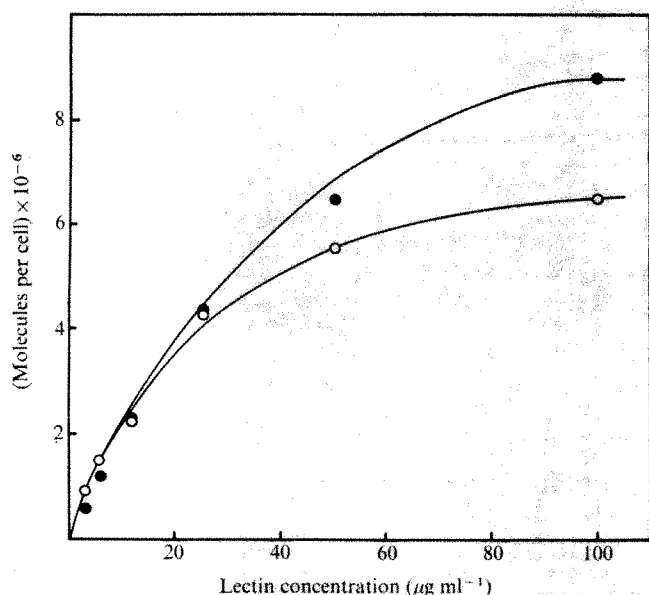


Fig. 1 Binding of ^{14}C -succinyl con A (\circ), and ^{125}I -con A (\bullet) to SV3T3 cells. ^{125}I -con A (specific activity 7×10^5 c.p.m. μg^{-1}) was prepared by the method of McConahey and Dixon²⁹ and purified by affinity chromatography on Sephadex. ^{14}C -succinyl con A (specific activity 2.5 or 6×10^5 c.p.m. μg^{-1}) was prepared using ^{14}C -succinic anhydride (New England Nuclear). All lectin solutions were filtered through $0.45 \mu\text{m}$ Millipore filters just before use. Cells were grown to 1×10^5 – 2×10^5 cells per well in 16 mm plastic multi-well tissue culture plates (Linbro, Los Angeles). Cell monolayers were washed twice with PBS (1 ml) and incubated with lectin in 0.3 ml PBS for 50 min at 4°C . After incubation cells were washed five times with PBS (1 ml), removed from the wells by overnight incubation with 10% Triton X-100, and the radioactivity bound to the cells determined. Nonspecific binding to the cells was determined as the residual binding in the presence of 100 mM methyl α -D-mannoside. Nonspecific binding of ^{125}I -con A was less than 10% of the total radioactivity bound in the absence of methyl α -D-mannoside. Nonspecific binding of ^{14}C -succinyl con A was 10–20% of the total radioactivity bound. The results reported are corrected for nonspecific binding of the lectins to the cells. Other control experiments showed that in the absence of cells ^{125}I -con A and ^{14}C -succinyl con A bound to wells which had contained only culture medium at 37°C for 24 h. The bound lectin, which was directly proportional to the concentration of free lectin, was reduced by 100 mM methyl α -D-mannoside. Because it is difficult to estimate the contribution of the lectin binding to the plastic (or serum glycoproteins adsorbed to the plastic) in the presence of cells, we have not made a correction for nonspecific binding to the wells. The maximum contribution of lectin bound to the plastic was less than 10% of the total radioactivity measured for the binding of ^{125}I -con A and between 10–30% of the total radioactivity measured for the binding of ^{14}C -succinyl con A.

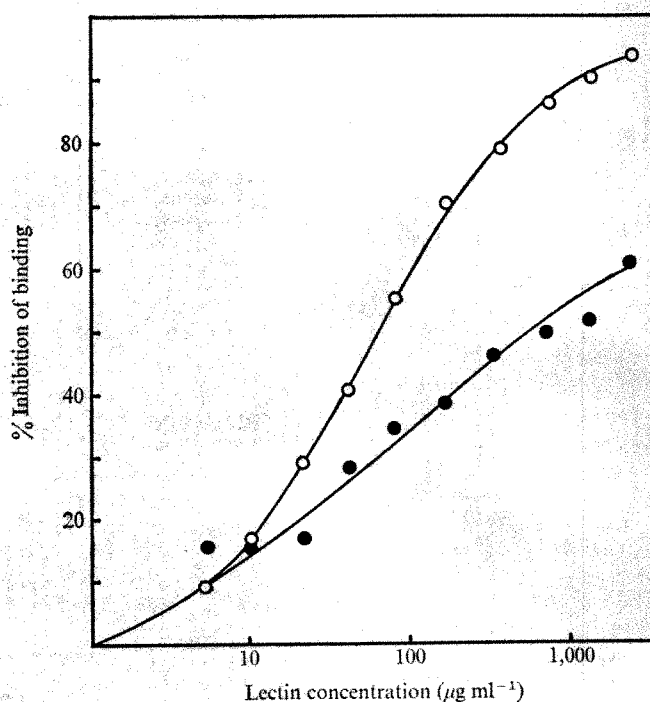


Fig. 2 Inhibition of the binding of ^{125}I -con A ($20 \mu\text{g ml}^{-1}$) to SV3T3 cells by succinyl con A (\bullet), and the native lectin (\circ). Competition binding studies were carried out as described for direct binding studies. ^{125}I -con A and competing lectin were added to the cells as a mixture in 0.3 ml PBS. No corrections for nonspecific binding were made.

50 mM methyl α -D-glucoside to prevent possible lectin-induced agglutination of the trypsinised cells.

The growth rate and maximum cell density of SV3T3 cells and 3T3 cells was slightly reduced by succinyl con A (Fig. 3). But SV3T3 cells treated with succinyl con A at a concentration of $100 \mu\text{g ml}^{-1}$ still grew to a high cell density before growth stopped and the cells began to die. 3T3 cells treated with succinyl con A stopped growing at a low density and maintained viability. The effects of

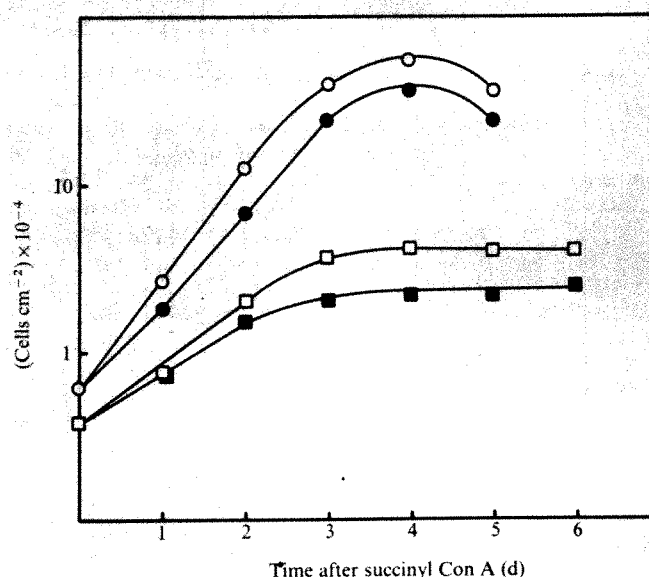


Fig. 3 Effect of succinyl con A ($100 \mu\text{g ml}^{-1}$) on the growth of SV3T3 and 3T3 cells. (\circ) SV3T3; (\bullet) SV3T3 + $100 \mu\text{g ml}^{-1}$ succinyl con A; (\square) 3T3; (\blacksquare) 3T3 + $100 \mu\text{g ml}^{-1}$ succinyl con A.

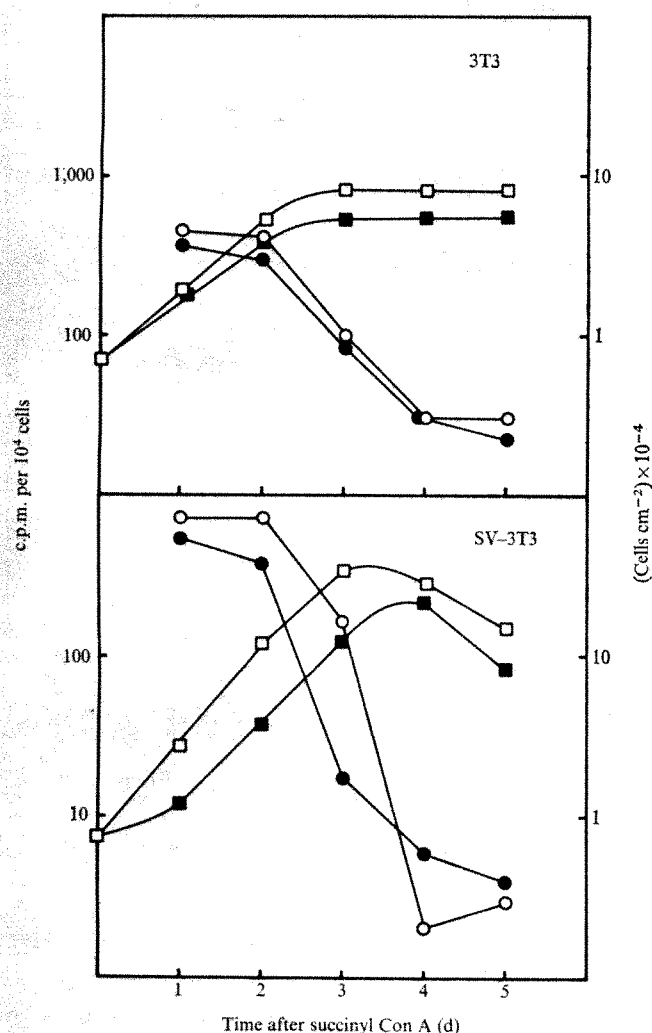


Fig. 4 Effect of succinyl con A ($100 \mu\text{g ml}^{-1}$) on DNA synthesis in SV3T3 and 3T3 cells. (O) ^3H -thymidine incorporation (c.p.m. per 10^4 cells) of cells grown without succinyl con A; (●) ^3H -thymidine incorporation of cells grown with $100 \mu\text{g ml}^{-1}$ succinyl con A; (□) cell density in the absence of succinyl con A; (■) cell density in the presence of succinyl con A. Cells were grown in 1 ml of culture medium which was not changed during the experiment.

succinyl con A on the growth of both cells could be abolished by 50 mM methyl α -D-mannoside in the culture medium demonstrating that specific binding of succinyl con A to the cells was necessary to produce the effects on growth. Succinyl con A had no detectable effect on either the morphology of individual cells or cell cultures.

In the experiment shown in Fig. 3 the culture medium was unchanged throughout the experiment. In other experiments with SV3T3, Py3T3 and 3T3 cells, medium was either partially or completely replaced daily with fresh medium containing the same concentration of succinyl con A. The results of these experiments were the same as those shown in Fig. 3, except that, as expected, all cells grew to higher cell densities. It is therefore unlikely that the reason succinyl con A has little effect on the growth of SV3T3 cells or 3T3 cells is because it is being rapidly degraded by proteolytic enzymes either of cellular origin or present in the serum. In experiments in which culture medium was replaced daily, SV3T3 cells, and to a lesser extent Py3T3 cells, treated with succinyl con A showed a variable tendency to detach from the dish immediately after the culture medium was changed so that experiments with these cells were sometimes difficult to follow for the desired time.

In growth experiments in which the succinyl con A dose was varied, $200 \mu\text{g ml}^{-1}$ succinyl con A (the highest dose tested) inhibited growth of 3T3 and SV3T3 cells only slightly more than $100 \mu\text{g ml}^{-1}$ succinyl con A. When the degree of growth inhibition was plotted as a function of the succinyl con A concentration a saturation curve was obtained which closely resembled the succinyl con A binding curve (Fig. 1). This provides further evidence that the inhibition of growth results from the binding of succinyl con A which occurs to an extent predicted by the binding assays. In contrast to the effects of trypsinised con A on the growth of transformed and normal cells previously reported¹³, succinyl con A inhibited the growth of normal and transformed cells to the same extent at each concentration tested.

Cell cycle analysis

In some growth experiments the rate of DNA synthesis in cell cultures was estimated in replicate cultures by measuring the incorporation of ^3H -thymidine ($2.5 \mu\text{M}$; 1.0 Ci mmol^{-1}) into trichloroacetic acid-insoluble material during a 2 h period (Fig. 4). Daily determinations were made in triplicate. The rate of cellular DNA synthesis remained relatively constant during the first two days of culture when the cells were growing exponentially. As growth slowed there was a large decline in DNA synthesis in cultures of 3T3 cells and SV3T3 cells whether or not the cells were grown in the presence of succinyl con A.

Although DNA synthesis declined in cultures of both normal and transformed cells, microfluorometric cell cycle analysis^{20,21} revealed a profound difference in their growth pattern (Fig. 5). As 3T3 cells reached their saturation density and ^3H -thymidine incorporation dropped, there was a progressive reduction in the proportion of cells in S phase and an accompanying increase in the proportion of cells in G_1 phase. In contrast, no change in the distribution of SV3T3 cells throughout the cell cycle could be detected as their growth slowed. Succinyl con A had no effect on the cell cycle distribution of either cell type at any stage of growth.

The results from microfluorometric analysis and ^3H -thymidine incorporation are consistent with current knowledge of the growth patterns of SV3T3 cells and 3T3 cells. 3T3 cells stopped growing as serum factors are depleted and arrest in G_1 phase^{5,7}, whereas SV3T3 cells continue to grow and eventually die. The dramatic drop in ^3H -thymidine incorporation as SV3T3 cells reach saturation density represents an extreme example of the lengthening of all stages of the cell cycle as nutrients begin to limit their growth^{8,9}.

Our studies provide no evidence that succinyl con A, which binds to con A binding sites, restores the growth pattern of transformed cells to normal. These results are in conflict with the hypothesis that covering of the con A binding sites on virally transformed cells restores normal growth^{13,22,23}. This hypothesis was based upon the effects of trypsinised con A on the growth of Py3T3 cells. Although we used SV3T3 cells in most of our experiments, in other experiments Py3T3 cells treated with succinyl con A also grew to a high cell density. Thus the inability of succinyl con A to restore normal growth seems to be a general phenomenon.

Several explanations could account for the different effects of succinyl con A and trypsinised con A on cell growth. Because of the heterogeneity of the con A binding sites and the failure to completely inhibit ^{125}I -con A binding with succinyl con A, we cannot formally exclude the possibility that succinyl con A and trypsinised con A bind to different fractions of the total receptor population. It should be stressed, however, that this is an unlikely explanation because although no quantitative studies on

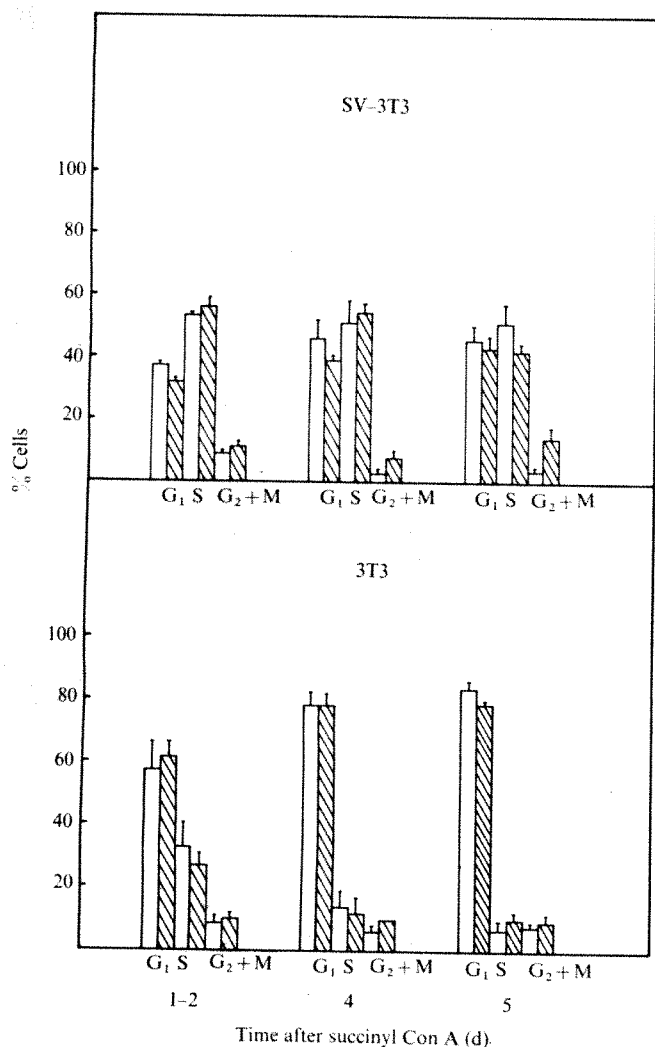


Fig. 5 Cell cycle analysis of SV3T3 and 3T3 cells grown with or without succinyl con A ($100 \mu\text{g ml}^{-1}$). Cells were prepared for flow microfluorometry as described^{20,21}. Cells were removed from the dishes, fixed with formalin and stored at 4°C until the end of the experiment. Cell samples were then stained with acroflavine, resuspended in water and analysed for DNA content using a flow microfluorometer equipped with an argon laser ($\lambda 488 \text{ nm}$)²¹. The vertical bars represent the percentage of the cell population in G_1 , S and G_2+M stages of the cell cycle during exponential growth (day 1 or 2) and as the cells reach saturation density (days 4 and 5). Open bars: untreated cells; hatched bars: cells treated with succinyl-con A. The results are the average values from three experiments ± 1 s.e.

the binding of trypsinised con A to the surface of transformed cells have been reported, Burger and Noonan¹³ observed a graded response of cell growth to different doses of trypsinised con A, $10 \mu\text{g ml}^{-1}$ trypsinised con A significantly reducing the saturation density of Py3T3 cells. Binding experiments (Fig. 1 and ref. 24) show that even the native lectin at a concentration of $10 \mu\text{g ml}^{-1}$ saturates less than 25% of the con A binding sites. No differences in the binding of succinyl con A and con A were observed until a lectin concentration of $50 \mu\text{g ml}^{-1}$ when more than 50% of the con A binding sites were occupied.

Alternatively succinyl con A may bind to the same sites as trypsinised con A but after binding behave differently. For example, succinyl con A, in contrast to the native lectin, neither induces clustering of its own receptors nor inhibits clustering of immunoglobulin receptors on the surface of mouse lymphocytes¹⁷. If this should be the case then succinyl con A will be invaluable in identification of the biologically significant events which occur after the binding of trypsinised con A.

A third possibility is that normal growth control is not restored by treating transformed 3T3 cells with trypsinised con A but that trypsinised con A, like the native lectin²⁵, differentially kills transformed cells. In the original experiments of Burger and Noonan¹³ none of the experimental results unambiguously distinguishes between the possible toxicity of trypsinised con A and its ability to induce transformed cells to arrest in G_1 phase and maintain viability. In particular, our experiments indicate that a decline in thymidine incorporation in cultures of transformed cells is insufficient evidence to justify the assumption that the cells are arresting in G_1 phase.

We conclude because of the lack of effect of succinyl con A on the growth pattern of transformed cells that it is unlikely that the covering of con A binding sites on these cells is alone sufficient to restore normal growth.

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- ¹ Dulbecco, R., *Science*, **166**, 962 (1969).
- ² Stoker, M. G. P., *Proc. R. Soc.*, **B181**, 1 (1972).
- ³ Todaro, G. J., Lazar, G. K., and Green, H., *J. cell. comp. Physiol.*, **66**, 325 (1965).
- ⁴ Holley, R. W., and Kiernan, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 300 (1968).
- ⁵ Nilausen, K., and Green, H., *Expl Cell Res.*, **40**, 166 (1965).
- ⁶ Todaro, G. J., Green, H., and Goldberg, B. D., *Proc. natn. Acad. Sci. U.S.A.*, **51**, 66 (1964).
- ⁷ Holley, R. W., and Kiernan, J. A., in *Growth Control in Cell Cultures CIBA Foundation Symposium*. (edit. by Wolstenholme, G. E. W., and Knight, J.), 3 (Churchill Livingstone, London, 1971).
- ⁸ Auer, G., and Zetterberg, A., *Expl Cell Res.*, **67**, 240 (1971).
- ⁹ Paul, D., Henahan, M., and Walter, S., *J. natn. Cancer Inst.* (in the press).
- ¹⁰ Wallach, D. F. H., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 868 (1968).
- ¹¹ Pardee, A. B., *In Vitro*, **7**, 95 (1971).
- ¹² Holley, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2840 (1972).
- ¹³ Burger, M. M., and Noonan, K. D., *Nature*, **228**, 512 (1970).
- ¹⁴ Goldstein, I. J., Hollerman, C. E., and Merrick, J. M., *Biochim. biophys. Acta*, **97**, 68 (1965).
- ¹⁵ Goldstein, I. J., Hollerman, C. E., and Smith, E. E., *Biochemistry*, **4**, 876 (1965).
- ¹⁶ Cunningham, B. A., Wang, J. L., Pflumm, M. N., and Edelman, G. M., *Biochemistry*, **11**, 3233 (1972).
- ¹⁷ Gunther, G. R., Wang, J. L., Yahara, I., Cunningham, B. A., and Edelman, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1012 (1973).
- ¹⁸ Trowbridge, I. S., *Proc. natn. Acad. Sci. U.S.A.* (in the press), (1973).
- ¹⁹ Taylor, R. B., Duffus, W. P. H., Raff, M. C., and de Petris, S., *Nature new Biol.*, **233**, 225 (1971).
- ²⁰ Van Dilla, M. A., Trujillo, T. T., Mullaney, P. F., and Coulter, J. R., *Science*, **163**, 1213 (1969).
- ²¹ Kraemer, P. H., Deaven, L. L., Crissman, H. A., and Van Dilla, M. A., in *Advances in Cell and Molecular Biology* (edit. by Dupraw, E. P.), **2**, 47 (Academic Press, New York, 1972).
- ²² Burger, M. M., *Fedn Proc.*, **32**, 91 (1973).
- ²³ Levine, A. J., and Burger, M. M., *J. theor. Biol.*, **37**, 435 (1972).
- ²⁴ Ardnt-Jovin, D., and Berg, P., *J. Virol.*, **8**, 716 (1971).
- ²⁵ Shoham, J. M., Inbar, M., and Sachs, L., *Nature*, **227**, 1244 (1970).
- ²⁶ Ozanne, B., and Sambrook, J., *Lepetit Colloq. Biol. Med.*, **2**, 248 (1971).
- ²⁷ Nicolson, G. L., and Lacorbiere, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1672 (1973).
- ²⁸ Henkart, P., and Humphreys, T., *Expl Cell Res.*, **63**, 224 (1971).
- ²⁹ McCahahey, P. G., and Dixon, F. J., *Int. Archs Allergy appl. Immun.*, **29**, 185 (1966).

letters to nature

Redshift of a galaxy near 4C11.50

NEAR the position of the radio source 4C11.50, Wampler *et al.*¹ have found a pair of QSOs separated by 4.8 arc s, with redshifts of 0.4359 and 1.901, respectively. Hazard *et al.*² have noted the presence of a 19-mag galaxy, 10 arc s west of the brighter, lower redshift QSO (4C11.50a); a plate obtained with the 224-cm telescope at Mauna Kea shows that this 'galaxy' is actually a close group of three galaxies. Several spectrograms have been obtained at 190 and 50 Å mm⁻¹, with the two brighter galaxies aligned along the slit at position angle 33°. There is a continuum break downwards to the blue at about 5,700 Å, and an emission line at 5,344.9 Å; these features can be identified with the long wavelength edge of the H and K lines, and with the [O II] λ3,727 doublet, respectively. Assuming an average wavelength of 3,727.4 Å for the [O II] doublet, the redshift is 0.4340 (not corrected for galactic rotation). The two galaxies on the slit are separated by about 2.5 arc s and are not well resolved on the spectrogram, so it is not certain to which one the redshift refers.

The redshift of 4C11.50a itself, determined from the sharp [O III] λλ4,959, 5,007 lines, is found to be 0.4358, in good agreement with the value of 0.4359 given by Wampler *et al.*¹. The difference in radial velocity between the QSO and the galaxy, in the local rest frame of the QSO, is 376 km s⁻¹. This observation is consistent with a physical association between 4C11.50a and the galaxy, and supports a cosmological interpretation of the redshift of the QSO.

Burbidge³ has pointed out that the sort of galaxy which observers tend to select for this sort of exercise is normally in the magnitude range which will give it a redshift similar to that of the nearby QSO. Although this may be true in a rough sense, there cannot be very much accuracy with this kind of preselection. Even if the dispersion in the magnitude of the galaxies is ignored, these magnitude estimates of faint galaxies from casual inspection of the Palomar Sky Survey prints are unlikely to have accuracies greater than ± 0.5 mag. That introduces an uncertainty of approximately ± 20% in the redshift, or ± 15,000 km s⁻¹ in the relative velocity for redshifts near 0.4. 4C11.50a is the third reported case in which an intrinsically bright (on the cosmological interpretation of the redshift) QSO has a redshift within a few hundred km s⁻¹ of the nearest galaxy visible on the Palomar Sky Survey prints. The other two are PKS2251 + 11 (refs 4 and 5) and 4C37.43 (ref. 6).

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¹ Wampler, E. J., Baldwin, J. A., Burke, W. L., Robinson, L. B., and Hazard, C., *Nature*, **246**, 203–205 (1973).

² Hazard, C., Jauncey, D. L., Sargent, W. L. W., Baldwin, J. A., and Wampler, E. J., *Nature*, **246**, 205–208 (1973).

³ Burbidge, G. R., *Nature phys. Sci.*, **246**, 17–25 (1973).

⁴ Gunn, J. E., *Astrophys. J. Lett.*, **164**, L113–L118 (1971).

⁵ Robinson, L. B., and Wampler, E. J., *Astrophys. J. Lett.*, **171**, L83–L86 (1972).

⁶ Stockton, A., *Nature phys. Sci.*, **246**, 25 (1973).

Search for optical pulsations from Cen X-3

THE optical system recently identified¹ with the X-ray source Cen X-3 is an obvious candidate for high frequency optical studies because it provides the possibility of observing optical effects related to the 4.842 s X-ray pulsations². Observations of this object were made at Cerro Tololo using an S-20 photomultiplier with no filter and making 1-ms integrations on to magnetic tape. The data were analysed by Fourier techniques similar to those used in the HZ Her study³. No clear harmonic activity emerged, and the average upper limits of the optical pulsations for three sets of data were found to be 0.031%, 0.11%, and 0.14% (Table 1).

Table 1 Time series observations of Cen X-3

Date	Telescope (cm)	Starting UT	Phase*	Length (min)	Upper limit (%)
January 24/25, 1974	152	0528	0.691	50	0.031
February 21/22, 1974	91	0121	0.024	400	0.11
February 22/23, 1974	91	0133	0.508	400	0.14

* See ref. 2.

There is, however, a tantalising peak at exactly the Doppler-shifted frequency of pulsation, at the 1.3 σ level. This appeared for an 8 min interval beginning at 0133 UT (phase = 0.508) on the night of February 22/23, 1974 (Table 1). This 'activity', corresponding to a fluctuation of 0.25%, should be regarded sceptically; it is reported here in recognition of the possibility that this object may, like HZ Her, display optical pulsations only rarely^{3,4}.

The detected optical pulsations in Her X-1 are of the same fractional order of magnitude as the observed upper limit for Cen X-3 reported here. The X-ray flux from Her X-1 (refs 5 and 6) is about 2.9 times fainter than that for Cen X-3 (refs 7 and 8). The corresponding optical flux—corrected for reddening taking $A_v = 0.3$ (ref. 9) and 4.3 (W. Krzeminski, personal communication) respectively—is 76 times fainter. Thus, if the pulsed optical emission in these kinds of objects is excited by the absorption of X rays, than significant optical activity at the present limit of detection would require a conversion efficiency about 26 times greater for Cen X-3 than for Her X-1. Although further observational work would increase the chances of detecting sporadic activity, a significant decrease from the present upper limit is not achievable with available instrumentation.

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¹ Krzeminski, W., *IAU Circ. No. 2612* Dec. 27 (1973).

² Schreier, E., Levinson, R., Gursky, H., Kellog, E., Tananbaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **172**, L79 (1972).

³ Davidsen, A., Henry, J. P., Middleditch, J., and Smith, H. E., *ibid.*, **177**, L97 (1972).

⁴ Groth, E. J., and Nelson, M. R., *ibid.*, **178**, L111 (1972).

⁵ Tananbaum, H., Gursky, H., Kellog, E. M., Levinson, R., Schreier, E., and Giacconi, R., *ibid.*, **174**, L143 (1972).

- ⁶ Ulmer, M. P., Baity, W. A., Wheaton, W. A., and Peterson, L. E., *ibid.*, **181**, L33 (1973).
⁷ Schreier, E., Levinson, R., Gursky, H., Kellog, E., Tananbaum, H., and Giacconi, R., *ibid.*, **172**, L79 (1972).
⁸ Baity, W. A., Ulmer, M. P., Wheaton, W. A., and Peterson, L. E., *ibid.*, **187**, L341 (1974).
⁹ Crampton, D., *Astrophys. J.*, **187**, 345 (1974).

Dynamic evidence on massive coronas of galaxies

A LONGSTANDING unresolved problem in galactic astronomy is the mass discrepancy observed in clusters of galaxies. The virial mass of the cluster per galaxy and the mass-luminosity ratio are considerably larger than the corresponding quantities for individual galaxies. This discrepancy cannot be a result of expansion or be because of the recent origin of clusters; these ideas contradict our present knowledge of the physical evolution and ages of galaxies¹. Therefore it is necessary to adopt an alternative hypothesis: that the clusters of galaxies are stabilised by hidden matter.

The discovery of the emission of X rays from clusters of galaxies can be explained in terms of hot intracluster gas. The mass of this gas, however, is insufficient to stabilise clusters².

Another possible way of removing the mass discrepancy is to suppose that the masses of individual galaxies have been underestimated. This hypothesis is supported by the fact that there is a very slow decrease in the rotational velocity of spiral galaxies, which indicates the pressure of large amounts of matter in their outer regions³. On the other hand, the discovery of large halos in elliptical galaxies^{4,5} indicate that these also contain previously unseen matter.

There have been several estimates of the mass of galaxies, based on the new observational data mentioned^{6,7}. A critical analysis shows, however, that these estimates are very uncertain.

We here attempt to obtain more exact information on the distribution of mass in the outer regions of galaxies.

Consider a body moving in a circular orbit of radius R , with a velocity V_c , around the centre of the galaxy. If mass distribution is spherical, then

$$M(R) = RV_c^2/G$$

is the mass of the galaxy inside the sphere of radius R , where G is the gravity constant. The same formula can be used for a nonspherical mass distribution, neglecting an error of about 10%.

Spiral galaxies contain neutral hydrogen and HII regions moving in the plane of the galaxy in nearly circular orbits. Observations of these objects permit us to calculate the function $M(R)$ in some galaxies at rather large distances from the centre. We have calculated this for five galaxies of different mass. The results for one galaxy (IC342) are given in Fig. 1.

For all galaxies we have also found the mass distribution $M_s(R)$ of known stellar populations—the bulge, the halo and the disk. We assume that these populations are physically homogeneous, in particular that the mass-luminosity ratio of stars is constant for the whole population. The relevant parameters (luminosity, L ; effective radius R_0 ; mass-luminosity ratio, f ; axial ratio of equidensity ellipsoids, ϵ) for all of the populations, have been determined from combined photometric, spectrophotometric, kinematical and radio observations, using previously described methods^{7,8}.

The resulting mass distribution of known stars, $M_s(R)$, deviates considerably from the mass distribution, $M(R)$, derived from the rotation curve. Rejecting the possibility of large systematic deviations from the circular velocity in the outer regions of galaxies, we conclude that galaxies contain

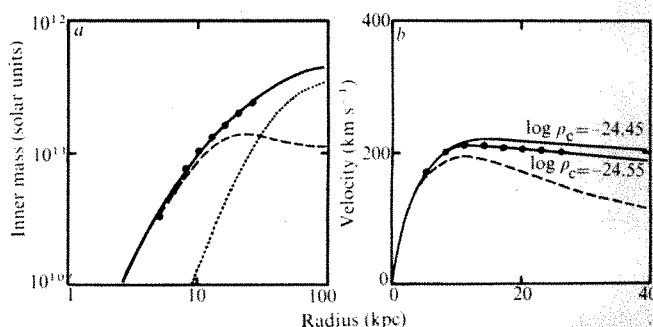


Fig. 1 The distributions of (a) the inner mass, $M(R)$, and (b) the circular velocity, V_c , in the galaxy IC342. Dots, observed values; dashed lines, model functions for known stellar populations; dotted lines, distributions for the corona; solid lines, total distributions. In Fig. 1b two variants of the total velocity distribution are given to demonstrate its dependence on the central density of the corona.

a previously unrecognised, massive population. It is convenient to call this population a 'corona'. We have estimated the parameters of coronas for the five galaxies (Table 1) adopting, for simplicity, an exponential density law

$$\rho(R) = \rho_c \exp(-R/R_0)$$

with spherical symmetry. (A small axial ratio, ϵ , would result in a large part of the kinetic energy being in the form of rotation, although the results of Ostriker and Peebles do not indicate this⁹.)

The available data permit us to derive the central density of the corona, ρ_c , with great accuracy. The length of the observed rotation curve, however, is insufficient to allow any determination of the mass and extent of the corona.

To determine total masses and dimensions of galactic coronas, more distant test bodies are needed. For this purpose isolated pairs of galaxies can be used, with secondary galaxies serving as test bodies for determining the mass distribution of primary galaxies¹⁰.

In the case of double galaxies we know the radial component of differential velocity, $v_r = \Delta V_r$. The projection factor, $p = V_c^2/v_r^2$, which depends on the orientation of the velocity vector and on the shape of the orbit, remains unknown, but its expected value, $\langle p \rangle$, can be calculated. Therefore, the mass distribution function can be found only statistically:

$$\langle M(R) \rangle = \langle p \rangle \langle Rv_r^2 \rangle / G,$$

where $\langle Rv_r^2 \rangle$ is the corresponding mean value for a sample of pairs of galaxies.

We have collected data on 105 pairs of galaxies with known types, radial velocities and estimates of magnitudes and dimensions^{11,12}. To prevent the sample from contamination by optical pairs, only those pairs with signs of interaction or with a small differential motion, $\Delta V_r/V_r < 0.23$, have been considered. The distances have been determined from the mean recession velocity, V_r , using the Hubble constant, $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. For some nearby pairs other distance indicators have been used. The pairs have been divided into five equal groups according to the radius, R . The resulting function $\langle M(R) \rangle$ is shown in Fig. 2.

We have also calculated the average mass distribution of known stellar populations, $M_s(R)$, and have determined the distribution of mass in the corona, $M_c(R)$ (Fig. 2). The resulting central density agrees well with our previous estimates from individual galaxies. The use of distant satellites as test bodies enables us to derive masses and effective radii of coronas with a sufficient accuracy. There are enough pairs in our sample to allow an estimation of the masses of coronas separately for primary elliptical galaxies and also for bright and intermediate spiral galaxies. The estimated parameters are given in Table 2. In the case of primary elliptical galaxies the total masses of coronas may be underestimated.

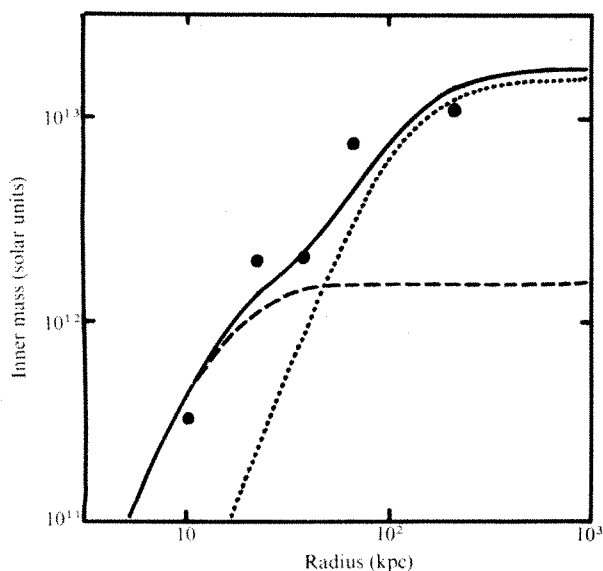


Fig. 2 The distribution of the mean inner mass, $\langle M(R) \rangle$, obtained from 105 pairs of galaxies. Symbols as in Fig. 1.

The mass of galactic coronas exceeds the mass of populations of known stars by one order of magnitude, as do the effective dimensions, by a similar factor. The central density of coronas is surprisingly constant: $\log \rho_c = -24.5 \pm 0.1$ (g cm^{-3}).

The presence of massive coronas in galaxies considerably reduces (if not removes) the virial mass discrepancy in clusters of galaxies. The mass-luminosity ratio rises to $f \approx 100$ for spiral and $f \geq 120$ for elliptical galaxies. With $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ this ratio for the Coma cluster is 170 (ref. 1).

According to new estimates the total mass density of matter in galaxies is 20% of the critical cosmological density.

The possible physical nature of galactic coronas will be discussed in a separate article.

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- ¹ Rood, H. J., Page, T. L., Kintner, E. C., and King, I. R., *Astrophys. J.*, **175**, 627 (1972).
- ² Holberg, J., Bowyer, S., and Lampton, M., *Astrophys. J. Lett.*, **180**, L55 (1973).
- ³ Roberts, M. S., in *Stars and Stellar Systems* (edit. by Sandage, A.), 9 (in the press).
- ⁴ de Vaucouleurs, G., *Astrophys. Lett.*, **4**, 17 (1969).
- ⁵ Arp, H., and Bertola, F., *Astrophys. Lett.*, **4**, 23 (1969).
- ⁶ Bertola, F., and Capaccioli, M., *Asiago Contr. No.* 234 (1970).
- ⁷ Einasto, J., *Tartu astr. Obs. Teated*, **40**, 3 (1972).
- ⁸ Einasto, J., and Kaasik, A., *Astr. Circ. USSR*, **790**, 1 (1973).
- ⁹ Ostriker, J. P., and Peebles, P. J. E., *Astrophys. J.*, **186**, 467 (1973).
- ¹⁰ Einasto, J., Saar, E., Kaasik, A., and Traat, P., *Astr. Circ. USSR*, **811** (1974).
- ¹¹ Karachentsev, I. D., *Astrofizika*, **2**, 81 (1966).
- ¹² Page, T., *Astrophys. J.*, **159**, 791 (1970).
- ¹³ Karachentsev, I. D., *Soobshch. spec. astr. Obs.*, **7**, 3 (1972).

Origin of QSO absorption lines

THE origin of the absorption lines in QSOs, with $z_{\text{abs}} \ll z_{\text{em}}$ has remained a puzzle. It has been suggested that they result from intervening matter lying along the line of sight but not associated with the QSO^{1,2}. It has also been suggested that the absorption lines arise 'locally' from matter expelled from the QSO core³⁻⁶. In particular, radiation pressure has been proposed as the driving mechanism³⁻⁵. It has, however, been shown that the final velocities required by the expelled matter ($v_f \sim c$) for z_{abs} to be $\ll z_{\text{em}}$, cannot be attained by radiation pressure⁷. Alternatively, matter could be accelerated to velocities $v_f \sim c$ by free electron scattering in the outer region of the QSO core⁷.

Any model that attempts to explain the formation of QSO absorption lines must explain the highly multiple z_{abs} spectra that are sometimes seen, and the distribution of the multiplicities. At least 10 z_{abs} per QSO are required to explain the spectra of the multiple z_{abs} QSOs PHL957, Ton1530, PKS0237-23 and 4C05.34 (refs 9-12).

The observed redshifts, $z_{\text{abs}} (\ll z_{\text{em}})$, in the local interpretation, correspond to final velocities $v_f \sim 100,000 \text{ km s}^{-1}$ and the absorption line widths correspond to velocity dispersions $\Delta v_f \sim 30 \text{ km s}^{-1}$ (refs 13 and 14). A particularly small Δv_f of $\sim 8 \text{ km s}^{-1}$ has recently been observed¹⁵. The basic problem of the local interpretation is to ascertain how a cloud be accelerated to final velocities $v_f \sim c$ yet have a final velocity dispersion $\Delta v_f/v_f \sim 10^{-4}$.

So far the existence of 'plasmoids', consisting of relativistic particles in a magnetic field, is the only mechanism in the local interpretation that has been suggested⁶ to decrease the relatively large possible macroscopic or bulk motions in QSO clouds (velocity dispersions). In the plasmoid mechanism there are several assumptions⁶. First, that an individual plasmoid typically expands adiabatically from $\sim 5 \times 10^{17} \text{ cm}$ to $\sim 10^4 \text{ pc}$; second, that $\sim 10^4$ plasmoids form a single cloud of dimension $\sim 10^4 \text{ pc}$; third, that inhomogeneities in the magnetic field of the single cloud cause density enhancements such that separate regions are created with different z_{abs} ; and fourth, that the different density enhancements have relativistic velocities with respect to one another, thus explaining the large differences in the observed z_{abs} .

As a possible alternative to that plasmoid mechanism, we show here that certain large relative bulk motions in QSO clouds can be dissipated without the presence of strong magnetic fields and without large adiabatic expansions. The essence of the argument is that a cloud with convergent internal velocities can coalesce inelastically because the internal kinetic energy can be dissipated and radiated away.

It is easy to show this when using the known data of QSO absorption clouds. I assume that the cloud, with convergent internal velocities, extends from a distance R_1 to R_2 from the QSO core, and has a velocity profile that decreases with increasing radius ($v(R_1) > v(R_2)$). An ion at R_1 has a relative velocity, \hat{v} , with respect to the neutral atoms in the cloud, where $0 < \hat{v} < \hat{v}_m$. The velocity, \hat{v}_m , is the maximum dispersion velocity of the cloud ($v(R_1) - v(R_2)$) and is taken to be large ($\hat{v}_m \gg 10^{-4}c$).

The average energy loss for an ion per neutral atom, $\epsilon(\hat{v})$, is approximately inversely proportional to $\hat{E}(\hat{v})$, where $\hat{E}(\hat{v})$ is the relative kinetic energy and \hat{v} is greater than a characteristic velocity, v_0 (ref. 16). Thus, $\hat{E}(\hat{v}) \epsilon(\hat{v}) \simeq \hat{E}(v_0) \epsilon(v_0)$. Because the relative velocity, \hat{v} , for an ion at R_1 varies from 0 to \hat{v}_m , the range of the ion in the cloud is of the order

$$\bar{\rho} \equiv [\rho E(v_0) \hat{v}_m^2 / v_0^2 \epsilon(v_0) / n(R_1)],$$

where f is the fraction of the atoms of the cloud which are neutral, and $n(R_1)$ is the particle density of the cloud. Assuming that the cloud thickness, $R_2 - R_1$ is comparable to R_1 , then

the condition for the dispersion velocity, \hat{v}_m , to be dissipated is

$$\bar{\rho} \ll R_1 \quad (1)$$

The density $n(R_1)$ can be related to the column density of the observed absorption clouds, $N_a \equiv n(R_a)\Delta R_a$, where R_a is the average distance of the absorption clouds from the QSO core, $n(R_a)$ is the particle density of the clouds at R_a , and ΔR_a is the shell thickness in which the observed absorption clouds are found. A high probability exists for finding absorption lines in QSOs with large z . This implies that the observed absorption clouds have a solid angle coverage approaching 4π sr. The mass of the clouds is $M = 4\pi N_a R_a^2$. We assume that $R_1 \ll R_a$. The time to expel the clouds of mass M (now at R_a), through a sphere of radius $R_1 < R_a$, is $< R_a/v_r$. It is also $\geq M/4\pi r^2 v_r R$. The density, $n(R_1)$, must therefore obey the relationship $n(R_1) \geq N_a R_a^2$.

The range, $\bar{\rho}$, in equation (1) thus obeys the relationship $\bar{\rho} < \bar{\rho}_m \equiv E(v_0) \hat{v}_m^2 / v_0^2 \epsilon(v_0) / N_a R_a$. Using existing experimental data we can evaluate $\bar{\rho}_m E(v_0)$ for protons in helium at ≈ 100 keV. The $\epsilon(v)$ for protons in helium has been measured and found to be $\sim 7.0 \times 10^{-15}$ cm² eV at 100 keV, and $\sim 6.5 \times 10^{-15}$ cm² eV at 40 keV (refs 17-20). Helium atoms in the absorption cloud will be the first atoms to recombine, and so $\epsilon(v_0) \approx 7.0 \times 10^{-15}$ cm² eV can be taken for the evaluation of $\bar{\rho}_m$. Burbidge and Chan²¹ evaluated the column densities of the QSOs PHL938, PKS0237-23 and Ton1530. They find that $N_a \approx 10^{19}$ cm⁻², which we assume to be true. Bahcall and Goldsmith¹⁰ placed a lower limit of 10^3 pc on R for the QSO 4C05.34, which we also assume to be true. We then have $\bar{\rho}_m \approx 10^{-4} \hat{v}_m^2 R_a^2 / v_0 f$, where \hat{v}_m and v_0 are in units 10^3 km s⁻¹ and R_1 is in pc.

Reasonable estimates of \hat{v}_m , R_1 and f give $\bar{\rho}_m/f \ll 1$ (for example $\hat{v}_m \sim 10^{-2} c$, $R_1 \sim 1$ pc, $f \sim 0.01$). The condition of equation (1) then seems to be satisfied.

It is of interest to estimate the final equilibrium velocity, \hat{v}_e , that the particles attain in the cloud. At very low energies, an important factor that must be taken into account is that the particles no longer travel in straight lines. Although traverse in a straight line are a good approximation when \hat{E} is large compared to atomic energies ($\sim I$), this assumption is not valid at very low energies (that is, when $\hat{E} \sim I$). When \hat{E} becomes comparable to I , large transfers of momentum can occur. Rather than scattering primarily in the forward direction, as at high energies, the scattering becomes approximately isotropic for $\hat{E} \sim I$. If ΔE is the average energy loss per collision, then the energy, ΔE , will be small, because most collisions at very low energies are elastic. The ratio of the range $\rho(\hat{E})$, of a particle, which occurs when large transfers of momentum are important to the range of the particle travelling in a straight line, $\rho_s(\hat{E})$, is $\rho(\hat{E})/\rho_s(\hat{E}) \sim N^{1/2}$, where $N \sim E/\Delta E$. For example, if $\Delta E \sim 0.1$ eV and $E \sim 100$ eV, then the ratio is $\sim 1/30$.

Particles in the cloud will lose energy at an appreciable rate as long as $\hat{E} > \bar{E}_i$, where \bar{E}_i is the average ionisation energy ($\bar{E}_i < I$). The inelastic cross section will rapidly decrease when $\hat{E} < \bar{E}_i$. Thus, an approximately stable equilibrium velocity occurs when $\hat{E}(\hat{v}_e) \sim \bar{E}_i$. Using $\bar{E}_i \sim 13$ eV gives $\hat{v}_e = (2\bar{E}_i/M)^{1/2} = 10^{-2}$, where M , the average atomic mass, is ~ 1 . It is interesting that this value is approximately equal to the observed dispersion velocity of absorption line clouds, $\Delta v_r (\sim 10^{-4} c)$.

There are thus indications that observed QO absorption clouds may have their origin in clouds with internal velocities which are convergent, even through the initial velocity dispersion, $\hat{v}_m \gg \Delta v_r \approx 10^{-4} c$, where Δv_r is the observed velocity dispersion. It might be argued, however, that clouds with internal velocities which are divergent, and with $\hat{v}_m \gg 10^{-4} c$ are as likely to occur as convergent clouds. Although this is true it should be noted that if such divergent clouds do exist it is very difficult to observe them. Because of the breadth of the absorption lines, the ratio of the magnitude of a given absorption line from such a cloud, to the magnitude of the line from a con-

vergent cloud, is $\ll 1$. For example, if $\hat{v}_m \sim 10^{-2} c$ the ratio is $< 1\%$.

The gas in a convergent cloud will be very highly ionised. In our discussion we have tried to be conservative and we therefore used $f \approx 0.01$, that is, the cloud is 99% ionised. The cloud will be heated further because of, for example, adiabatic compression ion-atom collisions and the formation of shock waves. The mechanism described for the formation of a cloud with a narrow velocity dispersion, requires that the cooling time is shorter than the dynamical scale of time. The question whether the heat can be radiated away before the cloud has time to rebound can only be answered by a detailed analysis of the dynamical equations.

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- ¹ Bahcall, J. N., and Salpeter, E. E., *Astrophys. J.*, **14**, 847 (1966).
- ² Bahcall, J. N., and Spitzer, L., *Astrophys. J. Lett.*, **15**, L63 (1969).
- ³ Scargle, J. D., Caroff, L. F., and Noerdlinger, P. D., *Astrophys. J. Lett.*, **161**, L115 (1970).
- ⁴ Mushotsky, R. F., Solomon, P. M., and Strittmatter, P. A., *Astrophys. J.*, **174**, 7 (1972).
- ⁵ Scargle, J. D., *Astrophys. J.*, **179**, 705 (1973).
- ⁶ Rees, M., *Astrophys. J. Lett.*, **160**, L29 (1970).
- ⁷ Opher, R., *Astrophys. J.*, **187**, 5 (1974).
- ⁸ Cohen, J. G., *Nature*, **237**, 273 (1972).
- ⁹ Lawrence, J. L., Morton, D. C., Zucchino, P., Oke, J. B., and Schmidt, M., *Astrophys. J.*, **171**, 233 (1972).
- ¹⁰ Bahcall, J. N., and Goldsmith, S., *ibid.*, **170**, 17 (1971).
- ¹¹ Bahcall, J. N., Osmer, P. S., and Schmidt, M., *Astrophys. J. Lett.*, **156**, L1.
- ¹² Lynds, R., *ibid.*, **164**, L73 (1971).
- ¹³ Morton, D. C., and Morton, W. A., *Astrophys. J.*, **174**, 237 (1972).
- ¹⁴ Burbidge, E. M., and Chan, Y. M. T., *ibid.*, **167**, 23 (1971).
- ¹⁵ Brown, R. L., and Roberts, M. S., *Astrophys. J. Lett.*, **184**, L7 (1973).
- ¹⁶ Bethe, H. A., *Annln. Phys.*, **5**, 325 (1930).
- ¹⁷ Reynolds, H. K., Dunbar, D. N. F., Wengel, W. A., and Whaling, W., *Phys. Rev.*, **92**, 742 (1953).
- ¹⁸ Phillips, J. A., *ibid.*, **90**, 532 (1953).
- ¹⁹ Weyl, P. K., *ibid.*, **91**, 289 (1953).
- ²⁰ Brolley, J. E., and Ribe, F. L., *ibid.*, **98**, 112 (1955).
- ²¹ Burbidge, E. M., and Chan, Y. W. T., *Astrophys. J.*, **167**, 213 (1971).

Absorption of gamma rays in intense X-ray sources

It has been proposed that intense X-ray sources could also be candidates for γ -ray sources, but there has been no experimental evidence. This can mean either that no γ rays are produced or that they are absorbed before they leave the source. Under certain assumptions about the geometry of the X-ray source one can show that the γ rays are strongly absorbed as a result of pair production with the X-ray photons. On the other hand, measurements on hard photons above the X-ray band may allow us to derive information on accretion sources by virtue of their absorption mechanism.

The importance of photon-photon absorption in astrophysics was first mentioned by Nikishov¹. He calculated the

absorption of high energy γ rays (10^{12} eV) on thermal photons in intergalactic space. Jelley² considered the same absorption mechanism within possible γ -ray sources. McBreen³ pointed out that γ rays from the Crab nebula should already be absorbed for γ -ray energies > 50 MeV as a result of pair production with the X-ray photon field. The following calculations describe the absorption of γ rays in some intense X-ray sources using recent data.

Two photons of energy E_x , E_γ can produce an electron-positron pair in a collision provided that

$$E_x E_\gamma \geq 2(mc^2)^2 / (1 - \cos \alpha) \quad (1)$$

where m is the electron mass and α is the angle between the directions of motion of the two photons. For a fixed γ energy E_γ the cross section σ is a function of the X-ray energy E_x (ref 4). The cross section σ rises steeply from a threshold $E_x = E_s$ given by equation (1), it has a maximum value at $E_x = 2E_s$ and falls off as E_x^{-2} for X-ray energies $E_x \gg E_s$.

We describe the absorption of the γ rays by calculating the dimensionless optical depth τ . The γ -ray intensity then varies as $I_\gamma = I_{\gamma 0} \exp(-\tau)$. Detailed calculations of τ assuming an isotropic radiation field are given by Gould and Schröder⁵. For simplicity it can be written

$$\tau = r \int \sigma n_x dE_x \quad (2)$$

taking the cross section σ and n_x , the density of X-ray photons per unit energy, to be constant throughout the source.

Both σ and n_x are functions of the X-ray energy E_x . r is the length along which absorption takes place. Furthermore, we assume that the angle α in equation (1) is 90° . If we approximate σ by a rectangular function with height $\sigma_0 = 1.7 \times 10^{-25}$ cm² and width $2.5E_s$ we get from equation (2)

$$\tau \approx r \cdot \sigma_0 n_x(2E_s) \cdot 2.5E_s \quad (3)$$

where we have chosen n_x at the energy $2E_s$, corresponding to σ_{\max} .

Equation (3) gives τ as a function of the γ -ray energy E_γ because from (1) we have

$$E_s = 2(mc^2)^2 / E_\gamma \quad (4)$$

To estimate n_x we use observational data⁶⁻⁸. If f is the flux of X-ray photons per unit energy measured on Earth we have approximately

$$n_x = f 4\pi R^2 / 4\pi r^2 c \quad (5)$$

where R is the distance to the X-ray source and r the size of the source which we take to be equal to the length where we have absorption.

To get reasonable values for r we refer to current models of X-ray sources. We calculated the optical depth for three different X-ray sources, Her X-1, Cyg X-1 and the Crab pulsar. For Her X-1 we take $r = 10^5$ cm, the size of the magnetic pole region on the surface of an accreting neutron star⁹. For Cyg X-1 we have $r = 10^6$ cm assuming an accretion disk around a black hole as the X-ray source⁹. In the case of the Crab pulsar we take $r = 3 \times 10^7$ cm an upper limit for the γ -ray source if the observed γ pulse has a halfwidth of about 1 ms.

Figure 1 shows τ as a function of the γ -ray energy E_γ .

It can be seen from Fig. 1 that we cannot expect γ rays above 10 MeV depending on the parameters of the source. On the other hand the electron-positron pairs produced by the absorption of the γ rays can be partly stopped and they then annihilate. This 511 keV radiation is no longer absorbed by the X-ray photon field and can probably escape depending on the matter density present.

If there are pulsed γ rays from the Crab above 10 MeV as claimed by some authors, this would not contradict the above result. From equation (1) we see, that the threshold energy E_s goes to infinity in the case of beamed radiation ($\alpha = 0$).

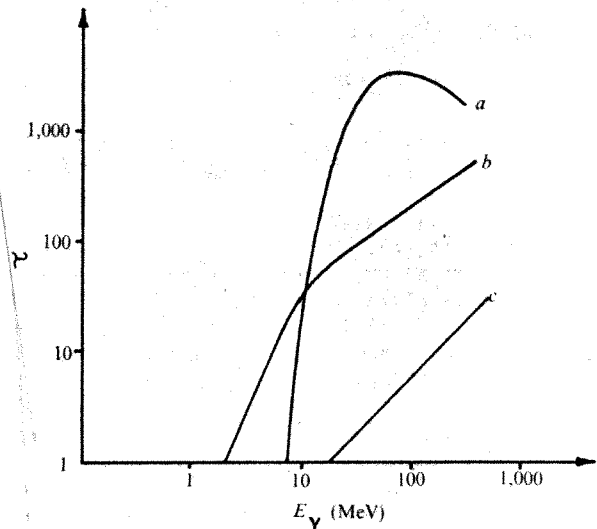


Fig. 1 τ as a function of the γ -ray energy E_γ . a, Her X-1; b, Cyg X-1; c, Crab.

One can speculate whether there exist other sources where the optical depth $\tau(E_\gamma)$ might be less than 1. From equations (3) and (5) we get

$$\tau \propto L_x / r \quad (6)$$

with $L_x = 4\pi R^2 f$. τ can be small for large r and small L_x .

Examples of the first case are Seyfert galaxies. If we take $r = 10^{-25}$ c, $L_x = 10^{42}$ erg s⁻¹ and compare with the corresponding parameters of Her X-1 ($r = 10^5$ cm, $L_x = 10^{37}$ erg s⁻¹) taking from Fig. 1, we obtain a value for τ , in the case of Seyfert galaxies, much less than 1.

An example of the second case could be an optically thin relativistic plasma of say $kT = 10$ MeV becoming thermally unstable. Ninety per cent of the energy would then be radiated within the energy range 1 MeV–10 MeV. Certainly, the temperature of such a plasma cannot exceed 20 MeV because of cooling caused by the production of electron-positron pairs¹¹. If there is not much X radiation compared with the γ radiation this also has some influence on the Eddington limit which sets an upper limit for the mass flow in an accreting object. The corresponding maximum luminosity is proportional to σ^{-1} , the cross section in the interaction between the infalling matter and the outgoing radiation field. Thus, we can have higher luminosities for γ -ray sources than for X-ray sources. This factor could be as large as 150 for γ energies above 20 MeV.

I thank Professor Martin Rees for drawing my attention to this problem. The work was done during a visit to the Institute of Astronomy in Cambridge, England.

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¹ Nikishov, A. I., *Soviet Phys. JETP*, **14**, 393 (1962).

² Jelley, T. V., *Nature*, **211**, 472 (1966).

³ McBreen, B., *Nature*, **224**, 893 (1969).

⁴ Jauch, J. M., and Rohrlich, F., *The theory of photons and electrons* (Addison-Wesley Publishing Company, Reading, Massachusetts and London, 1955).

⁵ Gould, R. J., and Schröder, G. P., *Phys. Rev.*, **155**, 1404 (1967).

⁶ Ulmer, M. F., Baity, W. A., Wheaton, W. A., Peterson, L. E.,

Astrophys. J. Lett., **181**, L33 (1973).

⁷ Haymes, R. C., and Harnden, F. R. jun., *Astrophys. J.*, **159**, 1111 (1970).

⁸ Hillier, R. R., Jackson, W. R., Murray, A., Redfern, R. M., and Sale, R. G., *Astrophys. J. Lett.*, **162**, L177, (1970).

- ⁹ Pringle, J. E., and Rees, M. J., *Astr. Astrophys.*, **21**, 1 (1972).
¹⁰ Pringle, J. E., Rees, M. J., and Pacholczyk, A. G., *Astr. Astrophys.*, **29**, 179 (1973).
¹¹ Bisnovatyi-Kogan, G. S., Zel'dovich, Ya.B., and Syunyaev, R. A., *JETP Lett.*, **12**, S45 (1970).

Energy source for comet outbursts

A COMET nucleus is generally recognised as an icy conglomerate, as was originally proposed by Whipple¹. The Orbiting Astronomical Observatory observations of Comet Tago-Sato-Kosaka² and Comet Bennett³ support the current ideas that H₂O is a major component of comets. If comets were indeed formed through an accretion mechanism at distances of many AU from the Sun, what is the nature of the resulting form of water ice?

A number of studies on the deposition of water vapour at low pressures and temperatures indicate that amorphous ice is formed. The reported physical properties include: a density of 2.3 g cm⁻³ (ref. 4), a specific heat 25% greater than that of ordinary hexagonal ice, and a latent heat for the phase transition from amorphous to cubic ice of 24 ± 2 calorie g⁻¹ (ref. 5). The transition occurs at a temperature near 140 K (ref. 6). The presence of impurities seems to enhance the growth of clathrate hydrate ices, but both the clathrates and the amorphous water ice can apparently coexist at densities of 1.4–1.7 g cm⁻³ (ref. 4). Clathrate compounds have densities which are typically 0.3–0.5 g cm⁻³ (ref. 7).

Observational evidence indicates that comet outbursts require an internal energy source⁸. If at least the surface of a comet nucleus contains a substantial percentage of amorphous ice, then the phase transition of the amorphous ice to a cubic structure provides a release of energy which may be responsible for the outbursts observed in many comets. In addition, if the density of amorphous ice is indeed about 2 g cm⁻³, then a 'pulverising' mechanism would exist because of the abrupt stresses introduced by the volume change in the solid as the density of the ice changes by a factor of two.

The total energy released during a cometary outburst is of the order of 10²¹ erg with an accompanying mass loss of 10¹² g (ref. 8). The resulting energy requirement of 10⁹ erg g⁻¹ compares favourably with the 10⁹ erg g⁻¹ released during the amorphous–cubic phase change.

Any theory to explain cometary outbursts must consider the spatial distribution of the phenomenon. Figure 1 reports the results of a study in which the positions of the comets are shown at the time of outburst⁹. Although some observational selection effects may be present, a definite clustering is apparent within 2.5 AU from the Sun. Calculations¹⁰ have shown that celestial bodies consisting of water ice with an albedo of 0.6, at a distance of 2.5 AU from the Sun, have expected surface temperatures ranging from 150 K for a rapidly rotating sphere, to 180 K for a non-rotating sphere (see Figs 1 and 2 of ref. 10). The phase transition from amorphous to cubic ice requires a temperature near 140 K. Higher temperatures are necessary if the surface has an insulating layer such as that predicted in the model we shall present here.

Any volume element which undergoes this phase transition increases its temperature by about 45 K. Therefore, the heat released can trigger the surrounding material so that the phase change is, in effect, a self feeding mechanism which propagates to a distance where the local temperature is near 100 K.

The outburst of a comet can therefore be envisaged as occurring in a series of consecutive steps: initiation, propagation, pulverisation, sublimation, ejection, and insulation.

Depending upon the condition of the surface of the comet, the amorphous–cubic ice phase transition can be achieved at different solar distances. If the surface is amorphous ice, an outburst is most probable at about 2.5 AU from the Sun. If the surface is covered with an insulating layer, however, a

closer approach to the Sun is required. On the other hand, if amorphous ice is in heat exchange with material with a temperature which increases faster than that of the ice, then an outburst can be expected at greater distances. If the temperature of the ice is slightly below the transition temperature, thermal spikes provided by solar activity could trigger the outburst.

Once the phase transition has been initiated, it will propagate within a region where the temperature of the ice is greater than 100 K. The volume into which the transition can propagate can be estimated. Our calculations consider a spherical comet with a radius of 5 km. For a rough estimate we assume that the surface temperature around the subsolar point decreases as a cosine function. For a subsolar temperature of 140 K, the surface area with a temperature above 100 K is bounded by a circle and contains 14% of the total surface area of the comet. Following Sommerfeld's¹¹ development, it is possible to estimate the depth at which the phase transition terminates 1 m below the subsolar point. For this calculation the thermal diffusivity of glass, 2×10^{-2} cm² s⁻¹, is used. As this depth is reduced to zero at the perimeter of the previously computed area, an average depth of 50 cm is assumed. The resulting volume which undergoes the phase transition is 2.2×10^{13} cm³. The density of amorphous ice is about 2 g cm⁻³ and so the mass involved is 5×10^{13} g.

The transformation of amorphous ice into cubic ice with a density of 0.94 g cm⁻³, must induce severe strains of the order of 20–30% which will pulverise the ice. It is useful to estimate the particle size which results from the fracturing process. For a stress, S , of about 10⁴ pound inch⁻², W is proportional to S^{-4} , where W is the average mass of the ten largest particles that result when the stressed material fractures. If this relationship holds in general for higher stresses, then an extrapolation to the anticipated stress encountered for the amorphous–cubic ice transformation ($\sim 10^6$ pound inch⁻² for strains of 20–30%), shows that W is of the order of 4×10^{-9} g. This implies that the largest particle has a size of about 10 μ m.

This mechanism therefore automatically provides a source of particulate matter which creates a huge surface area. Consequently, the equilibrium vapour pressure between the particulates will be rapidly established. With the mass (5×10^{13} g)

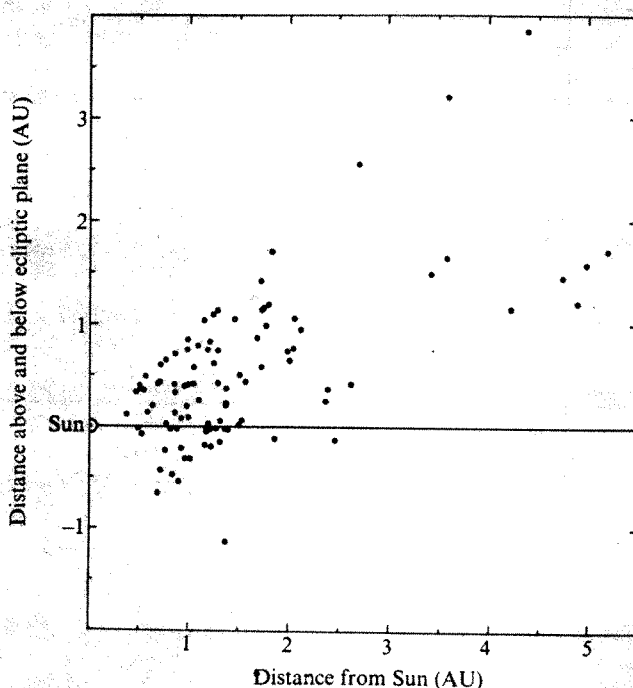


Fig. 1 Positions of comets at the time of outbursts. ○, Hypothetical outbursts preceding discovery; ●, observed outbursts after discovery⁹. (Courtesy of the IAU.)

involved in the phase transition the energy, E , which is released, is given by:

$$E = 5 \times 10^{13} \text{ g} \times 24 \text{ calorie g}^{-1} \\ = 10^{15} \text{ calorie}$$

This can generate water vapour of mass, M :

$$M = E/H = 10^{15} \text{ calorie}/650 \text{ calorie g}^{-1} \\ = 1.5 \times 10^{12} \text{ g}$$

where H is the heat of sublimation. This means that 3% of the generated particulate matter is sublimated. This gas must certainly expand into the vacuum of space at a few times the velocity of sound, carrying with it, at least to an order of magnitude, a comparable mass of dust. This mass is consistent with observed values for typical comet outbursts⁸.

A large fraction of the fractured material remains on the surface. This effectively creates an insulating layer with a high albedo which tends to prevent further outbursts for some time.

This picture of comet outbursts can be subjected to many refinements, such as variations of comet sizes, or of rotation rates and inclinations, impurities and inhomogeneities in the ice, and orbital parameters. The general features of this theory are, however, consistent with observations and in our opinion provide for a more plausible source of energy than has been previously suggested. Those suggestions have included the vaporisation of pockets of methane and/or carbon dioxide⁸, explosive radical reactions¹², and collisions with interplanetary boulders.

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¹ Whipple, F. L., *Astrophys. J.*, **111**, 375 (1950).

² Jenkins, E. B., and Wingert, D. W., *Astrophys. J.*, **174**, 697 (1972).

³ Code, A. D., Houck, T. E., and Lillie, C. F., *The Scientific Results from the Orbiting Astronomical Observatory (OAO-2)*, NASA SP-310, 109 (1972).

⁴ Delsemme, A. H., and Wenger, A., *Science*, **167**, 44 (1970).

⁵ Ghormley, F. A., *J. chem. Phys.*, **46**, 1321 (1966).

⁶ McMillan, F. A., and Los, S. C., *Nature*, **206**, 806 (1965).

⁷ Delsemme, A. H., and Wenger, A., *Planet. Space Sci.*, **18**, 709 (1970).

⁸ Whitney, C., *Astrophys. J.*, **122**, 190 (1955).

⁹ Pittich, E. M., *Splitting and Sudden Outbursts of Comets as Indicators of Nongravitational Effects*, (Forty-fifth IAU Symposium, Leningrad, 1970), 283 (Reidel, Dordrecht, 1972).

¹⁰ Watson, K., Murray, B. C., and Brown, H., *Icarus*, **1**, 317 (1963).

¹¹ Sommerfeld, A., *Partial Differential Equations*, 68 (Academic Press, New York, 1949).

¹² Donn, B., and Urey, H. C., *Astrophys. J.*, **123**, 339 (1956).

Ratio of the temperatures of the quiet Sun and the centre of the new moon at $\lambda = 1.3 \text{ mm}$

As radio wave spectral line observations are made at ever shorter wavelengths, the need for precise calibration increases. This is because, as a larger portion of the frequency spectrum becomes available for observation, an increasing number of multiple molecular transitions will be detected. To obtain reliable excitation parameters from these detections they must be calibrated accurately at each frequency. Linsky¹ has proposed the Moon as a radiometric standard for observations of extended sources in the infrared, millimetre and microwave portions of the spectrum. We therefore measured the ratio of the quiet Sun temperature to that of the centre of the new Moon

at 231 GHz ($\lambda = 1.3 \text{ mm}$).

The 36-foot telescope of the National Radio Astronomy Observatory (NRAO) was used for the measurements, on June 30, 1973. At $\lambda = 1.3 \text{ mm}$, using a 1.21λ by 1.77λ feed horn, the telescope has a beam size of 44'' × 51'' of arc as measured on Jupiter. A single ended mixer receiver was used that incorporated a Schottky-barrier diode as the nonlinear element². The noise temperature of the double-sideband receiver was 6,000 K, and the intermediate frequency bandwidth was 60 MHz, giving a ΔT r.m.s. of $\sim 1.5 \text{ K}$ with a time constant of 0.25 s.

At this frequency, antenna calibration is rather uncertain and atmospheric attenuation can be quite high, and so it was decided to carry out the observations when the Sun and Moon were close together in the sky. This allowed a comparison of brightness temperatures without any first order dependence on either the antenna or the atmospheric calibration. June 30, 1973 was the date of a total solar eclipse and during our measurements the centres of the Sun and Moon were separated by 6° 45' in the sky: the elevation of the Sun was 41.7° and that of the Moon was 47.7°.

The absolute temperature scale of the receiver was calibrated by placing absorbers, alternately at room temperature and liquid nitrogen temperature, in front of the feed of the antenna. Sky dipping data gave an average value of $\sim 1.2 \text{ db}$ attenuation/atmosphere (clear weather; 27° C; 35% relative humidity) which implied a differential attenuation of 0.18 db between the central solar and lunar positions. The Sun was observed through the dome of the telescope which had been previously measured (using the Moon) to have an attenuation of 2.87 db.

Antenna temperatures referred to an elevation angle of 41.7°, which, if corrected for dome and differential atmospheric attenuation would be 2,481.5 K and 53.2 K for the centres of the Sun and Moon respectively; a value of 46.6 for their ratio. Assuming that the temperature of the quiet Sun is 5,800 K at $\lambda = 1.3 \text{ mm}$, a value of 124.5 K is derived for the central lunar temperature at new Moon.

An examination of H α and K spectroheliograms of the Sun taken on the day of our observations by the McMath-Hulbert Observatory, showed that except for a small flare close to the eastern limb of the Sun, the solar disk, particularly the central region where our observations were taken, was free from any major disturbance. This was confirmed by drift scans of the Sun during the observations. Thus, we are confident that the ratio quoted does refer to the quiet Sun.

Because of our method of observation, the value we have derived for the ratio of the quiet Sun to that of the new Moon is relatively insensitive to any errors in the absolute calibrations of the atmospheric attenuation, the antenna or the receiver. The value mainly depends on the linearity of the detector and the noise on the signal. Thus, we calculate that the error on the derived ratio—46.6—is ± 0.4 .

Bastin *et al.*³ quote the error on their solar measurement as $\pm 400 \text{ K}$. Consequently, the value we have derived for the central lunar temperature at new Moon—124.5 K—has an uncertainty of $\pm 8.6 \text{ K}$.

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¹ Linsky, J. L., *Astrophys. J. Suppl. Ser.*, **25**, 163 (1973).

² Schneider, M. V., and Wrixon, G. T., *Proc. Int. Microwave Conf. Atlanta*, 1974.

³ Bastin, J. A., Gear, E. A., Jones, G. O., Smith, H. J., and Wright, P. J., *Proc. R. Soc.*, **A278**, 543 (1964).

Oldest and largest lunar basin?

MANY questions in lunar science have not yet been answered satisfactorily.

What, for example, is the origin of the nearside-farside asymmetry of the Moon? Why are Oceanus Procellarum and Mare Imbrium richer in KREEP (basalt rich in trace elements) than any other region on the Moon? Why are the lunar Appenines apparently not composed of anorthositic highland crust? I suggest here a possible solution to these, and other, problems.

The nearside-farside asymmetry is evident from the dark mare material which fills the circular basins on the nearside. Although there are similar basins on the farside, they are apparently not filled with mare basalt. Laser altimeter data suggest that this may be a result of the greater thickness of low density crust on the farside. The surface of the farside is largely elevated from a sphere of radius 1,738 km, centred on the Moon's centre of gravity, and the nearside is correspondingly depressed¹. Although this may explain why Maria occur only on the nearside, the differences in crustal thickness are still unaccounted for. Either the Moon accreted heterogeneously, or else low density crust was, at some early stage of lunar evolution, transferred from the nearside to the farside. A possible mechanism for the transfer of vast quantities of crustal material could involve a very major impact on the nearside.

Measurements with γ -ray spectrometers have revealed that the levels of natural radioactivity (which results from U, Th and K) are considerably higher in Oceanus Procellarum and Mare Imbrium than anywhere else on the lunar surface². These regions are therefore presumed to be richer in KREEP material. It is usually assumed that this distribution of KREEP arose as a result of the Imbrium impact. It has, however, been pointed out that if KREEP was ejected by the Imbrium event then the Haemus Mountains to the east of the Imbrium basin should be as radioactive as the Fra Mauro formation to the south³. This has not been found to be the case.

The age of the Imbrium event is generally taken to be about 3.9 Gyr (refs 4 and 5) whereas the whole rock 'isochron' ages for KREEP rocks are much older⁶, suggesting that KREEP basalts were extruded long before the Imbrium impact. The present day distribution of KREEP may, in fact, largely represent the extent of these original lava flows. The subsequent redistribution by the Imbrium impact may only have been a second order effect.

Before the Apollo 15 mission it had been expected that the lunar Appenines would consist of anorthositic crust, supposedly uplifted by the Imbrium impact. When samples were returned from the Appenine front, however, they were found to have predominantly a KREEP composition, and there were few truly anorthositic rocks. This lack of anorthositic crust in the region has since been confirmed by the results of the X-ray fluorescence experiments. The measured Al:Si ratio from the Appenines is about half the value obtained from the Descartes highlands⁷. These results suggest that the original crust in this region was removed and replaced with KREEP basalts, before the formation of the Imbrium Basin.

I propose that soon after the formation of the lunar crust the Moon collided with a planetesimal which was even larger than the projectile which formed the Imbrium Basin. This collision produced a basin 1,200 km in radius centred at 23°N 29°W (Fig. 1). Its size and position are defined by the highlands to the west of Oceanus Procellarum and to the north of Mare Frigoris, and by the western edge of the Southern Central Highlands. There is no evidence for the presence of any appreciable amount of anorthositic crust within this region. It must therefore have been largely removed, and at least some of it may even have been deposited on the lunar farside, con-

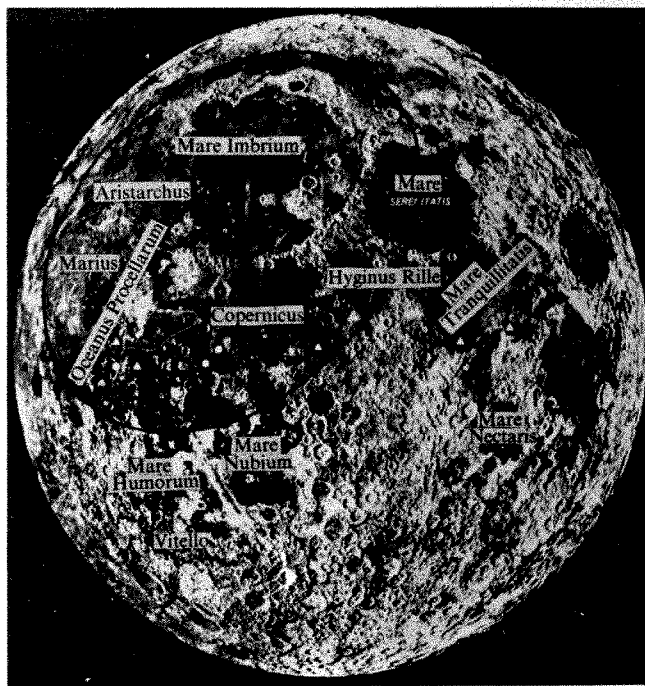


Fig. 1 The location of the 'Gargantuan Basin' on the lunar nearside.

tributing to the observed excess crustal thickness there. It is not unreasonable to suppose that this basin was subsequently filled with KREEP basalt lavas. This volcanic activity probably occurred about 4.3 Gyr ago; a date based on the whole rock 'isochrons' for Apollo 14 and 15 KREEP, and Apollo 16 VHA basalts⁶. Alternatively, the activity may have been as recent as 4.14 Gyr if only the Apollo 14 and 15 results are considered. About 3.9 Gyr ago the Imbrium impact redistributed these basalts, in the form of KREEP rich breccias, to the Fra Mauro formation and elsewhere^{4,5}. At the same time the lunar Appenines, largely composed of KREEP, would have been uplifted (see Fig. 2).

All of the large impact basins on the lunar nearside have circular, positive gravity anomalies, or mascons, associated with them⁸. The existence of the mascons, which are usually attributed to the presence of thick lenses of high density mare basalt, implies hydrostatic disequilibrium and provides a powerful constraint on any model of the thermal evolution of the Moon. The circular basin proposed here, however, does not have an associated mascon. If this basin was indeed filled with KREEP basalt 4.3 Gyr ago, in sufficient quantities to produce a mascon, the lunar crust at this time cannot have been rigid enough to support it. The absence of a mascon corresponding to this 'Gargantuan Basin' thus provides a very strong constraint for lunar thermal models.

If the Gargantuan Basin exists, the collision which produced it could have occurred before lunar capture by the Earth, during lunar capture, or after the Moon was in Earth orbit. One speculative possibility is that the Moon was originally in an eccentric solar orbit, possibly between Mars and Jupiter. Following a collision with a minor planet, that original orbit became perturbed into an orbit which crossed the Earth's, and the Moon was subsequently captured by the Earth. A second possibility is that the planetesimal which formed the basin was a minor terrestrial satellite which collided with the Moon as it passed near the Earth at a high velocity. Collision with a minor Earth satellite is a mechanism which could allow efficient lunar capture⁹. If the impact did indeed result in the capture of the Moon by the Earth, then the other large basins must all have formed when the Moon was in Earth orbit. Either the projectiles which produced them were themselves in

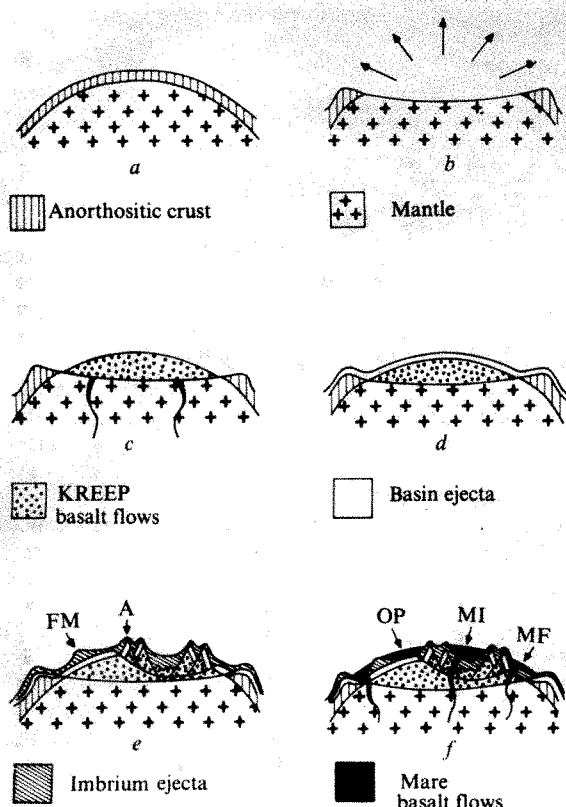


Fig. 2 The evolution of the lunar nearside: *a*, Differentiation of the lunar crust (4.6 Gyr); *b*, formation of the 'Gargantuan Basin', and deposition of excavated crust on the lunar farside; *c*, eruption of KREEP basalts (4.3 Gyr); *d*, deposition of ejecta from major basins (such as Serenitatis, 4.3-3.9 Gyr); *e*, deposition of the Fra Mauro formation (FM) and uplift of lunar Apennines (A) by the Imbrium impact (3.9 Gyr); *f*, eruption of mare basalts (3.8-3.2 Gyr). OP, Oceanus Procellarum; MI, Mare Imbrium; MF, Mare Frigoris.

Earth orbit and were collected as the Moon receded from the Earth because of tidal friction, or they may have constituted a family of asteroids in highly eccentric orbits which crossed the Earth's orbit. The inferred low impact velocity of the Imbrium projectile¹⁰ supports the first possibility. In either case it is difficult to explain why the projectiles were not all finally accreted until at least 400 Myr after lunar capture.

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- ¹ Kaula, W. M., Schubert, G., Lingenfelter, R. E., Sjogren, W. L., and Wollenhaupt, W. R., *Proc. fourth Lunar Sci. Conf.*, 3, 2811-2819 (Pergamon Press, Oxford, 1973).
- ² Metzger, A. E., Trombka, J. I., Peterson, L. E., Ready, R. C., and Arnold, J. R., *Science*, **179**, 800-803 (1973).
- ³ Schonfeld, E., and Meyer, C., jun., *Proc. fourth Lunar Sci. Conf.*, 1, 125-138 (Pergamon Press, Oxford, 1973).
- ⁴ Papanastassiou, D. A., and Wasserburg, G. J., *Earth Planet. Sci. Lett.*, **12**, 36-48 (1971).
- ⁵ Turner, G., Huneke, J. C., Podosek, F. A., and Wasserburg, G. J., *Earth planet. Sci. Lett.*, **12**, 19-35 (1971).
- ⁶ Nyquist, L. E., Hubbard, N. J., Gast, P. W., Bansal, B. M., Wiesmann, H., and Jahn, B., *Proc. fourth Lunar Sci. Conf.*, 2, 1823-1846 (Pergamon Press, Oxford, 1973).
- ⁷ Adler, I., et al., *Proc. fourth Lunar Sci. Conf.*, 3, 2783-2791 (Pergamon Press, Oxford, 1973).
- ⁸ Sjogren, W. L., Wimberley, R. N., and Wollenhaupt, W. R., *The Moon*, **9**, 115-128 (1974).
- ⁹ Kaula, W. M., and Harris, A. W., *Nature*, **245**, 367-369 (1973).

¹⁰ Morgan, J. W., Laul, J. C., Krähenbühl, U., Ganapathy, R., and Anders, E., *Proc. third Lunar Sci. Conf.*, 2, 1377-1395 (MIT Press, Boston, 1972).

Can Einstein's theory of gravitation be tested beyond the geometrical optics limit?

AN upper limit of a few $\times 10^{-3}$ arc s has been placed¹ on the differential deflection of right and left circularly polarised (r.c.p. and l.c.p., respectively) radiation which passes by the Sun from the quasar 3C273. The separation of quasar images in r.c.p. and l.c.p. radiation permits a measurement of the effect of gravitation on polarised radiation. This seems to be the first experiment in which Einstein's theory of gravitation is tested beyond the geometrical optics approximation. Here, I estimate the new effect (that is, the differential gravitational deflection of polarised radiation), and point out that though, using present techniques, it is too small to be measurable for the Sun, it is, however, potentially observable for gravitationally collapsed objects.

Electromagnetic waves in a gravitational field can be thought of as propagating in flat spacetime but in the presence of a 'medium' with properties which are given by conformally invariant quantities constructed from the metric tensor of the Riemannian spacetime, in Cartesian coordinates (ref. 2). Here, Greek indices run from 0 to 3, and Latin indices from 1 to 3. Gravitational units, in which $G=c=1$, are used throughout. The electromagnetic field tensor is decomposed in a Cartesian frame, $(t=x^0, x_i=x^i)$ $F_{\mu\nu} \rightarrow (E, B)$, $(-g)^{1/2} F^{\mu\nu} \rightarrow (-D, H)$ to obtain the usual Maxwell's equations in a medium, together with the relationships²

$$D_i = \epsilon_{ij} E_j - (G \times H)_i \quad (1)$$

$$B_i = \mu_{ij} H_j + (G \times E)_i \quad (2)$$

where, $\epsilon_{ij} = \mu_{ij} = -(-g)^{1/2} g^{ij} / g_{00}$, and $G_i = -g_{0i} / g_{00}$. If the vectors $F^\pm = E \pm iH$ and $S^\pm = D \pm iB$, $S_i^\pm = \epsilon_{ij} F_j^\pm \pm i(G \times F^\pm)_i$ are introduced, then the equations

$$(1/i) \nabla \times F^\pm = \pm (\partial/\partial t) S^\pm \quad (3)$$

$$\nabla \cdot S^\pm = 0 \quad (4)$$

determine the properties of waves in a gravitational field³. The coordinate transformation, $t' = t + \phi(x)$, induces the gauge transformation, $G'_i = G_i + \partial\phi/\partial x^i$, and $\epsilon'_{ij} = \epsilon_{ij}$, under which F^\pm remains invariant, $F'^\pm(t', x) = F^\pm(t, x)$, a property which will be used later. G and $U_{ij} = \delta_{ij} - \epsilon_{ij}$, can be interpreted as gravitational vector and tensor potentials, respectively.

In the scattering of electromagnetic radiation from a gravitational field (asymptotically flat spacetime with no other electromagnetic fields present) the r.c.p. radiation ($F^- = 0$, polarisation properties determined at infinity) decouples from l.c.p. radiation ($F^+ = 0$) according to equations (3) and (4). The helicity of the photon is thus conserved. Moreover, if the gravitational field is free of matter, and spherically symmetrical, then the scattering amplitude is independent of the photon helicity and the gravitational field does not alter the state of polarisation of the incident wave. For more general fields, such as the Kerr field or the exterior field of a rotating body, the scattering amplitude is dependent upon the helicity. The physical reason for this is easily seen by writing the equation for waves of frequency ω in the exterior field of a slowly rotating body of mass, m , and angular momentum, $J = J\hat{z}$:

$$[(1/i) \nabla - \omega G] \times F^\pm = \mp i \omega N F^\pm \quad (5)$$

where, $F^\pm = f^\pm \exp(-i\omega t)$; $G = 2J \times r / r(r-m/2)^2$; and $N = (1+m/2r)^3 / (1-m/2r)$. The index of refraction, N , causes a general deflection for any wave packet⁴. The photon interacts with the vector potential G ; not only with its gravitational 'charge' (energy) but also with its spin, because a rotating body produces a gravitational 'magnetic' field. Moreover, $r \gg m$, $G \approx 2J \times r / r^3$, which is the vector potential caused by a gravitational 'magnetic moment' $2J$. The spin of the

photon is parallel (or antiparallel) to its momentum, and therefore the path of a photon in the exterior field of a slowly rotating body depends on its helicity. It should be emphasised that in the geometrical optics limit where terms of the form $\lambda |\partial g_{ij} / \partial x_k|$ are neglected, the rays follow

$$(\nabla S)^2 - 2\omega \mathbf{G} \cdot \nabla S = \omega^2 N^2 \quad (6)$$

regardless of their polarisation. Thus, wave packets with different polarisation states are expected to follow separate paths which differ from equation (6) in the first approximation by terms of the order of $\lambda |\partial G_{ij} / \partial x_k|$, or the amount by which the vector potential varies over the wavelength of the radiation.

The path of a wave packet of frequency ω , $\omega m \gg 1$, moving nearly parallel to the y axis in the y - z plane far away from the rotating object, is expected to be influenced by the gravitational field of the object at distances close to D along the z axis, where $\mathbf{G} \approx \eta \times \mathbf{r}$, $\eta = 2J/D^3 \ll 1$ and $N \approx 1 + 2m/r$. The dimensions of the packet are assumed to be very small compared with D . The new effects are connected with the presence of \mathbf{G} , it is therefore of interest to consider the solutions of the wave equation

$$[(1/i)\nabla - \eta^2 \times \mathbf{r}] \times \Phi \pm = \mp i\omega \Phi \pm \quad (7)$$

which, under the gauge transformation $\Psi \pm = \Phi \pm \exp(i\omega \eta x)$, is transformed into

$$[(1/i)\nabla - \omega \mathbf{A}] \times \Psi \pm = \mp i\omega \Psi \pm \quad (8)$$

with $A_x = A_z = 0$, and $A_y = 2\eta x$. Solutions of equation (8) can be found in the form $\Psi \pm = \Psi \pm(x) \exp(iK_y y + iK_z z)$, together with the dispersion relation

$$\omega^2 = (K_z \pm)^2 + 2\eta K_z \pm + 2\eta \omega (2n \pm + 1) \quad (9)$$

where, $n^+ = 0, 1, 2, \dots$. This relationship implies that for r.c.p. and l.c.p. photons in the same state, $\Psi, n^+ = n^-$, and moving in essentially the same directions:

$$K_z^- - K_z^+ = 2\eta \quad (10)$$

Thus, the paths of r.c.p. and l.c.p. photons propagating in a direction other than the direction of rotation, split because of the interaction of the photon helicity with the angular momentum of the body, as is evident from equation (9). If the waves propagate along the direction of rotation of the body then equation (10) amounts to different indices of refraction for r.c.p. and l.c.p. waves, so that the plane of linear polarisation rotates in the same direction as the body, in agreement with the results of Skrotskii⁵. Therefore, the wave packet under discussion is focused differently, depending on its state of polarisation, and the differential deflection angle is given by

$$\delta = \omega^{-1} |K_z^+ - K_z^-| = \gamma \lambda J / D^3 \quad (11)$$

where $\lambda = 2\pi/\omega$ and γ is a numerical factor which is expected to be of the order of unity.

Harwit *et al.*¹ used 8 GHz radio waves which passed by the Sun at a distance of $\sim 7.8 R_\odot$. For the Sun, $\delta_\odot < 10^{-18} \gamma$ arc s, which is too small to be detectable, even with the improved techniques of very long base line interferometry, which are likely in the near future. The effect is, however, potentially observable for collapsed objects, and may provide a means of measuring the angular momentum of the body.

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¹ Harwit, M., Lovelace, R. V. E., Dennison, B. K., Jauncey, D. L., and Broderick, J., *Nature*, **249**, 230-233 (1974).

² Volkov, A. M., Izmet'sev, A. A., and Skrotskii, G. V., *Zh. eksp. teor. Fiz.*, **59**, 1254 (1970); *Soviet Phys. JETP*, **32**, 686-689 (1971).

³ Mashhoon, B., *Phys. Rev.*, **D7**, 2807-2814 (1973).

⁴ Weyl, H., *Raum, Zeit, Materie*, fifth ed., 258 (Springer, Berlin, 1923).

⁵ Skrotskii, G. V., *Dokl. Akad. Nauk SSSR*, **114**, 73 (1957); *Soviet Phys. Dokl.*, **2**, 226-229 (1957).

Qnd mantle creep

EXPERIMENTS on model materials show that the acoustic properties of the low velocity zone (LVZ) can arise from the formation of intergranular fluid or melt with no significant reduction in creep strength. Consequently, only very limited bounds on the asthenosphere can be inferred from seismic data.

The effect of partial melting on the acoustic velocity, v , and attenuation, Q^{-1} , of a material is sensitive to the dihedral angle, θ , of the melt in the solid grain boundaries. Relevant data are summarised in Figs 1 and 2. When $\theta = 0$, the melt wets the grain boundaries and a small volume fraction of fluid is sufficient to decrease greatly v and Q^{-1} . There is no theory which accounts for this. In the interpretation of seismic data, however, $\theta = 0$ is the case of greatest interest because the glass formed by quenching samples of partially melted crystalline rock always wets the grain boundaries. The volume fraction of melt required to account for the LVZ is estimated from the data (Figs 1 and 2) to be about 0.01.

In a multicomponent system with a solidus temperature T_s , and liquidus temperature, T_L , partial melting occurs when $T_s < T < T_L$. The presence of a small quantity of fluid at a temperature above T_s means that grain boundaries no longer offer resistance to plastic deformation and that a liquid path is available for diffusion. If deformation because of diffusion through the fluid is not rate-controlling, the creep strength of a partially melted polycrystal in the absence of tensile stress is hardly affected by the fluid. The creep strength reflects the strength of the individual solid grains, and depends on T/T_m , where T_m is the melting temperature of the pure solid. It should, then, be possible to put a material in which T_m/T_s is large, and $\theta = 0$, in a state of partial melting such that the acoustic attenuation and creep strength are both large and the sound velocity is low.

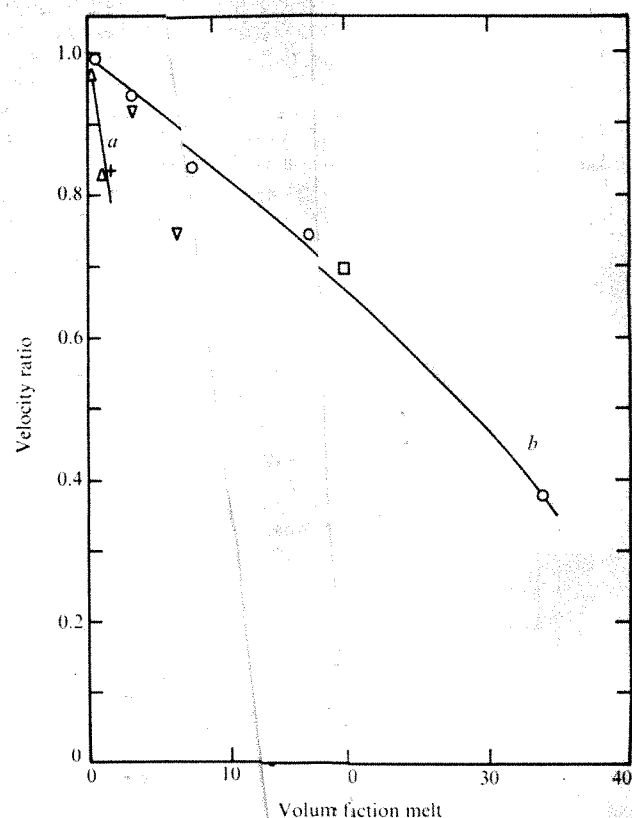


Fig. 1 Ratio of extensional wave velocity in partial melts at the solidus to that in the solid phase as a function of the volume fraction of melt. a, $\theta = 0^\circ$; b, $\theta = 50^\circ$. Materials are: \square , W-Ag (ref. 6); \circ , Cu-Pb (ref. 7); ∇ , inverted Δ , NaCl-H₂O (ref. 8); +, KCl-AgCl (ref. 9); \triangle , Au-Ag (ref. 7).

This hypothesis has been tested with experiments on an Al-2 at % Ga alloy. In this alloy $T_s = 29^\circ\text{C}$ and $\epsilon = 0$; T_m for pure aluminium is 660°C . Above 29°C the alloy attains about 1% melt; it has no tensile strength and can be separated easily into individual grains, each coated by a liquid film. The velocity and attenuation of sound in the Al-Ga alloy have been measured above and below T_s using the method of driven extensional resonance (Table 1). When T_s is exceeded the attenuation increases by a factor of 40 and the velocity drops by 4%. Creep strength measurements have been made on bars of the same alloy loaded in compression at constant stress (Fig. 3). Initially, there is a rapid decrease in strain rate, $\dot{\epsilon}$, identified with transient creep. When steady state creep has been established, after about 10 h at 20°C , $\dot{\epsilon}$ decreases at a constant rate because of the increase in cross section that results from compression. In the experiment illustrated, the temperature was increased from 20 to 33°C after the test had run for 38 h. There was a transient increase in $\dot{\epsilon}$, but after 10 h the creep rate had again become steady and was about the same as that of the alloy containing no melt. Partial melting resulted in no increase in the steady state creep rate.

Table 1 Acoustic properties of Al-Ga alloys

Temperature ($^\circ\text{C}$)	Velocity (km s^{-1})	Attenuation (Q^{-1})
22	5.35	1.0×10^{-4}
32	5.13	39×10^{-4}

If a material is to offer little resistance to steady state creep deformation, then $T \rightarrow T_1$. If T_1 is much less than T_m the small resistance arises because there is enough fluid to disaggregate the solid structure if $T_1 \rightarrow T_m$ the grains of the solid have an intrinsically low creep resistance, regardless of the detailed nature of the operative creep mechanism¹. It is not necessary that $T \rightarrow T_1$ for the acoustic attenuation to be high and the sound velocity anomalously low. These acoustic characteristics can be produced either by heating slightly above T_s when $(T_1 - T_s)/T_1$ is large or, in rock by the presence of

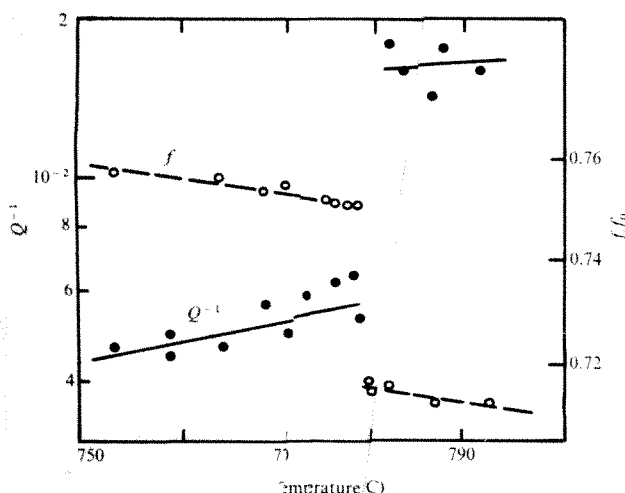


Fig. 2 Internal friction, Q^{-1} and frequency of extensional resonance relative to the room temperature, f/f_0 , for a Cu-8.13 weight % Ag alloy heated through T_s (ref. 7). At T_s there is a discontinuous change in both Q^{-1} and the frequency ratio. The volume fraction of liquid formed is 1.4×10^{-3} .

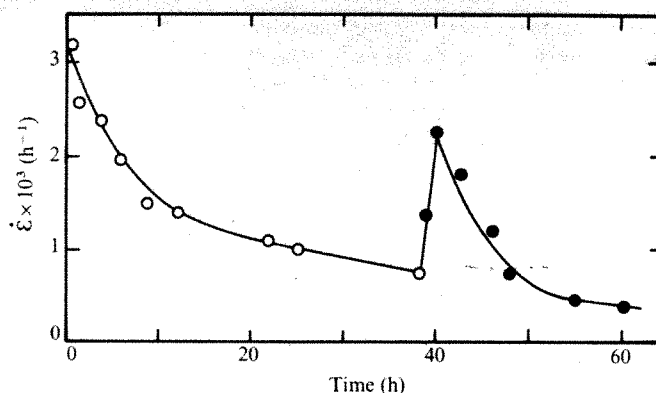


Fig. 3 Creep rate of an Al-Ga alloy under constant compressive load, initially at 20°C , and after 38 h, at 33°C .

small quantities of intergranular fluid rich in water. (Open cracks can also cause the acoustic characteristics of the LVZ but are unlikely to occur under high hydrostatic pressure.) The existence of a LVZ is not, therefore, evidence for the presence of an asthenosphere. A gradual variation of mantle strength properties with depth, as predicted on the basis of solid state data²⁻⁴, is fully consistent with the seismic structure of the upper mantle.

Seismic data fail to establish bounds for either the temperature or the strength properties of the upper mantle. They can, however, place a bound on the properties of the lower mantle. All polycrystalline solids with acoustic properties which have been tested to high temperature in the laboratory show a rapidly increasing 'background' internal friction as $T \rightarrow T_m$ (ref. 5). This is thought to be a result of the coupled, stress-induced motion of dislocations and solute atoms, but conclusive evidence is lacking. In order for the acoustic attenuation of the lower mantle to be as low as the observed value of 10^{-3} , the temperature must be below that at which significant background internal friction appears. Comparison with laboratory data on the high temperature background internal friction of polycrystalline ceramics indicates that in the lower mantle, $T < (0.5-0.75)T_m$.

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¹ Sherby, O. D., and Burke, P. M., *Prog. Mater. Sci.*, **13**, 325-390 (1968).

² Stocker, R. L., and Ashby, M. F., *Rev. Geophys. Space Phys.*, **11**, 391-426 (1973).

³ Gordon, R. B., *J. geophys. Res.*, **70**, 2413-2418 (1965).

⁴ Weertman, J., *Rev. Geophys. Space Phys.*, **8**, 145-168 (1970).

⁵ Nowick, A. S., and Berry, B., *Anelastic Relaxation in Crystalline Solids* (Academic Press, New York, 1972).

⁶ Umekawa, S., Kotfila, R., and Sherby, O. D., *J. Mech. Phys. Solids*, **13**, 229-235 (1965).

⁷ Stocker, R. L., thesis, Yale Univ. (1973).

⁸ Spetzler, H., and Anderson, D., *J. geophys. Res.*, **73**, 6051-6060 (1968).

⁹ Goetze, C., thesis, Harvard Univ. (1969).

Origin of iron ore by diagenetic replacement of calcareous oolite

MANY non-uniformitarian¹ or quantitatively improbable² hypotheses have been offered to explain the origins of extensive sedimentary rocks rich in iron. Ironstones and iron formations³, here termed non-cherty and cherty iron ores, have, however, two consistent characteristics which suggest that their origins were fundamentally similar and involved processes which are still operative at present⁴. The first is the occurrence of mudrocks, or their metamorphosed equivalents, overlying individual ore beds within a few metres, with the ore beds themselves covered by non chemical sedimentary rock and not eroded. The second is the occurrence of oolite texture in unmetamorphosed deposits which are not closely associated with volcanic rock or shales bearing pyroclastic material. The internal structures of well preserved ferriferous ooids closely resemble those of Recent calcareous ooids. Some Recent ferriferous spherules are concentrically layered⁵ but, unlike the ooids in iron ores, they contain clay minerals other than ferrous septechlorite, and occur collectively only as moderately iron-rich sediments, no thicker than a few tens of centimetres. Extensive beds of ferriferous oolite thicker than 1 m have formed repeatedly throughout the history of the Earth, even within the last 5 Myr (ref. 6).

The problem is how can the precipitation from solution of major quantities of iron and silicon, with or without substantial aluminium, phosphorus and manganese, give rise to extensive beds which contain ferrous minerals and shallow water sedimentary structures. A study of the mid-Cainozoic Paz de Rio iron ore of north-eastern Columbia⁴ has resulted in a genetic model which is most clearly illustrated in reference to Upper Pliocene ore under, and adjacent to, the Sea of Azov (Fig. 1).

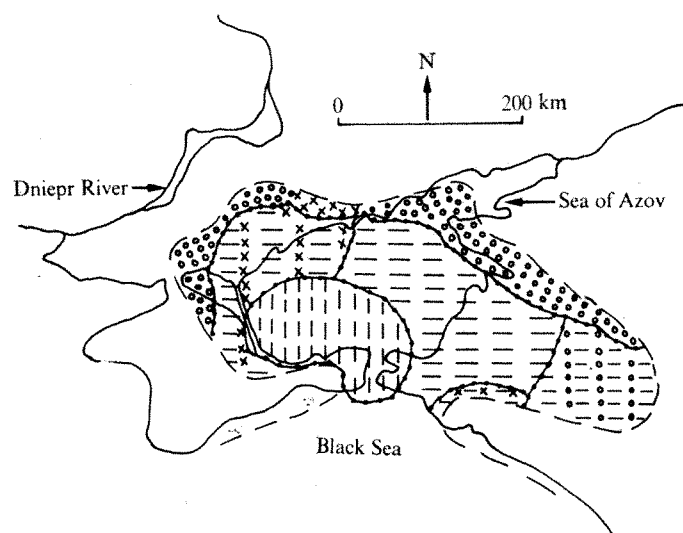


Fig. 1 Map 20 of Upper Pliocene lithofacies in the former Sea of Azov. Circles, non-ferruginous sand; crosses, ferruginous sand; horizontal dashes, shale; vertical dashes, ferriferous oolite with overlying shale. Interbedding is represented by alternation of symbols.

Like all other non-cherty ores, the Azov deposit is predominantly oolitic, and the average thickness of continuous iron ore is only a few metres, although thicknesses do reach 52 m locally. The most abundant ore mineral is ferrous, septechlorite rich in aluminium (chamosite), which is partially replaced by siderite⁶. Goethitic oolite has formed by the oxidation of chamositic oolite above the present water table in the Kerch Peninsula. Fossil shells and shell molds are common, and barite pseudomorphs of wood are present locally. Soviet investigators have suggested that one

possible origin is the dissolution resulting from bacterial action, of terrigenous sediment on the seafloor, and the precipitation of the derived solutions as chamositic oolite at the sediment-water interface⁷. The concentrations of dissolved aluminium in modern marine water⁷, 0.001 parts per million (p.p.m.), and fluviolacustrine water⁸, < 1 p.p.m., are, however, too low to supply sufficient quantities to an extensive region of hypothetical chamositic sedimentation. There is more aluminium in the Azov ore chamosite⁶ than there is dissolved in all of the oceans of the world. Moreover, the absence of dissolved oxygen, which is required if ferrous iron is to be rendered soluble, is incompatible with transport in very shallow, turbulent water.

I therefore propose that the iron, aluminium, and silicon were dissolved by organic, acid-rich groundwater from the interdistributary clay of a delta which prograded over calcareous oolite deposits. Like calcareous oolite sediment in the modern Caspian and Aral Seas⁹, Upper Pliocene oolite in the Sea of Azov apparently formed far from major river discharge and was circumvented by terrigenous sediment (Fig. 1). Shale covering the oolite is interpreted as representing subsequent deltaic progradation. Rain and flood water trapped between natural levees on the delta would have drained from interdistributary swamps rich in organic material, through the highly permeable oolite, up to distributaries (Fig. 2). Aluminium would have been transported in solution as metallo-organic complexes at concentrations of between one and a few p.p.m., as in some British bogs¹⁰. This has been done experimentally at room temperature in 0.01M humic acid solution of clays¹¹. Organic complexing would have greatly enhanced the solubility of iron, but not that of silicon. The experimentally determined Al:Si molar ratio for solutions of clay minerals in dilute, strongly complexing acids, is close to unity¹¹, similar to the molar ratio in chamosites⁴.

If only free ferric oxide and amorphous aluminosilicates were dissolved under interdistributary swamps, over 20 times (ref. 12) as much deltaic mud as oolite would have been leached before the completion of replacement. Migrating distributaries would have continually eroded weathered silt and clay toward the Black Sea. The duration of replacement may be estimated by assuming a deltaic sedimentation rate of 50 million tons a year (about one-tenth that of the Mississippi River) on to the area of oolite illustrated in Fig. 1, and leaching of a volume of deltaic sediment 30 times greater than that of the oolite replaced. Alternatively, the duration can be estimated using Darcy's Law, by assuming the precipitation of 20 p.p.m. of iron from groundwater, a permeability of 100 darcy for oolitic sediment, and a head loss of 1m between interdistributary swamps and distributary channels (Fig. 2). Both calculations suggest that roughly 10⁵ yr are required for the replacement of 5 m of oolite⁴.

The dissolution of silicates by solutions rich in organic material and the subsequent replacement of calcareous oolite, have been experimentally simulated by Gruner², who found that internal ooid structures were preserved in the ferriferous replacement. The preservation of the internal structure is here attributed to the presence of primary carbonaceous layers within calcareous ooids, which act like templates, as in the replacement of aragonite by calcite¹³. Oxidic ooid layers which alternate with chamositic layers in well preserved, mid-Cainozoic ore in Colombia, formed by the oxidation of selected chamositic layers⁴, probably because of differences in the content of organic matter. The greatest loss of oolitic texture in this ore has occurred at the top, possibly as a result of initial rapid dissolution by descending groundwater⁴.

Those who oppose the idea that ferriferous oolite may have originated by the replacement of calcareous oolite, have noted the common occurrence of unreplaced fossil shells beside ferriferous ooids in Phanerozoic ores. Recent

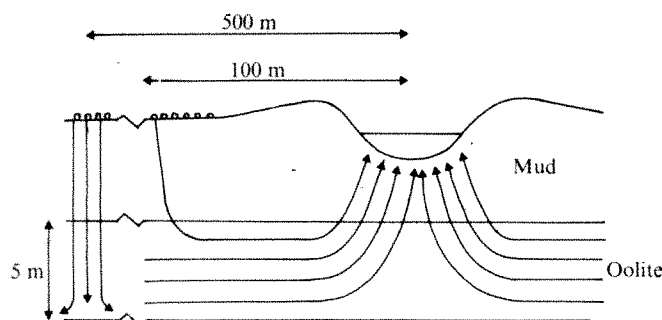


Fig. 2 Hypothetical cross section of groundwater flow from an interdistributary swamp through oolite to a distributary.

oolids are, however, relatively porous aggregates of minute, aragonitic crystallites¹³ which would react readily. Gruner² found that small, calcareous shells were not replaced by solutions which replaced thin sections of calcareous oolite. Opponents to the idea have also noted the lack of lateral gradation from ferriferous oolite to calcareous oolite. But deltaic sedimentation sufficiently potent to fill a lagoon landward from a shallow bank of calcareous oolite, like that in the Aral Sea⁹, would readily cover the entire surface of an oolite bank and cause uniform replacement.

Chamositic oolite has been forming for at least the past 2×10^9 Myr (ref. 14) and cherty, ferriferous oolite has formed at least as recently as the early Jurassic¹⁵. Lower Proterozoic chamositic iron ore is interpreted as recording organic complexing in groundwater which drained interdistributary swamps. The common association of cherty, iron ores poor in aluminium³, of all ages, with volcanic rock or shale containing pyroclastic material¹⁶ suggests that much of the silica and iron was leached from the overlying pyroclastics. A lesser degree of organic complexing during rapid weathering of pyroclastics would have resulted in the decreased solubility of aluminium, whereas iron would have been relatively soluble in a reducing environment under swamps.

The ratio of iron ore to other sedimentary rock is typically very high in Lower Proterozoic ore-bearing sequences, although the total thicknesses within the Phanerozoic of some shallow water sedimentary sequences which include thin ore beds, are comparable to those of the Lower Proterozoic^{4,17}. The difference in the proportion of iron ore is attributed to a difference in the proportion of non-shelly, calcareous sedimentation within slowly subsiding, shallow, restricted basins like the Sea of Azov. Thick ores are attributed to cyclical transgressions of oolite over exposed areas of pyroclastics and/or deltaic sediments which were largely or entirely eroded by fluvial action and marine transgression. Non-calcareous sedimentation unaccompanied by basin subsidence may have lasted longer than calcareous sedimentation without leaving much record.

Cherty ores display a wide variety of textures and structures. Layers of ferrous septeclorite poor in aluminium (greenalite) in some siliceous oolids from the Lower Proterozoic Gunflint Formation of Ontario, are as fine and regular as aragonitic layers in Recent oolids. Elsewhere, the oolitic texture has been partially destroyed during the diagenetic development of compositionally distinct bands¹⁸. Banding is characteristic of most cherty oolite, even where it is non-ferriferous¹⁹, and must be related to the process of silicification. Laterally similar properties of the primary calcareous sediment may have permitted the planar flow of groundwater from which laterally continuous chert layers precipitated.

I therefore conclude that ferriferous oolite is calcareous oolite that has been covered by deltaic sediment and/or pyroclastics which were exposed to weathering and leached.

Water, rich in solutes, drained laterally through the underlying oolite, replacing aragonitic oolids by chamosite if it was rich in aluminium, or by greenalite and chert if it was poor in aluminium. I predict that oxygen isotopic ratios of chamosite in marine ores will indicate precipitation from fresh water. Extensive ferriferous oolite could readily form within the next 10^9 yr by the diagenetic replacement of calcareous oolite in the north-western portion of the Aral Sea⁹.

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- ¹ Cloud, P. E., *Econ. Geol.*, **68**, 7, 1135 (1973).
- ² Gruner, J. W., *Econ. Geol.*, **17**, 6, 408 (1922).
- ³ James, H. L., *Prof. Pap. US geol. Surv.*, 440W (1966).
- ⁴ Kimberley, M. M., thesis, Princeton Univ. (1974).
- ⁵ Lemoalle, J., and Dupont, B., *Int. Union geol. Sci.*, **A3**, 167 (1973).
- ⁶ Sokolova, E. I., *Physicochemical Investigations of Sedimentary Iron and Manganese Ores and Associated Rocks* (Daniel Davey, New York, 1964).
- ⁷ Turekian, K. K., *Oceans* (Prentice-Hall, New Jersey, 1968).
- ⁸ Beck, K. C., Reuter, J. H., and Perdue, E. M., *Geochim. cosmochim. Acta*, **38**, 341 (1974).
- ⁹ Dickey, P. A., *Bull. Am. Ass. Petrol. Geol.*, **52**, 2396 (1968).
- ¹⁰ Sparling, J. H., *J. Ecol.*, **55**, 1 (1967).
- ¹¹ Huang, W. H., and Keller, W. D., *Nature*, **239**, 149 (1972).
- ¹² Van Houten, F. B., *Bull. geol. Soc. Am.*, **83**, 2761 (1972).
- ¹³ Shearman, D. J., Twyman, J., and Karimi, M. Z., *Proc. geol. Soc.*, **81**, 561 (1970).
- ¹⁴ Wagner, P. A., *Mem. geol. Surv. Un. S. Afr.*, **26** (1928).
- ¹⁵ Latal, E., *XIX int. geol. Congr.*, **2**, 529 (1952).
- ¹⁶ LaBerge, G. L., *Econ. Geol.*, **61**, 572 (1966).
- ¹⁷ Hayes, A. O., *Mem. geol. Surv. Br. Can.*, **78** (1915).
- ¹⁸ Dimroth, E., and Chauvel, J.-J., *Bull. geol. Soc. Am.*, **84**, 111 (1973).
- ¹⁹ Choquette, P. W., *J. Geol.*, **63**, 337 (1955).
- ²⁰ Markevich, V. P., *Int. Geol. Rev.*, **2**, 367 (1960).

In vitro transcription of three adjacent *E. coli* transfer RNA genes

RECENT evidence suggests that the structural genes of some transfer RNA molecules are clustered in groups of two or three on the *Escherichia coli* chromosome¹⁻⁵. The arrangement of tRNA and spacer sequences for two such tRNA clusters have been described⁶⁻⁸. The pattern of transcription of tRNA genes in clusters is not known although the formation of larger precursors in tRNA biosynthesis has been reported^{9,10}. *In vitro* transcription of tRNA₁^{Tyr} gene carried by $\phi 80\text{psu}^+$ phage DNA by purified RNA polymerase was shown to result in the formation of pre-tRNA^{Tyr} molecules bigger in size than mature tRNA^{Tyr}. In the present communication we describe the transcription of a tRNA gene cluster (tRNA₂^{Gly}(su⁺), tRNA₂^{Tyr}, tRNA₃^{Thr}) carried by the DNA of transducing phage λ h80cI857S-t68dglyTsu⁺tyrTthrT (abbreviated λ h80T) recently isolated by Squires *et al.*⁵. *In vitro* transcription of this phage DNA by purified RNA polymerase is shown to produce pre-tRNA molecules of a molecular size consistent with the model of a polycistronic precursor.

The defective transducing phage λ h80T was prepared according to Squires *et al.*⁵ from a double lysogen of strain BF 266. Separation from the helper phage λ h80cI857S-t68 (λ h80H) was carried out by equilibrium density centrifugation.

E. coli ³²P-tRNA was annealed to λ h80T phage DNA in the presence of increasing amounts of unlabelled *E. coli* tRNA, purified tRNA^{Tyr} or a tRNA preparation devoid of tRNA^{Tyr}.

The hybridisation-competition curves presented in Fig. 1 show that unlabelled *E. coli* tRNA competes efficiently with the *E. coli* (^{32}P) tRNA, reducing its hybridisation almost completely. On the other hand, purified tRNA^{Tyr} and a tRNA preparation lacking tRNA^{Tyr} reduce ^{32}P -tRNA hybridisation by only 32 and 65% respectively. These data are in accord with the tRNA gene content described for the λ h80T phage DNA⁵. Due to the presence of three tRNA genes, tRNA^{Tyr} is expected to compete with only one third of ^{32}P -tRNA hybridisation to the phage DNA whereas a tRNA preparation lacking tRNA^{Tyr} should compete only with the hybridisation of the two other RNA species.

Phage λ h80T DNA was transcribed *in vitro* by DNA-dependent RNA polymerase in the presence or absence of ρ termination factor. In order to follow the transcription of the tRNA genes carried by λ h80T DNA we examined the ability of the *in vitro* synthesised RNA to compete with *E. coli* ^{32}P -tRNA for hybridisation sites on the phage DNA. An annealing experiment was carried out using a constant amount of λ h80T DNA and *E. coli* ^{32}P -tRNA in the presence of increasing amounts of *in vitro* synthesised λ h80T RNA. The results presented in Fig. 2a show that the *in vitro* synthesised λ h80T RNA contains polynucleotide chains homologous to those of *E. coli* tRNA. These results also imply that all three tRNA genes carried by λ h80T DNA were transcribed *in vitro* since the synthesised RNA competes efficiently with more than 90% of the hybridisation of *E. coli* ^{32}P -tRNA to λ h80T DNA. The specific production of tRNA₂^{Tyr} sequences was demonstrated using

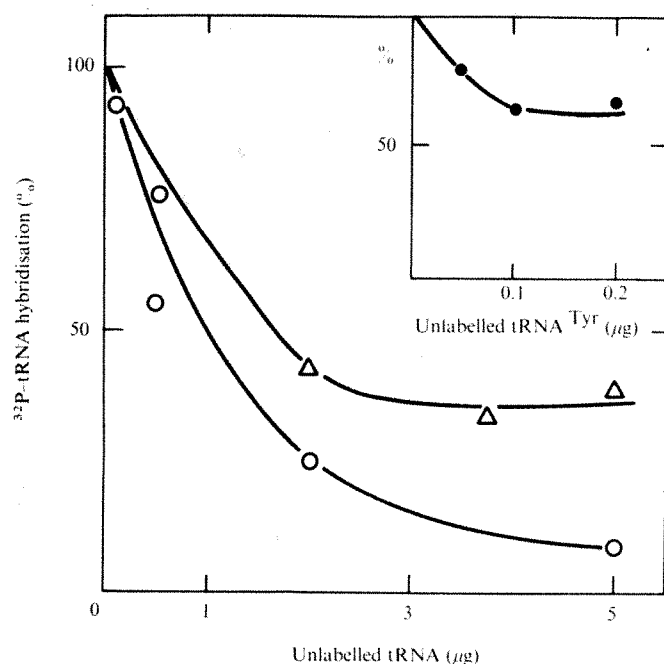


Fig. 1 Competition of *E. coli* ^{32}P -tRNA with unlabelled *E. coli* tRNA, tRNA^{Tyr} and tRNA devoid of tRNA^{Tyr} for hybridisation to λ h80T DNA. *E. coli* tRNA and ^{32}P -tRNA were prepared as described previously¹². tRNA^{Tyr} was prepared by fractionation of N-carbobenzoyloxy-(^{14}C)-tyr tRNA on a methylated albumin-silicic acid column¹³. The tRNA lacking tRNA^{Tyr} (containing only 10% of the original tRNA^{Tyr}) was eluted from such a column at low salt concentrations while the N-carbobenzoyloxy-(^{14}C)-tyr tRNA was eluted with 1 to 2 M NaCl. λ h80T DNA was prepared as previously described¹¹. Nitrocellulose filters containing 3.5 μg of denatured λ h80T DNA were immersed in hybridisation mixtures of 0.3 ml $2\times\text{SSC}$ (0.3 M NaCl, 0.03 M Na citrate) containing 1 μg of *E. coli* ^{32}P -tRNA (1.2×10^6 c.p.m. μg^{-1}) and increasing amounts of unlabelled *E. coli* tRNA (O—O), tRNA devoid of tRNA^{Tyr} (Δ — Δ) and tRNA^{Tyr} (●—●). After 18 h at 67° C the filters were removed, washed with $2\times\text{SSC}$, treated with pancreatic RNase (25 $\mu\text{g ml}^{-1}$ in $2\times\text{SSC}$), washed again and counted. The radioactivity remaining on the filters was expressed as percentage of control which contained no competing RNA.

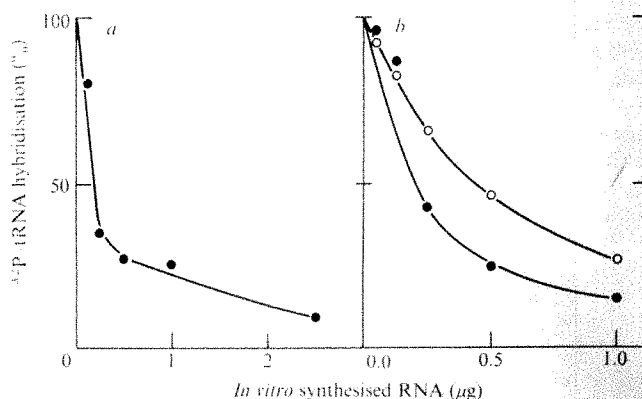


Fig. 2 a, Competition between *in vitro* synthesised λ h80T RNA and *E. coli* ^{32}P -tRNA for hybridisation with λ h80T DNA. λ h80T DNA was transcribed *in vitro* by *E. coli* RNA polymerase in the presence of ρ factor. DNA-dependent RNA polymerase was prepared from *E. coli* MRE-600 cells¹⁴ and further purified by low salt glycerol gradient centrifugation¹⁵. Termination factor ρ was prepared from *E. coli* MRE-600 cells as described by Roberts¹⁶. Transcription was carried out in a reaction mixture containing in 10 ml: 50 μmol Tris HCl (pH 7.9); 10 μmol MgCl₂; 1 μmol dithiothreitol; 75 μmol KCl; 0.4 μmol each of ATP, GTP, UTP and ^3H -CTP (4×10^5 c.p.m. nmol⁻¹); 300 μg of λ h80T DNA; 760 units RNA polymerase (2,300 U mg⁻¹) and 65 μg ρ factor. The synthesis was initiated, after 5 min pre-incubation at 38° C, by the addition of the nucleoside triphosphates. The reaction was stopped and RNA isolated as described elsewhere¹¹. The hybridisation mixture contained, in a volume of 0.5 ml $2\times\text{SSC}$, 10 μg denatured λ h80T DNA, 0.9 μg ^{32}P -tRNA (8.5×10^5 c.p.m. μg^{-1}) and increasing amounts of *in vitro* λ h80T RNA. The mixtures were incubated for 90 min at 70° C, loaded on filters and processed as described in legend to Fig. 1. The radioactivity remaining on the filters was expressed as percentage of control which contained no competing RNA. b, Competition between *in vitro* synthesised λ h80T RNA and *E. coli* ^{32}P -tRNA for hybridisation with $\phi 80\text{psu}_L^+$ L strand DNA. λ h80T DNA was synthesised *in vitro* by transcribing λ h80T DNA with RNA polymerase holoenzyme (O—O) or holoenzyme + ρ factor (●—●). $\phi 80\text{psu}_L^+$ DNA preparation and strand separation were as previously described¹¹. The hybridisation mixture contained in 0.3 ml $2\times\text{SSC}$, 2 μg $\phi 80\text{psu}_L^+$ L strand DNA, 0.9 μg ^{32}P -tRNA and increasing amounts of *in vitro* synthesised λ h80T RNA. The mixtures were incubated for 90 min at 70° C, loaded on filters and processed as described above.

the $\phi 80\text{psu}_L^+$ DNA. This transducing phage DNA carries on the L strand the tRNA₁^{Tyr} gene^{11,17} which differs in sequence from tRNA₂^{Tyr} gene by only two nucleotides¹⁸. The difference in sequence between tRNA₁^{Tyr} and tRNA₂^{Tyr} being very small, we assumed tRNA₂^{Tyr} to hybridise equally well to the tRNA₁^{Tyr} gene carried by $\phi 80\text{psu}_L^+$ DNA. Accordingly, we followed the *in vitro* synthesis of tRNA₂^{Tyr} on λ h80T DNA by its competition with *E. coli* ^{32}P -tRNA hybridisation to the L strand of $\phi 80\text{psu}_L^+$ DNA Fig. 2b. These results establish the presence of tRNA₂^{Tyr} chains among the tRNA-like polynucleotides synthesised *in vitro* on λ h80T DNA.

λ h80T RNA transcribed *in vitro* in the presence or absence of ρ factor was fractionated by centrifugation through a sucrose gradient. Figure 3 shows that the total RNA synthesised *in vitro* has a wide size distribution and that transcription of λ h80T DNA in the presence of ρ results in size reduction of RNA molecules having sedimentation constants of 16S or higher. The presence of tRNA₂^{Tyr}-like molecules along the gradient was determined, as described in Fig. 2b, by competition with *E. coli* ^{32}P -tRNA hybridisation to the L strand of $\phi 80\text{psu}_L^+$ DNA. The histograms presented in Fig. 3 show the distribution of the tyrosine specific tRNA-like chains in the gradient fractions. The tRNA₂^{Tyr}-like chains seem to be located in two main fractions (2 and 3) on the gradient and to possess sedimentation values higher than 4S. Once again it is observed that

the presence of ρ during transcription results in a specific increase in the relative amounts of tRNA^{Tyr}-like material possibly by causing termination outside the tRNA genes. Though the presence of ρ considerably reduced the size of λ h80T RNA it seems to have only a minimal effect on the size of tRNA-like material. The distribution of the total tRNA-like material transcribed on λ h80T DNA (tRNA^{Gly}, tRNA^{Tyr} and tRNA^{Thr}) in the gradient fractions was determined as described in Fig. 2a by competition with the hybridisation of *E. coli* ³²P-tRNA to λ h80T DNA and was found to be similar to that observed for tRNA^{Tyr} chains (data not shown). In order to determine more accurately the size of the *in vitro* transcribed tRNA molecules we have analysed fractions 2 and 3 from the sucrose gradient by polyacrylamide gel electrophoresis in the presence of molecular size markers (Fig. 4). From the distribution of the radioactivity of RNA in the polyacrylamide gel, average molecular weights of 2.3×10^5 and 1.2×10^5 are observed for sucrose gradient fractions 2 and 3 respectively.

Are the three tRNA genes of λ h80T DNA part of a single transcriptional unit? Evidence bearing on this question may be obtained by considering the transcription of the tRNA₂^{Tyr} gene. This gene was shown, by electron microscopy studies by Wu *et al.*⁸, to be located between tRNA₂^{Gly} (su⁺₃₆) and tRNA₃^{Thr} genes at a distance of 140 and 260 nucleotides respectively. If the tRNA₂^{Tyr} gene is transcribed in a continuous sequence together with the adjacent tRNAs and spacer regions, the transcript should possess a much higher molecular weight than that of a mature tRNA. The purified RNA polymerase and factors used in *in vitro* studies is a suitable system for the detection of such primary transcription products since it lacks the precursor tRNA processing and modifying enzymes.

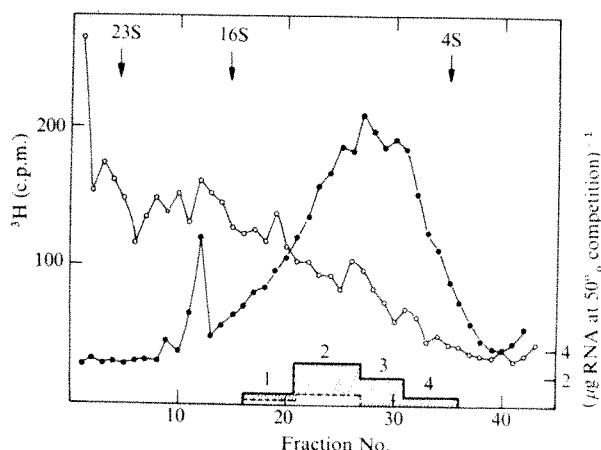


Fig. 3 Sucrose density gradient analysis of *in vitro* synthesised λ h80T RNA. λ h80T DNA (250 μ g) was transcribed *in vitro* by RNA polymerase holoenzyme in the presence (●—●) and absence (○—○) of ρ factor in reaction mixtures as described in legend to Fig. 2a. Each preparation of synthesised RNA was dissolved in 150 μ l of $1 \times$ SSC, layered onto a 5 ml of 5–20% sucrose gradient in $1 \times$ SSC and centrifuged for 4½ h at 50,000 r.p.m. and 4°C in SW 50.1 rotor Spinco model L centrifuge. Fractions of 7 drops were collected, aliquots of 1 μ l were precipitated with trichloroacetic acid, filtered through nitrocellulose filters and the radioactivity retained on the filters determined. In the same run, in a separate sucrose gradient, 23S, 16S, and 4S *E. coli* RNA were used as sedimentation constant markers. The histograms (ordinate at right) represent the tRNA₂^{Tyr} content of pooled fractions of RNA synthesised in the presence (|||||) and in the absence (□□□) of ρ factor. The tRNA₂^{Tyr} content was determined by competition with ³²P-tRNA hybridisation on ϕ 80psu⁺ L strand DNA as described in the legend for Fig. 2b. The relative amounts of tRNA^{Tyr}-like material are presented as the reciprocal of the *in vitro* synthesised RNA quantities which reduce by 50% the ³²P-tRNA hybridisation to ϕ 80psu⁺ L strand DNA.

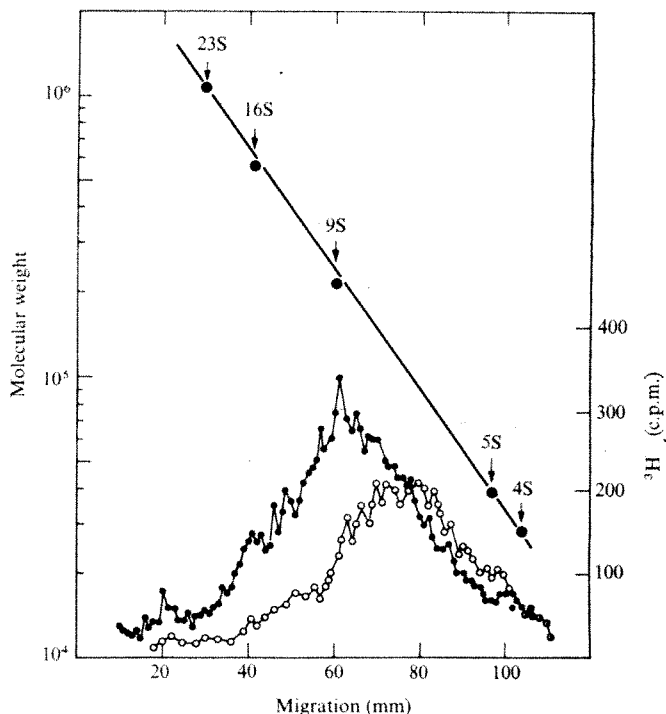


Fig. 4 Evaluation of the size of *in vitro* synthesised tRNA-like material by polyacrylamide gel electrophoresis. Fractions 2 (●—●) and 3 (○—○) of the λ h80T RNA synthesised *in vitro* by RNA polymerase in the presence of ρ factor (Fig. 3) were concentrated to 60 μ l and layered on to a 2% acrylamide, 0.5% agarose composite gel in Tris-EDTA-borate buffer (pH 8.3)¹⁹. 23S and 16S *E. coli* rRNA, 9S rabbit globin mRNA, 5S rRNA and 4S tRNA were used as markers. The electrophoresis was run for 2 h at 0°C and 350 V. At the end of the run the markers were located by staining the gel with 'Stains all' (Eastman Kodak) and the distribution of the ³H-RNA determined by counting 1 mm slices dissolved in 0.3 ml NCS-tissue solubiliser and 3 ml toluene scintillator solution.

The tRNA₂^{Tyr} sequences transcribed *in vitro* were found mainly in RNA molecules with an average molecular weight of 230,000, a value close to that expected for a transcript of three tRNA genes of 80 nucleotides each and spacer regions of 140 and 260 nucleotides. Our findings thus favour the hypothesis of a polycistronic precursor⁵.

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- Russell, R. L., Abelson, J. N., Landy, A., Gefter, M. L., Brenner, S., and Smith, J. D. *J. molec. Biol.*, **47**, 1–13 (1970).
- Brenner, D. J., Fournier, M. J., and Doctor, B. P., *Nature*, **227**, 448–451 (1970).
- Orias, E., Gartner, T. K., Lannan, J. E., and Betlach, M., *J. Bact.*, **109**, 1125–1133 (1972).
- Squires, C., and Carbon, J., *Nature new Biol.*, **233**, 274–277 (1971).
- Squires, C., Konrad, B., Kirschbaum, J., and Carbon, J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 438–441 (1973).
- Miller, R. C., jun., Besmer, P., Khorana, H. G., Fianndt, M., and Szybalski, W., *J. molec. Biol.*, **56**, 363–368 (1971).
- Wu, M., and Davidson, N., *J. molec. Biol.*, **78**, 1–21 (1973).
- Wu, M., Davidson, N., and Carbon, J., *J. molec. Biol.*, **78**, 23–34 (1973).
- Altman, S., and Smith, J. D., *Nature new Biol.*, **233**, 35–39 (1971).

- ¹⁰ Schedl, P., and Primakoff, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2091-2095 (1973).
¹¹ Daniel, V., Sarid, S., Beckmann, J. S., and Littauer, U. Z., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 1260-1266 (1970).
¹² Daniel, V., Lavi, S., and Littauer, U. Z., *Biochim. biophys. Acta*, **182**, 76-84 (1969).
¹³ Stern, R., and Littauer, U. Z., *Methods in Enzymology*, **20**, 83-98 (1971).
¹⁴ Chamberlin, M., and Berg, P., *Proc. natn. Acad. Sci. U.S.A.*, **48**, 81-94 (1962).
¹⁵ Burgess, R. R., *J. biol. Chem.*, **244**, 6160-6167 (1969).
¹⁶ Roberts, J. W., *Nature*, **224**, 1168-1174 (1969).
¹⁷ Andoh, T., and Ozeki, H., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 792-799 (1968).
¹⁸ Goodman, H. M., Abelson, J. N., Landy, A., Brenner, S., and Smith, J. D., *Nature*, **217**, 1019-1024 (1968).
¹⁹ Peacock, A. C., and Dingman, C. W., *Biochemistry*, **7**, 668-674 (1968).

Uptake of *Escherichia coli* DNA into HeLa cells enhanced by amphotericin B

MAMMALIAN cells in culture take up small amounts of DNA, presumably by pinocytosis^{1,2}. Unlike bacterial cells the mammalian cells do not seem to require a special state of competence for DNA uptake³; and since naked DNA of oncogenic viruses can successfully infect or transform cells⁴, at least some of the DNA taken up by the cells can be functional. In spite of these successful experiments, however, almost all attempts to achieve controlled DNA transformation in mammalian cells have failed. The failure has been attributed to two factors: (1) insufficient transport of added DNA by the pinocytic mechanism¹; and (2) degradation of the foreign DNA in lysosomes⁵.

Modifications of the input DNA by % irradiation or shear⁶⁻¹³ or partial degradation by nucleases¹⁴ have generally only reduced uptake. A few complex polycations, including DEAE-dextran¹⁵, protamine sulphate¹⁶, polylysine and polyornithine¹⁷, do enhance transport into cultured human cells. The enhancement is however at the most two to three-fold. Also the treatments and effects of these agents are difficult to reverse or control, and some of the agents form insoluble complexes with DNA^{18,19} that may not be easily assimilated by cells.

Encouraged by its capacity to promote uptake of a variety of antibiotics into fungal²⁰ and animal²¹ cells, we have tested the polyene macrolide antibiotic amphotericin B (AmB) as a potential agent to enhance uptake of DNA into HeLa cells. Here we report that while the levels of AmB required are high, the transport of DNA is increased 8 to 10-fold under optimal conditions without affecting subsequent cell viability or growth. The process is reversible and can be controlled, because the increased transport stops when the cells are washed free of AmB. Furthermore, the DNA taken up in the presence of AmB is less altered than that transported in its absence.

Figure 1 shows the kinetics of transport of *E. coli* DNA into HeLa cells in culture in the presence of different concentrations of AmB. Acid-precipitable radioactivity was measured in cells after they were washed with medium containing sodium iodoacetate to remove any nonspecifically bound DNA²². Samples incubated without AmB showed the low level of transport already reported²³, whereas samples that contained more than 20 $\mu\text{g ml}^{-1}$ of AmB showed a considerable enhancement in the transport of DNA. The transport was linear for 30 min, and reached a plateau value at 30 to 60 min. In some experiments, further incubation caused a loss of 10%-30% of the net internalised DNA, an observation similar to some earlier reports^{23,24}. After 3 h of incubation at the concentrations of AmB shown, 85-90% of the cells were still viable, as judged by trypan blue dye exclusion. Washed free of DNA and AmB, the

cells uniformly resumed growth and 24 h later had grown as well as untreated cells.

The interdependence of cell number, DNA concentration, and AmB concentration in determining the level of DNA uptake observed is comparable with that observed in the potentiation of antibiotic uptake into fungal cells by AmB (E. Battaner and B. V. K., unpublished work). Figure 2 shows this interdependence, where the ratio of drug to cell number is shown to be particularly important. This

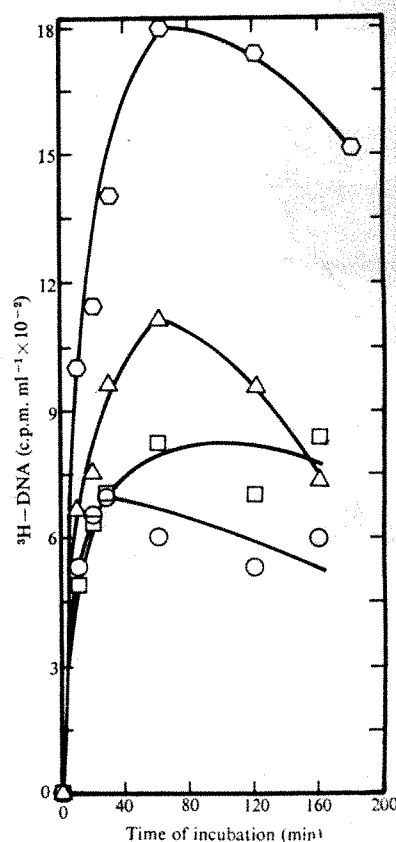


Fig. 1 Kinetics of transport of DNA by HeLa cells. Cells grown in spinner culture in Joklick modified Eagle's medium supplemented with 5% horse serum were centrifuged and suspended in the same medium, but with 10% calf serum. The dispersed cells were counted in a haemocytometer, and then dispensed in 4.5 ml portions into flasks at 37° C with different concentrations of amphotericin B and 77 μg of *E. coli* ³H-DNA. The *E. coli* DNA was prepared by labelling a culture of strain 15T⁻, a thymine auxotroph with ³H-thymine for several generations. DNA was extracted according to ref. 28. It had a specific activity of 12,000 c.p.m. μg^{-1} and showed a characteristic narrow sedimentation distribution in a sucrose gradient at 20° C. At specified time intervals portions of cell suspension were centrifuged and washed twice in incubation medium containing 0.025 M sodium iodoacetate in the cold at pH 7.3 (ref. 22). Each equivalent of 0.5 ml of cell suspension was precipitated with 5% CCl_3COOH . The precipitate was collected on a glass fibre filter and counted in a toluene-based scintillant in a Packard Spectrometer. Acid precipitable radioactivity was plotted per ml of cell suspension. Amphotericin B concentrations used were 200 (○), 100 (○) and 20 (□) μg per ml of cell suspension.

is probably because of the large number of sterol binding sites for AmB per cell²⁵. For example, in the conditions of Fig. 1 with 2.3×10^5 cells ml^{-1} , there was 3.3 times more DNA uptake in the presence of 200 $\mu\text{g ml}^{-1}$ AmB. In optimal conditions with 4.5×10^5 cells ml^{-1} as in Fig. 2, there was 8 to 10 times more DNA uptake.

Transport was inhibited 85-90% by 10 mM sodium cyanide (Fig. 2) which is consistent with the idea that

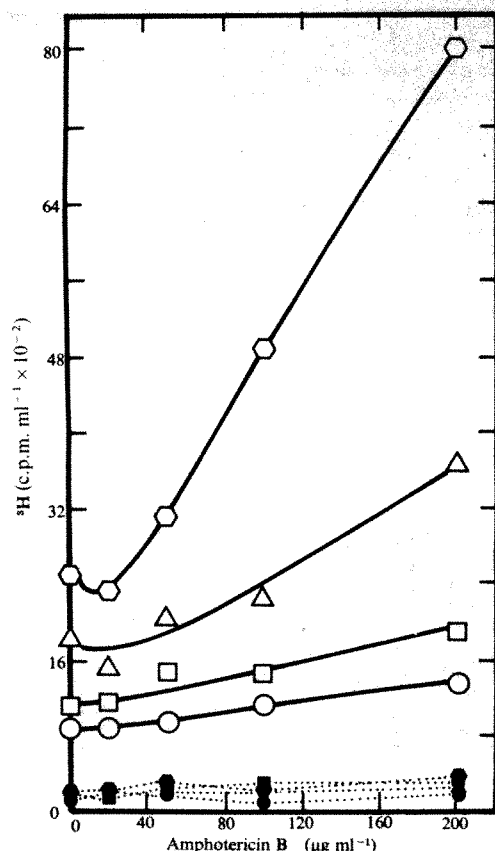


Fig. 2 Transport of DNA by HeLa cells. HeLa cells (4.5×10^5 cell ml^{-1}) were resuspended as in Fig. 1 and incubated in 0.5 ml portions at 37°C with specified concentrations of amphotericin B and *E. coli* ^3H -DNA. After 30 min, cells were washed and acid-precipitable radioactivity was measured as in Fig. 1. The response to AmB and DNA concentration is indicated. 4.5×10^5 cells ml^{-1} incubated with 2 (○), 4 (□), 8 (△) and 20 (○) μg of ^3H -DNA for 30 min at 37°C at the indicated concentrations of amphotericin B. A second set of samples incubated under identical conditions contained 10 mM sodium cyanide with 2 (●), 4 (■), 8 (▲) and 20 (●) μg of ^3H -DNA ml^{-1} .

uptake might depend on an active process such as pinocytosis²⁶. Also, uptake was temperature dependent: at 0°C , it was completely inhibited in the absence of AmB, and was inhibited 50–60% in its presence.

The effect of AmB on DNA uptake seems to require its simultaneous presence in the medium. For example, when cells were pretreated with AmB (Fig. 3) and washed free of AmB before incubation with DNA, they took up DNA only to the same extent as cells that had not been treated with AmB. This suggests that the binding of the drug to the cell membrane is reversible or temporary in its effect, and confirms that the short time of exposure of the cells to high concentrations of AmB is nontoxic and reversible.

When HeLa cells were incubated with different concentrations of AmB and *E. coli* DNA for 30 min at 37°C , then washed free of AmB and DNA and incubated further in fresh medium, it was found that the acid-precipitable radioactivity in the cells declined steadily. After 8 h only about two thirds of the DNA taken up in untreated cells, and one-third of that transported in presence of AmB was still recoverable from cells. But the DNA extracted from the AmB treated cultures at any one time was relatively more intact, as judged by the sedimentation behaviour of the two DNA samples on an alkaline sucrose density gradient (data not shown).

The possibility that bacterial DNA might be preserved intact longer in AmB treated cells was further supported by DNA-DNA hybridisation trials (Fig. 4). Labelled DNA re-extracted from cells treated with $200 \mu\text{g}$ of AmB ml^{-1} for 30 min reannealed to an extent indistinguishable from the input bacterial DNA; at this time, 70% of the label in cell incubated without AmB hybridised much more slowly. Very probably, it had already been degraded and the products reincorporated at random into HeLa cell sequences.

The apparently prolonged intact survival of DNA sequences taken up in the presence of AmB could increase the probability of it functioning in the recipient cell. The binding of AmB to membrane sterols may increase endocytosis in a way that avoids a direct route to lysosomal vacuoles; or alternatively, the number of new phagosomes simply saturate the lysosomal complement of the cell for some time. At any rate, the advantage of increased survival of intact DNA, combined with the increased levels of uptake and the easy reversibility of the AmB effect, makes AmB a promising agent for permeability control.

Though it is true that our arguments for the incorporation of the DNA into the cells are indirect, two kinds of data support the inference that AmB truly promotes uptake rather than a loose ionic association of DNA with the cell membrane. First, these concentrations of AmB potentiate uptake of a variety of antibiotics^{20,21,27} and of macromolecules that include RNase, DNase and even latex beads $1.1 \mu\text{m}$ in diameter (to be published). Second, the results satisfy the criteria for uptake in previous studies with DNA, including the requirement for energy and the resistance to extensive washing with iodoacetate. We are now testing

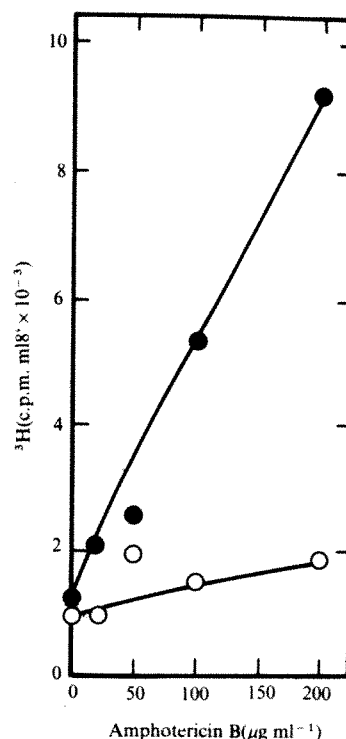


Fig. 3 Effect of pretreatment of cells with amphotericin B on the uptake of DNA. HeLa cells (5.0×10^5 cells ml^{-1}) were washed as in Fig. 1 with medium containing sodium iodoacetate and incubated at 37°C for 30 min in 0.5 ml fractions with specified concentrations of amphotericin B. After incubation, cells were washed free of amphotericin B and resuspended in the same volume with $4.4 \mu\text{g}$ of ^3H -DNA (specific activity $24,500$ c.p.m. μg^{-1}). Incubation was then resumed in the presence (●) or absence (○) of amphotericin B at the indicated levels. Cells were then washed and assayed for incorporated DNA as in Fig. 1.

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- De BOER, J., and MANG, H. J. (edited by). *Proceedings of the International Conference on Nuclear Physics*, Munich, August 27-September 1, 1973. Vol. 1: Contributed Papers. Pp.lxx+739. Vol. 2: Invited Papers. Pp.xii+837. ISBN-0-7204-0257-3. (Amsterdam and London: North-Holland Publishing Company; New York: American Elsevier Publishing Company, Inc., 1973.) n.p.

EHRENREICH, Henry, SEITZ, Frederick, and TURNBULL, David (edited by). *Solid State Physics: Advances in Research and Applications*, Vol. 28. Pp.xiv+430. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £27.50.

FEDOSEEV, V. A. (edited by). *Advances in Aerosol Physics*, No. 4. Pp.vi+148. ISBN-0-7065-1272-3. (New York and Toronto: John Wiley and Sons; Jerusalem: Israel Program for Scientific Translations, 1973.) n.p.

HASS, Georg, FRANCOMBE, Maurice H., and HOFFMAN, Richard W. (edited by). *Physics of Thin Films: Advances in Research and Development*, Vol. 7. Pp.xiv+421. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £31; £14.90.

HOUSTON, W. V., and PHILLIPS, G. C. *Principles of Quantum Mechanics: The Non-Relativistic Theory with some Illustrative Applications*. Pp.xi+376. ISBN-0-7204-0215-8. (Amsterdam: North-Holland Publishing Company, 1973.) Dfl.110; \$44.

ISRAEL, H., in collaboration with DOLEZALEK, H. *Atmospheric Electricity*. Vol. 2: Fields, Charges, Currents. Authorized and revised translation from the second German edition. (Problems of Cosmic Physics, Vol. 29.) Pp.318-796. (Jerusalem: Israel Program for Scientific Translations; Springfield, Virginia: US Department of Commerce, National Technical Information Service, 5285 Port Royal Road, 1974.) \$6.

KITAIGORODSKII, S. A. *The Physics of Air-Sea Interaction*. Translated from Russian. Pp.v+237. ISBN-0-7065-1281-2. (Jerusalem: Israel Program for Scientific Translations, 1973.) n.p.

KOVACS, I., and ZSOLDOS, L. *Dislocations and Plastic Deformation*. (International Series of Monographs in Natural Philosophy, Vol. 60.) Pp.xii+343. ISBN-0-08-017062-5. (Oxford and New York: Pergamon Press, 1973.) £3.50.

MARTON, L. (edited by). *Advances in Electronics and Electron Physics*, Vol. 34. Pp.x+362. (New York and London: Academic Press; a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £29.50.

MASSEY, H. S. W., and GILBODY, H. B. *Recombination and Fast Collisions of Heavy Particles*. (Electronic and Ionic Impact Phenomena, Vol. 4.) Second edition. Pp.xxv+2115-3122+38. ISBN-0-19-851253-8. (Oxford: Clarendon Press; London: Oxford University Press, 1974.) £29 net.

MULLER, E. W., and TSONG, T. T. *Field Ion Microscopy, Field Ionization and Field Evaporation*. (Progress in Surface Science, Vol. 4, Part 1.) Pp.viii+140. ISBN-0-08-017779-4. (Oxford and New York: Pergamon Press, 1973.) n.p.

REIMANN, Arnold L. *Physics*. Vol. 3: Modern Physics. Pp.xiv+459+32. ISBN-06-045363-X. (New York: Harper and Row, Inc., 1973.) £3.80.

SCHWINGER, Julian. *Particles, Sources, and Fields*, Vol. 2. Pp.459. ISBN-0-201-06783-8. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1973.) n.p.

SHOTTON, E., and RIDGWAY, K. *Physical Pharmaceutics*. Pp.xiii+409. ISBN-0-19-859708-8. (Oxford: Clarendon Press; London: Oxford University Press, 1974.) £15 boards; £5.75 paper.

STACEY, Jr., Weston M. *Variational Methods in Nuclear Reactor Physics*. (Nuclear Science and Technology: a Series of Monographs and Textbooks.) Pp.viii+181. ISBN-0-12-662060-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) £18.50; £9.

SUBE, Ralf und EISENREICH, Gunther. *Physik: Englisch, Deutsch, Französisch, Russisch*. A-M. Pp.1-1026. N-Z. Pp.1027-2012. Register. Pp.2013-2896. (Technik-Wörterbuch.) (Berlin: Veb Verlag Technik, 1973.) 290M.

V. DEINSE, P. A. *Vraagteken bij de Relativiteit van Einstein*. Pp.45. (Utrecht: Uitgegeven door Techniek en Spel, 1974.) n.p.

VAN DEN HANDEL, J. (edited by). *Selected Papers in Physics*. (In Honour of C. J. Gorter.) Pp.xi+359. (Reprinted from *Physica*, Vol. 69, No. 1.) ISBN-0-7204-0289. (Amsterdam: North-Holland Publishing Company, 1973.) Dfl.60; \$21.80.

WALLIS, R. F. *Lattice Dynamics of Crystal Surfaces*. (Progress in Surface Science, Vol. 4, Part 3.) Pp.viii+233-367. ISBN-0-08-017798-0. (Oxford and New York: Pergamon Press, 1973.) £3.50.

WEST, R. N. *Positron Studies of Condensed Matter*. (Taylor and Francis Monographs on Physics.) Pp.123. ISBN-0-85066-070-X. (London: Taylor and Francis, Ltd., 1974.) £3.

Chemistry

BARNES, Dorothy S. *Environmental Chemistry in the Laboratory*. Pp.vii+166. ISBN-0-06-380438-7. (New York and London: Canfield Press, a Department of Harper and Row, Inc., 1973.) £3.25.

BRECK, Donald W. *Zeolite Molecular Sieves: Structure, Chemistry and Use*. Pp.ix+771. ISBN-0-471-09985-6. (New York and London: Wiley-Interscience, John Wiley and Sons, 1974.) £18.

BRIMACOMBE, J. S. (senior reporter). *Carbohydrate Chemistry*, Vol. 6. (A Specialist Periodical Report. A Review of the Literature Published during 1972.) Pp.xii+620. ISBN-0-85186-052-4. (London: The Chemical Society, 1973.) £8.

CHAMBERS, R. D. *Fluorine in Organic Chemistry*. (Interscience Monographs on Organic Chemistry.) Pp.xv+391. ISBN-0-471-14330-8. (New York and London: Wiley-Interscience, John Wiley and Sons, 1973.) £10.75.

CHRISTIE, William W. *Lipid Analysis: Isolation, Separation, Identification and Structural Analysis of Lipids*. Pp.xiv+338. ISBN-0-08-017753-0. (Oxford and New York: Pergamon Press, 1973.) £6.

COFFEY, S. (edited by). *Rodd's Chemistry of Carbon Compounds: a Modern Comprehensive Treatise*. Vol. 3, Part B: Aromatic Compounds. Second edition. Pp.xix+519. ISBN-0-444-40971-8. (Amsterdam, London and New York: Elsevier Scientific Publishing Company, 1974.) Dfl.160; \$58.20.

CROMPTON, T. R. *Chemical Analysis of Organometallic Compounds*. Vol. 1: Elements of Groups I-III. (The Analysis of Organic Materials.) Pp.x+258. ISBN-0-12-197301-8. (London and New York: Academic Press, 1973.) £5.80; \$16.25.

DAUREN, William G. (editor-in-chief). *Organic Reactions*, Vol. 20. Pp.ix+494. ISBN-0-471-19621-5. (New York and London: John Wiley and Sons, 1973.) £12.50.

DOLLISH, Francis R., FATELEY, William G., and BENTLEY, Freeman F. *Characteristic Raman Frequencies of Organic Compounds*. Pp.xviii+443. ISBN-0-471-21769-7. (New York and London: Wiley-Interscience, John Wiley and Sons, 1974.) £12.60.

DREW, Thomas B., COKELET, Giles R., HOOPES, Jr., John W., and VERMEULEN, Theodore (edited by). *Advances in Chemical Engineering*, Vol. 9. Pp.xiii+312. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) £29.50; £13.85.

FACKLER, Jr., John P. (edited by). *Symmetry in Chemical Theory: Application of Group Theoretical Techniques to the Solution of Chemical Problems*. (Benchmark Papers in Inorganic Chemistry.) Pp.xv+508. ISBN-0-87933-018-X. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc.; Chichester: John Wiley and Sons, Ltd., 1973.) £14.

FLUENDY, M. A., and LAWLEY, K. P. *Chemical Applications of Molecular Beam Scattering*. (Studies in Chemical Physics.) Pp.xi+400. ISBN-0-412-11810-6. (London: Chapman and Hall, 1973. Distributed in the USA by Halsted Press, a Division of John Wiley and Son, Inc., New York.) £8 net.

FREEMAN, Stanley K. Applications of Laser Raman Spectroscopy. Pp. xi+336. ISBN-0-471-17788-6. (New York and London: Wiley-Interscience, John Wiley and Sons, 1974.) £9.75.

FRIEDMAN, Mendel. The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins. Pp.viii+485. ISBN-0-08-016845-0. (Oxford and New York: Pergamon Press, 1973.) £7.50.

GOODISMAN, Jerry. Diatomic Interaction Potential Theory. Vol. 2: Applications. (Physical Chemistry: A Series of Monographs, Vol. 31-2.) Pp. xiii+410. ISBN-0-12-290202-5. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £34; £16.30.

HAMMES, Gordon G. (edited by). Investigation of Rates and Mechanisms of Reactions. Part 2: Investigation of Elementary Reaction Steps in Solution and Very Fast Reactions. (Techniques of Chemistry, Volume 6.) Third edition. Pp.xiii+665. ISBN-0-471-93127-6. (New York and London: Wiley-Interscience, John Wiley and Sons, 1974.) £15.50.

JANZ, G. J., and TOMKINS, R. P. T. Nonaqueous Electrolytes Handbook, Vol. 2. Pp.xiii+933. ISBN-0-12-380402-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$60; £28.20.

KATRITZKY, A. R., and BOULTON, A. J. (edited by). Advances in Heterocyclic Chemistry, Vol. 16. Pp.ix+349. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) \$35; £16.80.

LAWLER, R. G. Chemically Induced Dynamic Nuclear Polarization. (Progress in Nuclear Magnetic Resonance Spectroscopy, Vol. 9, Part 3.) Pp. 145-210. (Oxford and New York: Pergamon Press, 1973.) £2.25.

LIM, Edward C. (edited by). Excited States, Vol. 1. Pp.xii+347. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) \$24.50; £11.75.

MUROV, Steven L. Handbook of Photochemistry. Pp.xi+272. ISBN-0-8247-6164-2. (New York: Marcel Dekker, Inc., 1973.) \$27.

MUSTAFA, Ahmed. Benzofurans. (The Chemistry of Heterocyclic Compounds: A Series of Monographs.) Pp.xvi+514. (New York and London: Interscience, John Wiley and Sons, 1974.) £25.

MUZZARELLI, R. A. A. Natural Chelating Polymers: Alginic Acid, Chitin and Chitosan. (International Series of Monographs in Analytical Chemistry, Vol. 55.) Pp.xii+254. ISBN-0-08-017235-0. (Oxford and New York: Pergamon Press, 1973.) £4.95.

NARGOLWALLA, Sam S., and PRZYBYLOWICZ, Edwin P. Activation Analysis with Neutron Generators. (Chemical Analysis: A Series of Monographs on Analytical Chemistry and Its Applications.) Pp.xvii+662. ISBN-0-471-63031-4. (New York and London: Wiley-Interscience, John Wiley and Sons, 1973.) £16.25.

NONHEBEL, D. C., and WALTON, J. C. Free-radical Chemistry: Structure and Mechanism. Pp.xv+572. ISBN-0-521-20149-7. (London: Cambridge University Press, 1974.) £15 net; \$35.

REUBEN, B.G., and BURSTALL, M. L. The Chemical Economy: a Guide to the Technology and Economics of the Chemical Industry. Pp. xix+530. ISBN-0-582-46307-6. (London: Longman Group Limited, 1973.) £6.95 net.

TAYLOR, William I., and FARNSWORTH, Norman (edited by). The Vinca Alkaloids: Botany, Chemistry, and Pharmacology. Pp.xx+357. ISBN-0-8247-6129-4. (New York: Marcel Dekker, Inc., 1973.) \$32.75.

TIKHONOV, V. N. Analytical Chemistry of Aluminium. Translated by J. Schmorak. (Analytical Chemistry of the Elements.) Pp.x+303. (New York, Toronto and Chichester: Halsted Press, John Wiley and Sons; Jerusalem: Israel Program for Scientific Translations, 1973.) £9.55.

WAUGH, John S. (edited by). Advances in Magnetic Resonance, Vol. 6. Pp.xii+310. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$27.50; £13.20.

WESTBERG, Karl. CHENG, Robert T. RIPPERTON, A., et al. The Chemistry of Air Pollution. Pp.200. ISBN-0-8422-7152-X. (New York: MSS Information Corporation, 1973.) \$15.

Technology

ALLAN, W. B. Fibre Optics: Theory and Practice. (Optical Physics and Engineering.) Pp.vi+247. ISBN-0-306-30735-9. (London and New York: Plenum Press, 1973.) \$26.

BARGELLINI, P. L. (edited by). Communications Satellite Systems. (Progress in Astronautics and Aeronautics, Vol. 32.) Pp.xv+480. ISBN-0-262-02100-5. (Cambridge, Mass. and London: The MIT Press, 1974.) \$20.

BARGELLINI, P. L. (edited by). Communications Satellite Technology. (Progress in Astronautics and Aeronautics, Vol. 33.) Pp. xv+411. ISBN-0-262-02101-3. (Cambridge, Mass. and London: The MIT Press, 1974.) \$20.

DUMMER, G. W. A., and WINTON, R. C. An Elementary Guide to Reliability. Second edition. Pp.vii+57. ISBN-0-08-017821-9. (Oxford and New York: Pergamon Press, 1974.) £1.

HARRIS, John (edited by). The Karl Weissenberg 80th Birthday Celebration Essays. Pp. xv+171. (Kampala, Nairobi and Dar-es-Salaam: East African Literature Bureau, 1973.) n.p.

JARZESKI, Z. M. Oxide Semiconductors. Translated from the Polish by B. Grzybowski-Swierkosz. Translation edited by Brian Randall Pamplin. (International Series of Monographs in the Science of the Solid State, Volume 4.) Pp.xi+285. ISBN-0-08-016968-6. (Oxford and New York: Pergamon Press; Warsaw: Wydawnictwa Naukowo-Techniczne, 1973.) £6.

METCALFE, Arthur G. (edited by). Interfaces in Metal Matrix Composites. (Composite Materials, Vol. 1.) Pp.xiv+421. ISBN-0-12-136501-8. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) \$29; £13.90.

PYKE, Magnus. Catering Science and Technology. Pp.xi+237. ISBN-0-7195-2898-4. (London: John Murray (Publishers), Ltd., 1974.) £3.50 net.

REID, C. N. Deformation Geometry for Materials Scientists. (International Series on Materials Science and Technology, Vol. 11.) Pp.viii+211. (Oxford and New York: Pergamon Press, 1973.) £3.80 hard cover; £2.50 flexi-cover.

REYNOLDS, A. J. Turbulent Flows in Engineering. Pp.xvii+462. ISBN-0-471-71782-7. (London and New York: Wiley-Interscience, John Wiley and Sons, 1974.) £10.

SCANNING ELECTRON MICROSCOPY: Systems and Applications 1973. (Proceedings of a conference organized by the Electron Microscopy and Analysis Group of the Institute of Physics, University of Newcastle upon Tyne, 3-5 July 1973.) Pp.x+339. (Conference Series No. 18.) ISBN-0-85498-108-X. (London and Bristol: The Institute of Physics, 1973.) £15; \$37.50.

SMITH, T. C., and PAO, Y. C. Introduction to Digital Computer Plotting. Pp.86. (New York, London and Paris: Gordon and Breach, Science Publishers, 1973.) £2.50 paper.

SWANN, P. R., HUMPHREYS, C. J., and GORINGE, M. J. (edited by). High Voltage Electron Microscopy. (Proceedings of the Third International Conference.) Pp.xi+475. ISBN-0-12-678750-6. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) £7.

ULTRASONICS INTERNATIONAL 1973: Conference Proceedings, 27-29 March 1973 Imperial College, London. (Sponsored by the journal *Ultrasonics*) Pp.344. ISBN-0-902852-26-4. (Guildford, Surrey: IPC Science and Technology Press, Ltd., 1973.) £11.

WOOD, W. G., and MARTIN, D. G. Experimental Method: a Guide to the Art of Experiment for Students of Science and Engineering. Pp.106. (London: The Athlone Press, University of London, 1974. Distributed by Tiptree Book Services, Ltd., Tiptree, Essex.) £1.50.

Earth sciences

BARRETT, Eric C., and CURTIS, Leonard F. (edited by). Environmental Remote Sensing: Applications and Achievements. (Papers presented at the Bristol Symposium on Remote Sensing, Department of Geography, University of Bristol, 2 October 1972.) Pp.vi+309. ISBN-0-7131-5696-1. (London: Edward Arnold (Publishers), Ltd., 1974.) £8.50 net.

BRYSON, Reid A., and HARE, F. Kenneth (edited by). Climates of North America. (World Survey of Climatology, Vol. 2.) Pp.x+420. ISBN-0-444-01062-7. (Amsterdam, London and New York: Elsevier Scientific Publishing Company, 1974.) n.p.

CASEY, R., and RAWSON, P. F. (edited by) The Boreal Lower Cretaceous. (The Proceedings of an International Symposium organized by Queen Mary College (University of London) and the Institute of Geological Sciences, 17-30 September 1972.) (Geological Journal Special Issues, No. 5.) Pp.448. (Liverpool: Seel House Press, 1973.) £9.75.

FEIGELSON, E. M. Radiant Heat Transfer in a Cloudy Atmosphere. Translated from Russian. ISBN-0-7065-1276-6. (Jerusalem: Israel Program for Scientific Translations; Chichester: John Wiley and Sons, Ltd., 1973.) £8.60.

FRYE, Keith. Modern Mineralogy. Pp.ix+325. ISBN-0-13-595686-2. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1974.) n.p.

GOLDBERG, Edward D. (edited by). North Sea Science. Pp.xviii+500. ISBN-0-262-07056-1. (Cambridge, Mass. and London: The MIT Press, 1973.) \$18.95; £8.50.

HOOD, D. W., SHIELDS, W. E., and KELLEY, E. J. (edited by). Environmental Studies of Port Valdez. Pp.493. (Occasional Publication No. 3.) Data Volume I: Environmental Studies of Port Valdez. Pp.800. (Occasional Publication No. 3A.) (Fairbanks, Alaska: Institute of Marine Science, University of Alaska, 1973.) n.p.

MATTHEWS, Robley K. Dynamic Stratigraphy: an Introduction to Sedimentation and Stratigraphy. Pp.xii+370. ISBN-0-13-22273-6. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1974.) n.p.

MILLIMAN, J. D. Marine Carbonates. (Recent Sedimentary Carbonates, Part 1.) Pp.xv+375 (39 plates). ISBN-3-540-06116-9. (Berlin and New York: Springer-Verlag, 1974.) DM66; \$27.10.

RODRIGO, L. A., y COUMES, F. Manual de Sedimentología: Técnicas de Laboratorio. Pp.150. (La Paz Bolivia: Universidad Mayor de San Andres, 1973.) US \$5.

STACEY, Frank D., and BANERJEE, Subir K. The Physical Principles of Rock Magnetism. (Developments in Solid Earth Geophysics, Vol. 5.) Pp.ix+195. ISBN-0-444-01084-8. (Amsterdam, London and New York: Elsevier Scientific Publishing Company, 1974.) Dfl.60; \$21.19.

URANIUM EXPLORATION METHODS. (Proceedings of a Panel on Uranium Exploration Methods held in Vienna, 10-14 April, 1972. Panel Proceedings Series.) Pp.320. (Vienna: International Atomic Energy Agency; London: HMSO, 1973.) 270 schillings; £6.10; \$15.

WALLACE, William J. The Development of the Chlorinity/Salinity Concept in Oceanography. (Elsevier Oceanography Series, Vol. 7.) Pp.xii+227. ISBN-0-444-01118-6. (Amsterdam, London and New York: Elsevier Scientific Publishing Company, 1974.) Dfl.60; \$17.70.

WEDFPOHL, K. H. (executive editor). CORRENS, C. W., SHAW, D. M., TUREKIAN, K. K., and ZEMANN, J. (editorial board). Handbook of Geochemistry Vol. II/3. Pp.iv+845. ISBN-3-540-05125-2. (Berlin and New York: Springer-Verlag, 1972.) Loose-leaf-binder. DM258; \$99.40.

Biological sciences

ABOU-SABE, Morad (edited by). Microbial Genetics. (Benchmark Papers in Microbiology. Pp.xv+451. ISBN-0-87933-046-5. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., 1973.) n.p.

AHMADJIAN, Vernon, and HALE, Mason E. (edited by). The Lichens. Pp.xiv+697. ISBN-0-12-044950-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$35; £16.80.

ARAPAYANTS, E. Sh., and KONSTANTINOV, A. I. Echolocation in Animals. Translated from Russian. (Academy of Sciences of the USSR, Joint Scientific Council on Physiology of Man and Animals.) Pp.vi+309. ISBN-0-7065-1283-9. (Jerusalem: Israel Program of Scientific Translation; Chichester: John Wiley and Sons, Ltd., 1973.) £10.50.

ALTSCHUL, Siri von Reis. Drugs and Foods from Little-Known Plants. Notes in Harvard University Herbaria. Pp.xii+366. ISBN-674-21676-8. (Cambridge, Mass.: Harvard University Press, 1973.) \$10.

REETSCHEN, Jean-Claude. L'Embryologie. ("Que Sais-je?" No. 68.) Pp. 126. (Paris: Presses Universitaires de France, 1974.) n.p.

BOUGHEY, Arthur S. Readings in Man, The Environment, and Human Ecology. Pp.x+595. (New York: Macmillan Publishing Company, Inc.; London: Collier Macmillan Publishers, 1973.) £3.50.

BULLA, Jr., LEE A. (edited by). Regulation of Insect Populations by Microorganisms. (Annals of the New York Academy of Sciences, Vol. 217.) Pp.243. (New York: New York Academy of Sciences, 1973.) \$26.

CAYLEY, Neville W. Australian Parrots in Field and Aviary. Extensively revised and rewritten by Alan H. London. Pp.xxx+342+13 plates. ISBN-0-207-12424-8. (Sydney and London: Angus and Robertson, 1973.) £12.50.

COULTER, John Merle. Evolution of Sex in Plants. (Facsimile of the 1914 edition.) Pp.vii+140. ISBN-02-843230-4. (New York: Hafner Press, a Division of Macmillan Publishing Co., Inc.; London: Collier Macmillan, 1973.) £3.50.

DAVIS, Bernard D., DULBECCO, Renato, EISEN, Herman N., GINSBERG, Harold S., WOOD, Jr., W. BARRY, and McCARTY, Maelyn. Microbiology, including Immunology and Molecular Genetics. Second edition. Pp.xv+1562. ISBN-06-140683-X. (New York and London: Harper and Row, 1973.) £15.15.

DeGRUY, Inez V., CARRA, Jarrell, and GOYNES, Wilton R. The Fine Structure of Cotton: An Atlas of Cotton Microscopy. Edited and with an introduction by Robert T. O'Connor. (Fiber Science Series.) Pp.xi+222 (96 plates). ISBN-0-8247-6109-X. (New York: Marcel Dekker, Inc., 1973.) \$43.

ELLIOTT, Alfred M. (edited by). Biology of Tetrahymena. Pp.x+508. ISBN-0-87933-013-9. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc.; Chichester: John Wiley and Sons, Ltd., 1973.) £19.25.

GRAY, Peter (edited by). The Encyclopedia of Microscopy and Microtechnique. Pp.xi+638. ISBN-0-442-22812-0. (New York and London: Van Nostrand Reinhold Company, 1973.) £16.25.

GREENWOOD, P. H., MILES, R. S., and PATTERSON, Colin (edited by). Interrelationships of Fishes. (Supplement No. 1 to the *Zoological Journal of the Linnean Society*.) Pp.xiv+536. ISBN-0-12-300850-6. (New York and London: Academic Press, 1973. Published for the Linnean Society of London.) £11; \$31.

HILLS, B. A. Gas Transfer in the Lung. Pp.xiv+176. ISBN-0-521-20167-5. (London: Cambridge University Press, 1974.) £4.90 net; \$14.50.

HOBBS, Betty C., and CHRISTIAN, J. H. B. (edited by). The Microbiological Safety of Food. (Proceedings of the Eighth International Symposium on Food Microbiology, Reading, England, September 1972.) ISBN-12-350750-2. (London and New York: Academic Press, 1973.) £7.50; \$21.

JACOB, Francois. The Logic of Living Systems. Translated by Betty E. Spillmann, ISBN-0-7139-0360-0. (London: Allen Lane, a Division of Penguin Books, Ltd., 1974.) £4 net.

JENKINS, Jr., FARISH A. (edited by). Primate Locomotion. Pp.xii+390. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1974.) \$34; £16.30.

JERMY, A. C., CRABBE, J. A., and THOMAS, B. A. (edited by). The Phylogeny and Classification of the Ferns. Pp.xiv+284. (Supplement No. 1 to the *Botanical Journal of the Linnean Society*, Vol. 67.) ISBN-0-12-385050-9. (New York and London: Academic Press, 1973. Published for the Linnean Society of London.) £9; \$25.

JONES, O. A., and ENDEAN, R. (edited by). Biology and Geology of Coral Reefs. Vol. 2: Biology 1. Pp.xxi+480. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$42.50; £20.40.

KERKUT, G. A., and PHILLIS, J. W. (edited by). Progress in Neurobiology. Vol. 2, Part 3. Pp.205-288. ISBN-0-08-017812-X. (Oxford and New York: Pergamon Press 1973.) £2.50.

KOZLOWSKI, T. T. (edited by). Shedding of Plant Parts. (Physiological Ecology: A Series of Monographs, Texts, and Treatises.) Pp.xii+560. ISBN-0-12-424250-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) \$35; £16.80.

LASCELLES, June (edited by). Microbial Photosynthesis. (Benchmark Papers in Microbiology.) Pp.xiii+401. ISBN-0-87933-049-X. (Stroudsburg, Pennsylvania: Dowden, Hutchinson and Ross, Inc., Chichester: John Wiley and Sons, Ltd., 1973.) £11.

LESHEM, Ya'Acov. The Molecular and Hormonal Basis of Plant-Growth Regulation. Pp.xii+156. ISBN-0-08-017649-6. (Oxford and New York: Pergamon Press, 1973.) £2.50.

LEWIS, H. L. Butterflies of the World. Pp.xvi+312. ISBN-0-245-52097-X. (London: George G. Harrap and Co., Ltd., 1974.) £10 net.

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Applied biological sciences

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BARNELL, H. R. Biology and the Food Industry. (The Institute of Biology's Studies in Biology. No. 45.) Pp.59. ISBN-0-7131-2436-9. (London: Edward Arnold (Publishers), Ltd., 1974.) £1.50 boards; 75p paper.

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- STULMAN, Julius, and LASZLO, Ervin. Emergent Man: His Chances, Problems and Potentials. (World Institute Creative Finding.) Pp.x+185. ISBN-0-677-14930-1. (New York, London and Paris: Gordon and Breach, 1973.) £5.20.
- SZASZ, Thomas S. The Second Sin. Pp.xxi+121. ISBN-0-7100-7757-2. (London: Routledge and Kegan Paul, 1974.) £2.50 cloth; £1.25 paperback.
- THE PROBLEM OF CHEMICAL AND BIOLOGICAL WARFARE. Vol. 3: CBW and the Law of War. (A Study of the Historical, Technical, Military, Legal and Political Aspects of CBW, and Possible Disarmament Measures.) Pp.194. ISBN-91-85114-17-0. (Stockholm: Almqvist and Wiksell; New York: Humanities Press; London: Paul Elek, 1973.) 40 Sw.kr.; \$10; £5.
- TUAN, Yi-Fu. Topophilia: a Study of Environmental Perception, Attitudes and Values. Pp.x+260. ISBN-0-13-95248-7. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1974.) \$8.95 cloth; \$4.95 paper.
- VARLEY, G. C., GRADWELL, G. R., and HASSELL, M. P. Insect Population Ecology: an Analytical Approach. Pp.x+212. ISBN-0-632-08050-7. (Oxford and London: Blackwell Scientific Publications, 1973.) £2.75.
- VILLANUEVA, J. R., GARCIA-ACHA, Isabel, GASCON, S., and URU-RURU, F. (edited by). Yeast, Mould and Plant Protoplasts. (Proceedings of the Third International Symposium on Yeast Protoplasts held at Salamanca, Spain, in October 1972.) Pp.xxiv+381. ISBN-0-12-722160-3. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1973.) £7; \$19.75.
- WALTFR, Heinrich. Vegetation of the Earth in Relation to Climate and the Eco-Physiological Conditions. Translated from the second German edition by Joy Wieser. Pp.xiv+237. ISBN-0-340-17165-0. (London: The English Universities Press; New York and Berlin: Springer-Verlag, 1973.) £2.45.
- WHITTAKER, Robert H. (edited by). Ordination and Classification of Communities. (Handbook of Vegetation Science. Part 5.) Pp. 737. ISBN-90-6101-186-X. (The Hague: Dr. W. Junk, 1973.) Dfl. 160.
- WILKINSON, Gerald. Trees in the Wild and other Trees and Shrubs. Pp.158. (ISBN-0-903792-05-2. (London: Stephen Hope Books, 1973.) £3.50 net.
- ZAR, Jerrold H. Biostatistical Analysis. (Prentice-Hall Biological Sciences Series.) Pp.xiv+620. ISBN-0-13-076984-3. (Englewood Cliffs, NJ: Prentice-Hall, Inc., 1974.) \$25.95.

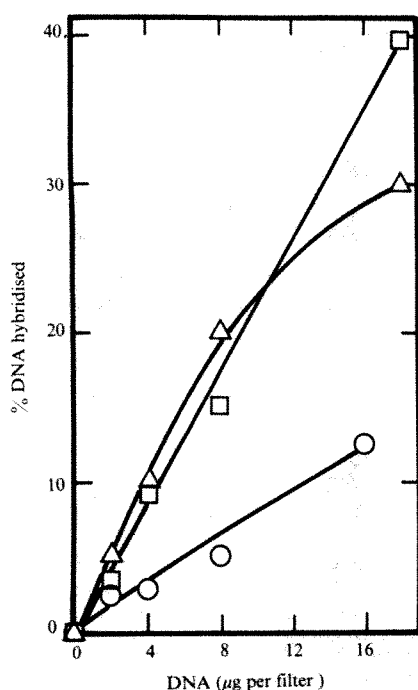


Fig. 4 Reannealing of DNA reisolated from HeLa cells which had taken up *E. coli* ³H-DNA. HeLa cells (2.5×10^5 cells ml^{-1}) were incubated with $200 \mu\text{g ml}^{-1}$ (Δ) or $0 \mu\text{g}$ (\square) of amphotericin B in the presence of $45 \mu\text{g}$ of *E. coli* ³H-DNA (specific activity $18,400$ c.p.m. μg^{-1}) at 37°C for 30 min. After incubation, cells (15×10^6) were washed and DNA was re-extracted²⁸. DNA was then heat denatured to single strands and DNA-DNA hybridisation was conducted essentially according to ref. 29. In a control experiment a comparable amount of *E. coli* ³H-DNA that had not been exposed to HeLa cells was denatured and its capacity to reanneal with itself was measured (\square). The reaction mixtures each contained $8 \mu\text{g}$ ($1,030$ c.p.m.) of labelled DNA and the specified amount of unlabelled *E. coli* DNA. Another set of control samples was incubated with HeLa cell DNA and a blank filter. The low levels of radioactivity nonspecifically absorbed to the control filters (about 5% of the experimental values) were subtracted from the test samples before calculating the percentage hybridisation.

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- ¹ Bhargava, P. M., and Shanmugam, G., *Prog. Nucleic Acid Res. molec. Biol.*, **11**, 103 (1971).
- ² Dobrzonski, T., *Wladysla. Postepy. Mikrobiol.*, **10**, 331 (1971).
- ³ Aaronson, S. A., and Martin, M. A., *Virology*, **42**, 848 (1970).
- ⁴ Spizizen, J., Reilly, B. E., and Evans, A. H., *A. Rev. Microbiol.*, **20**, 371 (1966).
- ⁵ Trouet, A., Campeneere, D. D., and De Duve, C., *Nature new Biol.*, **239**, 110 (1972).
- ⁶ Laval, F., and Malaise, E., *C. hebdom. Séanc. Acad. Sci. Paris*, **268**, 3119 (1969).
- ⁷ Laval, F., Laval, J., and Malaise, E., *C. hebdom. Séanc. Acad. Sci. Paris*, **268**, 3215 (1969).
- ⁸ Shimizu, T., Koyama, S., and Iwafuchi, M., *Biochim. biophys. Acta*, **55**, 795 (1962).

- ⁹ Sung, S., and Quastel, J. H., *Nature*, **200**, 781 (1963).
- ¹⁰ Meizel, S., and Kay, E. R. M., *Biochim. biophys. Acta*, **103**, 431 (1965).
- ¹¹ Meizel, S., and Kay, E. R. M., *Biochim. biophys. Acta*, **123**, 34 (1966).
- ¹² Weisberger, A. S., *J. Lab. clin. Med.*, **56**, 957 (1960).
- ¹³ Koyama, S., *Gann*, **54**, 11 (1963).
- ¹⁴ Sirotak, F. M., and Hutchinson, D. J., *Biochim. biophys. Acta*, **36**, 246 (1959).
- ¹⁵ Howard, B. V., Estes, M. K., and Pagano, J. S., *Biochim. biophys. Acta*, **228**, 105 (1971).
- ¹⁶ Amos, H., *Biochem. biophys. Res. Comm.*, **5**, 1 (1961).
- ¹⁷ Kanji, O., Oluf, G. L., and Raymond, M. A., *Can. J. Bot.*, **50**, 2077 (1972).
- ¹⁸ Wilczok, T., *Nature*, **196**, 1314 (1962).
- ¹⁹ Maes, R., Sedwick, W., and Vaheri, A., *Biochim. biophys. Acta*, **134**, 269 (1967).
- ²⁰ Kwan, C. N., Medoff, G., Kobayashi, G. S., Schlessinger, D., and Raskas, H. J., *Antimicrobial Agents and Chemotherapy*, **2**, 61 (1972).
- ²¹ Medoff, G., Kwan, C. N., Schlessinger, D., and Kobayashi, G. S., *Antimicrobial Agents and Chemotherapy*, **3**, 441 (1973).
- ²² Glick, J. L., *Cancer Res.*, **27**, 2350 (1967).
- ²³ Cocito, C., Prinzie, A., and DeSomer, P., *Experientia*, **18**, 218 (1962).
- ²⁴ Borenfreund, E., and Bendich, A., *J. biophys. biochem. Cytol.*, **9**, 81 (1961).
- ²⁵ Schroeder, F., Holland, J. F., and Bieber, L. L., *Biochemistry*, **11**, 3105 (1972).
- ²⁶ Loni, M. C., *Experientia*, **22**, 711 (1966).
- ²⁷ Medoff, G., Kwan, C. N., Schlessinger, D., and Kobayashi, G. S., *Cancer Res.*, **33**, 1146 (1973).
- ²⁸ Marmur, J., *J. molec. Biol.*, **3**, 208 (1961).
- ²⁹ Gelb, L. D., Kohn, D. E., and Martin, M. A., *J. molec. Biol.*, **57**, 129 (1971).

Effect of a protein-free diet on mitotic activity of transplanted splenic lymphocytes

THE graft versus host reaction (GVHR) is initiated by donor thymus-derived lymphocytes¹⁻³, and, the defence reaction of the host seems to be influenced by the extent of host lymphoid tissue^{4,5}. Dietary protein deprivation provokes an involution of the lymphoid organs, mainly of the thymus and of the thymus-dependent fraction of blood and lymphoid tissue lymphocytes^{6,7}. It is therefore of interest to know if a protein-free diet given to donors of a spleen graft inhibits the GVHR reactivity of the grafted lymphocytes, and if the same diet given to recipients lowers the capacity of these animals to react against the grafted cells.

The latter hypothesis has already been confirmed⁸. A local lymph node GVHR, produced in hybrid F_1 rats by injection of parental spleen lymphocytes, was reported to be much weaker in protein-deprived recipients than in normally fed ones. In contrast, the weight response of the popliteal lymph node after subplantar injection of lymphocytes from protein-deprived parental rats was not reduced. Here, I report the influence of protein deprivation of donors or recipients on the proliferation of grafted lymphocytes recognised by the Y chromosome.

Adult female (Sherman \times Wistar) F_1 hybrid rats were inoculated intraperitoneally with splenic lymphocytes from male parental (Sherman) rats. The cell suspensions were prepared as described previously⁸. The dose of injected viable cells (unstained by erythrosine) was 120×10^6 or 60×10^6 depending on whether the recipients were fed normally or had been fed on a protein-free diet for the previous two months. (For composition of diet see ref. 9.) The terminal body weight was about 210 g for the normal rats and 105 g for the deficient ones.

The donors also included normally fed and protein-deprived rats. Among the former, 12 rats had been thymectomised at 25 d of age. These were introduced into the study to see whether the thymic atrophy of protein-deprived donors could be responsible for the blocking of the multiplication of grafted cells derived from these rats.

Table 1 Mitoses of donor cells in spleen of recipient rats during first week of a graft *versus* host reaction

Strain and sex Donor	Sex Recipient	Diet		Time since transplantation					
		Donor	Recipient	6 h	6 h	48 h	48 h	7 d	7 d
				Total mitoses ‰ ± s.e.	Donor mitoses ‰ ± s.e.	Total mitoses ‰ ± s.e.	Donor mitoses ‰ ± s.e.	Total mitoses ‰ ± s.e.	Donor mitoses ‰ ± s.e.
Sherman	(Sherman × Wistar) F ₁	Normal	Normal	7.43 ±0.75 (7)	19.93 ±7.05	13.67 ±0.92 (6)	14.51 ±2.08	18.20 ±1.91 (5)	6.93 ±1.99
♂	♀	Normal	Protein-free	1.71 ±0.03 (7)	0	7.50 ±0.99 (6)	11.00 ±4.30	8.67 ±1.98 (6)	17.67 ±3.80
♂	♀	Protein-free	Normal	6.00 ±0.38 (7)	12.23 ±3.73	15.50 ±1.77 (6)	0	19.33 ±2.73 (6)	0.93 ±0.93
♂	♀	Protein-free	Protein-free	—	—	7.17 ±0.70 (6)	6.36 ±0.94	11.40 ±3.04 (6)	21.00 ±3.67
Thymect. Sherman ♂	♀	Normal	Normal	4.71 ±0.36 (7)	0.79 ±0.79	9.00 ±1.61 (5)	9.16 ±5.67	—	—
Sherman ♂	Sherman ♀ (Syngeneic controls)	Normal	Normal	—	—	9.60 ±0.93 (5)	2.00 ±1.23	4.75 ±0.85 (5)	—
Uninjected Sherman ♀		Normal		—	—	4.00 ±0.63 (5)	—	—	—

Values are means ± s.e. The numbers of rats are indicated in parentheses. All recipient rats had been injected intraperitoneally 1 h 30 min before killing with 0.25 mg per 100 g of colchicine. The total number of mitoses was established on chromosomal preparations performed according to ref. 13 and stained with Unna's Blue. The percentages of mitoses of the male donor cells were counted after construction of karyotypes from 100 selected mitotic figures.

The F₁ recipients were killed 6 h, 48 h or 7 d after the transplantation. Uninjected female Sherman controls or female Sherman rats injected with 120 × 10⁶ male Sherman lymphocytes and killed after 48 h or 7 d were also studied to exclude GvHR-independent mitotic activity.

As shown in Table 1, normally fed F₁ recipients inoculated with normal parental lymphocytes exhibited a progressive increase in the mitotic index between days 1 and 8, whereas the percentage of male (donor) mitoses dropped from about 20 to 7. These results agree with already published reports¹⁰⁻¹² indicating that cellular proliferation in the spleen, induced by GvHR, is mainly of host origin. In normal recipients inoculated with lymphocytes from protein-deprived donors, the total number of mitoses per 10³ cells showed the same changes as in the above group but the mitoses of the grafted cells disappeared almost completely after 48 h and 7 d. As shown by the total number of mitotic figures, the host reaction was at least as important as after inoculation of normal parental lymphocytes.

If the protein-free diet was given not to the donors but to the recipients, splenic mitoses were much less numerous than in normal recipients although their number increased between days 1 and 8. No donor cell mitoses were detected 6 h after transplantation but the initial inhibition of the grafted cells was only temporary and after 7 d the number of male mitoses became even significantly higher in protein-deprived than in normal recipients. Since the proliferation of the parental lymphocytes seems to be conditioned by continuous stimulation exercised by alloantigens of the host cells¹², it seems that the latter cells preserve their capacity of stimulating the donor lymphocytes, although (partly) losing their ability to defend themselves against their aggressors.

If both donors and recipients were deprived of proteins, the total number of mitotic figures was as low as in the preceding group, although higher than in uninjected rats. But the multiplication of donor cells from protein-deprived parents was not at all impaired after contact with cells of protein-deprived recipients while it was, as seen above, inhibited by lymphocytes of normally fed F₁ hybrids.

The inability of lymphocytes from protein-deprived donors to divide in the spleen of normal recipients could not be attributed exclusively to the low content of the graft in GvH-reactive T

lymphocytes because of the thymic atrophy of the donors. Indeed, if the transplanted lymphocytes came from normally fed but thymectomised parental rats, the multiplication of these cells in the recipients, although completely inhibited in all rats during the first hours and in some rats even 2 d after inoculation, reached normal levels in other rats after 2 d. Since thymic atrophy cannot be more harmful than late thymectomy, it may well be that the suppression of mitotic division of cells from protein-deprived donors may be due to inactivation of donor B lymphocytes as well as to inactivation of T lymphocytes.

Assuming that the GvHR-initiating role of the donor T cells consists in the recognition of foreign histocompatibility antigens of the host¹², and that this recognition is followed by production of antibodies by donor B lymphocytes against these antigens, it may be that protein deprivation of donor rats does not completely impair the capacity of their T lymphocytes to recognise alloantigens but that the immunological response of their B cells is prevented. This naturally facilitates the host reaction against the grafted lymphocytes, from protein-deprived donors, which may explain the blocking of proliferation of the latter cells.

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¹ Miller, J. F. A. P., and Osoba, D., *Physiol. Rev.*, **47**, 437-520 (1967).

² Potworowski, E. F., Zavallone, J. D., Glicker, J. C., and Lamoureux, G., *Rev. eur. Et. clin. biol.*, **16**, 155-157 (1971).

³ Cantor, H., *Cell. Immun.*, **3**, 461-469 (1972).

⁴ Field, E. O., and Gibbs, J. E., *Transplantation*, **3**, 634-668 (1965).

⁵ Bonney, W. W., and Felobush, T. L., *Transplantation*, **15**, 215-220 (1973).

⁶ Aschkenasy, A., *Nutrition et Hématopoïèse* (CNRS, Paris 1971).

⁷ Aschkenasy, A., *Ann. Nutr. Alim.*, **27**, 147-160 (1973).

⁸ Aschkenasy, A., *C. r. Soc. Biol.*, **167**, 430-434 (1973).

- ⁹ Aschkenasy, A., *Rev. fr. Et. clin. biol.*, **13**, 792-799 (1968).
¹⁰ Howard, J. G., Michie, D., and Simonsen, M., *Br. J. exp. Path.*, **42**, 478-485 (1961).
¹¹ Fox, M., *Immunology*, **5**, 489-495 (1962).
¹² Elkins, W. L., *Progr. Allergy*, **15**, 78-187 (1971).
¹³ Lam-Po-Tang, P. R. L. C., *Scand. J. Haemat.*, **5**, 158-160 (1968).

Neutral evolution and immunoglobulin diversity

THE origin of immunoglobulin diversity and species specificity continues to be a controversial topic. Proponents of the germ line theory have presented a limited gene expansion-contraction model in general terms¹⁻³; the mechanism has not yet been demonstrated to produce sufficiently rapid changes in a multi-gene system to account for the observed phenomena. Species divergence of immunoglobulin *V* regions continues to be cited as strong evidence against multiple germ line *V* genes^{4,5}. We report here computer simulation of random unequal crossing over within a system of multiple related genes and compare the results with the relationships observed between immunoglobulin *V* region sequences from different species. We conclude that the species-specific features of immunoglobulin *V* regions can arise as a result of such random processes operating in the multigene set.

We assume that the previous evolutionary history of the organism established a set of genes by repeated duplication and accumulation of point mutations such that the individual genes are homologous but not necessarily identical. The homology is compatible with continued unequal crossing over. This event and its consequences are illustrated in Fig. 1. In the absence of any meiotic distortion the probability of the progeny receiving a chromosome with one additional gene or with one less gene is equal. In the absence of selective pressures, the new duplication or deletion, in itself, would represent a neutral mutation and as such would become fixed in the population at a rate equal to the rate of occurrence of the event⁶.

We simulated the evolution of 50 related genes by unequal crossing over in the absence of selection. At each step of an iterative process, a gene occupying one of the positions in the linear array was selected on a random basis and was either duplicated in the array or deleted. At each event, only the duplicated or the deleted product was chosen at random and was then assumed to become fixed in the population as a neutral mutation before the next repetition. Figure 2 is the result of 200 repetitive cycles. Comparison of the evolved sequence with the initial sequence shows that some of the original genes have been eliminated and replaced by duplications of other original genes. The consequence is evolution of subgroups of closely related genes within a set of related genes. Point mutations and single codon additions or deletions will occur within genes of a subgroup during its evolutionary history. These events and the structure of the ancestral gene of each subgroup will give the characteristic features of the subgroup and provide variation within the individual members.

Speciation divides a common gene pool and permits random mutational events to act on the two resulting pools in an independent manner. This was simulated by taking the original set of 50 related genes and repeating the 200 cycles of unequal cross overs under separate random control. Figure 3 compares the results of independent evolution of the two gene pools. In most cases, the remaining genes in the second example differ from those in the first as do the subgroups and their distribution. Speciation and subsequent independent evolution by this mechanism in the absence of any selective forces does permit multiple homologous but nonidentical genes to evolve and produce species-specific subgroups which have descended from different ancestral genes.

The simulation has been presented in terms of 50 genes;

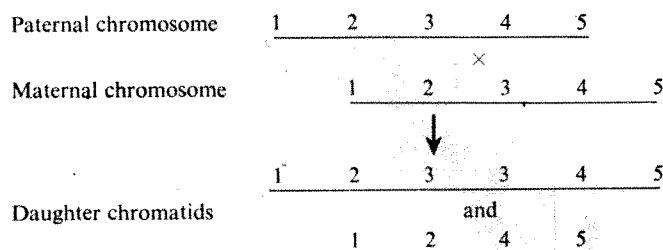


Fig. 1 Unequal crossing over between tandem sets of homologous genes and the products of the event.

however, the results are valid for larger sets, as shown in Fig. 4. Within the limits of the random process, the number of cross overs required for any given reduction in number of subgroups is directly proportional to the number of original genes. For example, 250 genes are reduced by 60% after 600 cross overs while 500 genes are reduced by 60% after 1,200 cross overs. Fifty genes are reduced to 1 subgroup after 2,000 cross overs and by extrapolation 1,000 genes would be reduced to 20 subgroups after 40,000 cross overs.

The number of subgroups present in any species will depend on the number of unequal cross overs which have been established following speciation (Fig. 4) and will also vary due to the random nature of the events involved. The model anticipates different numbers of subgroups in different species, such as appear to exist in mouse and human kappa chains^{1,7}. Although the total number of *V* genes tends to remain at a constant average level, random fluctuations about this mean value occur during any one simulation. The model could thus account for the differences in the proportion of kappa to lambda chains observed in different species. Similarly, variations in the proportions of the heavy chain subgroups in different species are expected⁴.

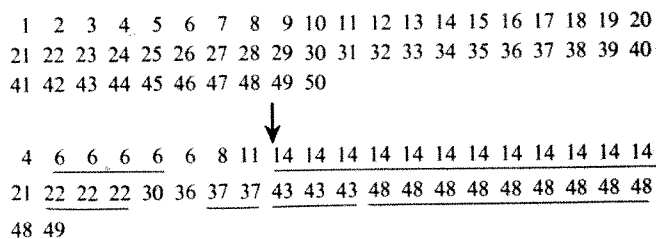


Fig. 2 The origin of subgroups by unequal crossing over. The top set of numbers represent 50 homologous but nonidentical genes arranged in tandem order. Gene deletion and duplication was simulated as shown in Fig. 1 with random selection of the gene involved and of the deleted or duplicated product. After 200 unequal cross overs the bottom set of numbers was obtained.

The model as presented in its simplest form can account for many of the documented facts concerning the evolution of *V* region sequences⁷. The model depends on cross overs which are ubiquitous genetic events⁸. Once the initial unequal cross over has taken place in the multiple gene system, subsequent recombinations involving either the deleted or duplicated product will also be unequal and the process will be self propagating⁹. The frequency of unequal crossing over could approach the frequency of recombination which has been estimated to be 2×10^{-3} for two markers separated by 1,000 tandem *V* genes¹. If the average mammalian generation time is 4 yr¹⁰, one neutral unequal cross over would be established every 2,000 yr where the rate of fixation is equal to the rate of occurrence⁶. If the kappa *V* gene pool contains 1,000 genes, the data in Fig. 4 suggest that 40,000 cross overs would be required to establish the present species divergence between mouse and human kappa

4	6	6	6	6	6	8	11	14	14	14	14	14	14	14	14	14	14	14	14
21	22	22	22	30	36	37	37	43	43	43	48	48	48	48	48	48	48	48	48
48	49																		
3	7	7	12	15	15	15	15	18	18	18	19	31	41	41	41	41	41	41	41
41	41	41	41	41	41	41	41	41	41	41	42	42	42	42	42	42	42	43	43
50	50	50	50	50	50	50	50												

Fig. 3 The effect of speciation on evolution by unequal crossing over. The top set of numbers are the product of 200 simulated cross overs as shown in Fig. 2. Speciation was simulated by taking the original set of 50 numbers and repeating the 200 unequal cross overs under separate random control. The bottom set of numbers is the result of the second series of unrelated random processes.

chains. This would take 8×10^7 yr which is the time estimated for evolution of the primates¹¹. The model is therefore compatible with a pool size of 1,000 genes and the evolutionary time scale.

Although the model has been tested in the absence of any selective pressures, it is obvious that these must exist. Simple determinants elicit production of several variable region sequences which suggests that considerable redundancy or degeneracy exists in the immune system¹²⁻¹³. A single V region sequence with the unique capacity to combine with a given pathogenic determinant⁷ is probably rare. We feel that the major role of selection will be to prevent deletion of the final copy of certain genes essential for immunological survival of the species in its indigenous pathogenic environment. Deletion of the corresponding gene would be lethal. Simulation of this situation gives a result which is not significantly different from the simulation without selection.

The model does not require extensive reduction or increase in gene number within the multiple gene system during evolution. Therefore, a fully functional immunological system will be available to the evolving species at all times. The past immunological experience of the species will be contained in the gene copies and may be subject to gradual elimination only if these genes are of no selective advantage to the present phenotype. The model permits a response to present immunological challenges based on past experience while maintaining adequate options to meet future attacks.

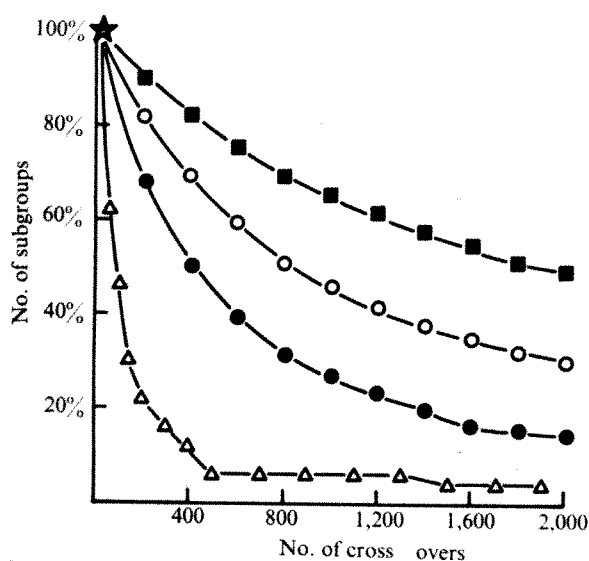


Fig. 4 The effect of the number of unequal cross overs and size of the initial gene set on the number of subgroups. The number of subgroups are expressed as a percentage of the original number of genes in the set. Δ , 50 initial genes; \bullet , 250 initial genes; \circ , 500 initial genes; \blacksquare , 1,000 initial genes. The star represents the convergence of all four curves at 100%.

Note added in proof. Smith (Cold Spring Harbor Symp., in the press) has also used computer simulation to reach conclusions similar to those presented here.

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- 1 Smith, G. P., Hood, L., and Fitch, W. M., *A. Rev. Biochem.*, **40**, 969-1012 (1971).
- 2 Hood, L., and Prahl, J., *Adv. Immun.*, **14**, 291-351 (1971).
- 3 Milstein, C., and Svasti, J., *Prog. Immun.*, **1**, 33-45 (1971).
- 4 Capra, J. D., Wasserman, R. W., and Kehoe, J. M., *J. exp. Med.*, **138**, 410-427 (1973).
- 5 Franek, F., and Novotny, J., *Eur. J. Biochem.*, **31**, 286-289 (1972).
- 6 Kimura, M., *Nature*, **217**, 624-626 (1968).
- 7 Hood, L., McKean, D., Farnsworth, V., and Potter, M., *Biochemistry*, **12**, 741-749 (1973).
- 8 Bodmer, W. F., and Parsons, P. A., *Adv. Genet.*, **11**, 1-100 (1962).
- 9 Gally, J. A., and Edelman, G. M., *A. Rev. Genet.*, **6**, 1-46 (1972).
- 10 King, J. L., and Jukes, T. H., *Science*, **164**, 788-798 (1969).
- 11 Simpson, G. L., *The meaning of evolution* (Yale University Press, 1949).
- 12 Hood, L., Eichmann, K., Lackland, H., Krause, R. M., and Ohms, J. J., *Nature*, **228**, 1040-1044 (1970).
- 13 Cohen, S., and Cooper, A., *Immunology*, **15**, 93-100 (1968).

Surface antigen(s) common to human astrocytoma cells

SINCE animal tumours induced by viruses exhibit common antigenic properties¹, the extent to which antigens are shared in human tumours is of obvious interest. Such antigenic cross reactions between human tumours of the same histological origin have been demonstrated by cell-mediated colony inhibition testing as well as by serological means^{2,3}. But few immunological studies of human brain tumours have been reported. Studying the cytotoxic effect of patients' lymphocytes on autologous or allogeneic astrocytomas, some workers have found antigenic cross reaction⁴ while others have not⁵. I report here the serological demonstration of surface antigen(s) common to cultured human astrocytoma cells.

Surgically removed human cerebral tumours were mechanically disaggregated and maintained in HEPES-buffered Eagle's Minimum Essential Medium with Earle's salts, 10% horse serum, 5% foetal bovine serum, glucose (3 mg ml⁻¹), glutamine (0.3 mg ml⁻¹), penicillin (50 units ml⁻¹), streptomycin (50 µg ml⁻¹) and Fungizone (2.5 µg ml⁻¹) (M. S. Lakshmi, personal communication.) The cells used for immunisation were those designated Astrocytoma 301, cultures of which were shown to be mycoplasma- and virus-free and of glial morphology by electron microscopy. The cells were collected in EDTA (0.02%) and washed in phosphate-buffered saline (PBS), pH 7.2. A New Zealand white rabbit was injected intravenously with 25×10^6 cells on days 0 and 14, and bled out on day 24. The serum was heated for 30 min at 56° C and its antibody activity assayed by dye exclusion cytotoxicity testing⁶ using cultured astrocytoma cells as targets and guinea pig serum as the source of complement. The antiserum was repeatedly absorbed with normal brain homogenate until further absorptions did not decrease the cytotoxicity against Astrocytoma 301 cells. This required four absorptions (Fig. 1a).

Brain homogenate was no more effective in decreasing cytotoxic activity than liver, spleen, kidney, myocardium or thyroid homogenates (Fig. 1a, b). On a volume per volume basis, brain was no more efficient in absorbing the original

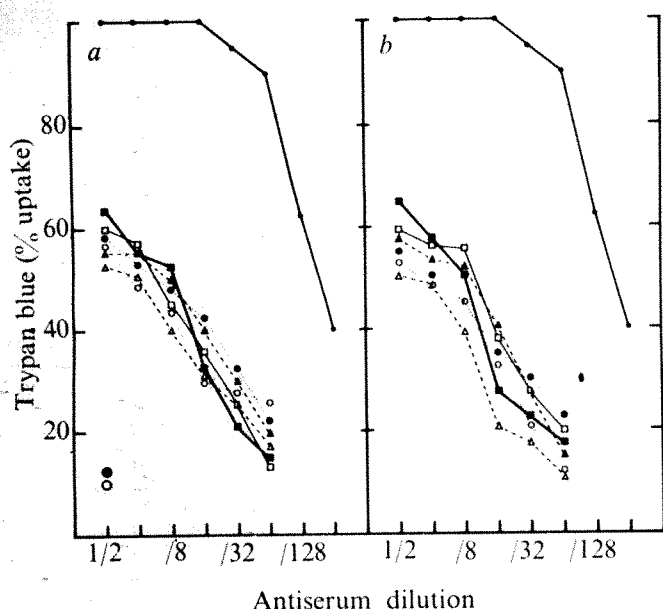


Fig. 1 Dye exclusion cytotoxicity tests showing the activity of unabsorbed anti-astrocytoma 301 serum (●—●) and of the antiserum absorbed *a*, $\times 4$ or *b*, $\times 6$ with human brain (■—■), liver (●—●), spleen (○—○), kidney (□—□), heart (△—△) and thyroid (▲—▲). Astrocytoma 301 cells, between third and sixth passage were collected in EDTA (0.02%), washed and suspended in phosphate-buffered saline (pH 7.2) with 0.1% bovine serum albumin (PBS) at 10^6 cells ml^{-1} . 25 μl of cells were incubated with 25 μl of doubling dilutions of antiserum for 30 min at 37°C ; after washing once, cells were resuspended in fresh unabsorbed guinea pig serum diluted 1:4 in PBS for 30 min at 37°C following which the number of dead cells was assessed using trypan blue staining. For absorption, tissues were homogenised in a Potter-Elvehjem homogeniser, and washed $\times 3$ in PBS. The antiserum was mixed with equal volume of packed homogenate (centrifuged $3,600g \times 10$ min) and absorbed for 30 min at 20°C with continuous shaking. ●, Normal serum; ○, complement.

Table 1 Absorption of anti-HAAA by non-astrocytoma cultures and homogenates

Culture	Passage no.	Absorbing material	Origin	% kill* of anti-HAAA			
				5	2.5	1.25	0.62
212	8	Meningioma		41	42	42	40
217	4	Meningioma		41	42	43	44
222	3	Meningioma		40	43	42	45
U2/18†	18	Breast epithelioma		46	45	43	43
U1/18†	18	Breast carcinoma		41	52	50	42
U3/15†	15	Breast carcinoma		43	43	44	42
U7/2†	2	Breast fibroadenoma		37	40	45	48
U9/16†	16	Breast fibroadenoma		39	40	45	47
MRC 5‡	—	Human diploid fibroblasts		40	42	45	47
HeLa‡	—	Carcinoma cervix		43	43	46	46
HFB.22	7	Foetal brain (22 week stage)		44	42	43	44
HFB.22	—	Foetal brain§ (homogenate)		50	49	46	46
HFB.12	—	Foetal brain§ (12 week stage)		48	47	47	45
Brain absorbed anti-HAAA serum				53	52	52	50

*25 μl anti-HAAA diluted 1:12, absorbed at 37°C as in Fig. 2 with doubling dilutions of cells ($\times 10^5$). Kill with normal rabbit serum was $< 10\%$.

† Cells donated by Dr P. A. Riley.

‡ Established line.

§ Doubling dilutions of 25 μl packed homogenate were used. Controls were used as in Figs 1 and 2.

antiserum than other tissues tested; it is therefore unlikely that there were any brain-specific antibodies in the original serum. Since the absorbed antiserum could not be further absorbed by a number of nonastrocytoma cells, but could be completely absorbed with cultured cells from each of seven different astrocytomas (Fig. 2, Table 1), I shall refer to the antigen(s) defined by it as human astrocytoma-associated antigen(s) (HAAA). When target cells were collected mechanically, by treatment with EDTA (0.02%) or trypsin (0.02%), similar results were obtained. Absorption of anti-HAAA activity by homogenates of two original astrocytoma (301 and A3) samples, stored at -165°C , indicates the presence of this antigen *in vivo*.

In view of the failure of cultured cells from foetal brain or normal brain homogenates to absorb the anti-HAAA activity (Fig. 2, Table 1) it seems unlikely that HAAA is an

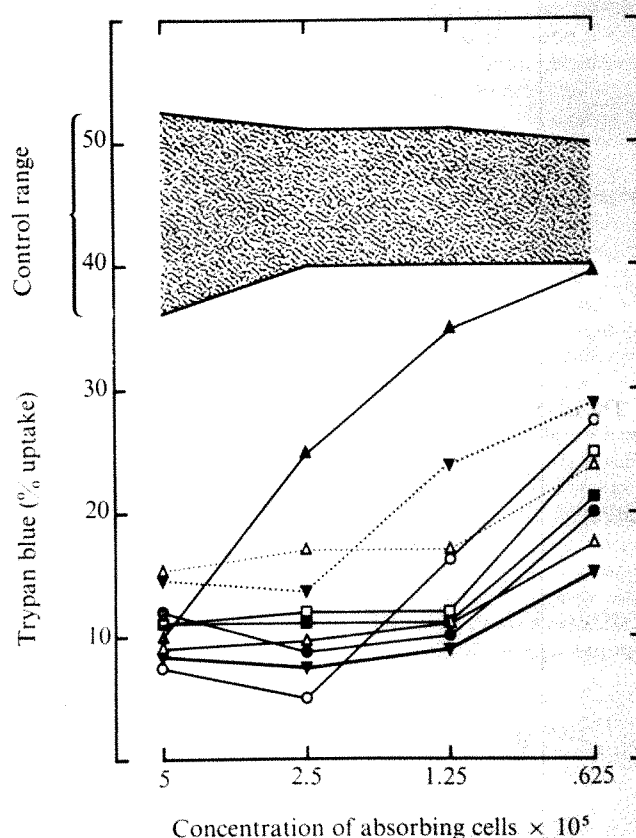


Fig. 2 Dye exclusion cytotoxicity tests showing the ability of cultured cells from seven different astrocytomas, A1 (●—●), A2 (○—○), A3 (▲—▲), A4 (△—△), A5 (■—■), A6 (□—□), and 301 (▼—▼) and homogenates of 301 (▼—▼) and A3 (▲—▲) to absorb anti-HAAA activity. Results of absorptions with various non-astrocytoma cells and homogenates fall within the cross-hatched area and are given in detail in Table 1. 25 μl of anti-HAAA diluted 1:12 in PBS was absorbed with doubling dilutions of a suspension of whole cells or homogenates (50% v/v) for 30 min at 37°C and the absorbed serum tested as in Fig. 1, with unabsorbed rabbit serum diluted 1:10 as complement.

oncofoetal antigen or an astrocyte differentiation antigen, although the former cannot be entirely excluded since HAAA expression could be restricted to a stage of embryogenesis not represented by the samples available here. It is also possible that absorption with normal adult brain tissue from more individuals would reveal that HAAA is normally expressed in a proportion of normal brains, making it an alloantigen analogous to the thymus-leukaemia (TL) antigen in mice⁷. The exact significance of the common antigen detected here remains in doubt but could suggest a common viral aetiology.

The data suggest that appropriately absorbed heteroantisera may be useful tools for defining surface antigens of human tumours, as they appear to be for some cytoplasmic antigens⁸. The demonstration of HAAA might stimulate renewed attempts at immunotherapy for gliomas⁹.

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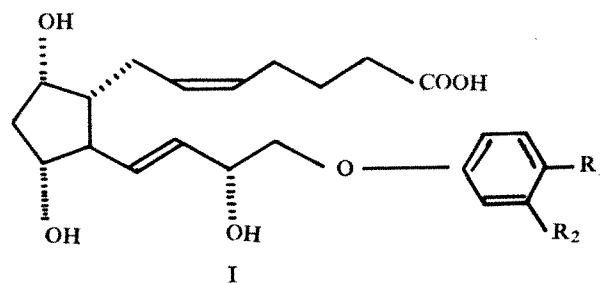
- ¹ Klein, G., *A. Rev. Microbiol.*, **20**, 223 (1966).
- ² Hellström, I., Hellström, K. E., Sjogren, H. O., and Warner, G. A., *Int. J. Cancer*, **7**, 1 (1971).
- ³ Morton, D. L., Malmgren, R. A., Hall, W. T., and Shidlovsky, G., *Surgery*, **66**, 152 (1969).
- ⁴ Kumar, S., Taylor, G., Steward, J. K., Waghe, M. A., and Morris-Jones, P., *Int. J. Cancer*, **12**, 194 (1973).
- ⁵ Levy, N. L., Mahaley, M. S., and Day, E. D., *Cancer Res.*, **32**, 477 (1972).
- ⁶ Boyse, E. A., Old, L. J., and Chuuroulinkov, I., *Methods in Medical Research*, IV, 39, (Year Book Medical Publishers, Chicago, 1964).
- ⁷ Boyse, E. A., and Old, L. J., *A. Rev. Genet.*, **3**, 269 (1969).
- ⁸ Bhattacharya, M., and Barlow, J. J., *Cancer*, **31**, 588 (1973).
- ⁹ Bloom, H. J. G., Peckham, M. J., Richardson, A. E., Alexander, P. A., and Payne, P. M., *Br. J. Cancer*, **27**, 253 (1973).

Potent luteolytic agents related to prostaglandin F_{2α}

EXPLOITATION of prostaglandin F_{2α} (PGF_{2α}) and E₂ (PGE₂) for the regulation of fertility in man and animals is limited by their side effects. Luteolytic agents which are much more potent than PGF_{2α}, without being correspondingly more toxic, have been found among the many analogues of this prostaglandin synthesised in these laboratories, notably among compounds of general structure I (ref. 1). We present here results obtained with three compounds—ICI 79,939, ICI 80,996 and ICI 81,008—which illustrate the variations in biological activity in this series.

The luteolytic activity of these compounds was assessed from their capacity to terminate pregnancy on administration (in phosphate buffer, pH 7.4) to hamsters and rats. They seem to be ineffective in this respect when given to hamsters before day 4, or rats before day 5 of pregnancy (vaginal sperm = day 1). But, when given subcutaneously (s.c.) to hamsters on days 4, 5 and 6, ICI 79,939 and ICI 80,996 are 200 times, and ICI 81,008 is 100 times as potent as PGF_{2α} in terminating pregnancy, as judged by the absence of embryos from the uterus at autopsy on day 8. The fully effective daily dose of ICI 79,939 or ICI 80,996 is 0.125 µg (~ 1.25 µg kg⁻¹), and this same dose is equally effective given only once on day 4. The potencies of the analogues are comparable whether given by intraperitoneal (i.p.) or subcutaneous (s.c.) injection. By mouth, the analogues (given on days 4–6) are only 1/20, and PGF_{2α} 1/40 as potent as when given s.c.

These substances are much less effective in interrupting early pregnancy in rats. Administration of an s.c. dose, at ~ 1700 h on day 5 and again at ~ 1000 h and 1700 h on day 6 proved to be



ICI 79,939	R ₁ =F;R ₂ =H (racemic)
ICI 80,996	R ₁ =H;R ₂ =Cl (racemic)
ICI 81,008	R ₁ =H;R ₂ =CF ₃ (racemic)

most effective as judged by the absence of implants at autopsy on day 9 and 11. Minimum fully effective doses (MFED) in groups of six rats are shown in Table 1a.

On this basis ICI 79,939 is ~ 200 times, and ICI 80,996 and ICI 81,008 are 100 times as potent as PGF_{2α} in rats.

In both hamsters and rats given effective doses of these analogues, daily administration of progesterone (4 mg, s.c.) maintains pregnancy. Moreover, in rats given a single dose of ICI 79,939 (10 µg) or ICI 80,996 (25 µg) on day 6 of pregnancy, reductions in plasma progesterone of over 60% were found 6 h later. We conclude that these substances owe their effect on early pregnancy in both species to luteolysis.

Administration of the analogues to rats in early pregnancy also results, within 48 h of the first dose, in the appearance of nucleated and/or cornified cells in the vaginal smear which is probably due to a decline in progesterone levels. It is seen also in intact or hysterectomised pseudopregnant rats dosed on day 6 and 7 of pseudopregnancy (oestrus = day 1) with ICI 80,996 (2.5 µg, s.c.) or ICI 81,008 (10 µg, s.c.). This also is attributed to the luteolytic action of the compounds which thus seems to be independent of the presence of embryos in the uterus—and even of the uterus itself.

Rats are more sensitive to PGF_{2α} (ref. 2) and the analogues around day 9 of pregnancy, when effective doses cause foetal resorption, and on day 19 or 20 when even smaller doses induce premature parturition. (Table 1b and c. Rats dosed on day 9 were autopsied on day 18. In rats dosed on day 20, parturition was taken to be premature if they littered before 1100 h on day 22—our rats normally litter between the afternoons of days 22 and 23.)

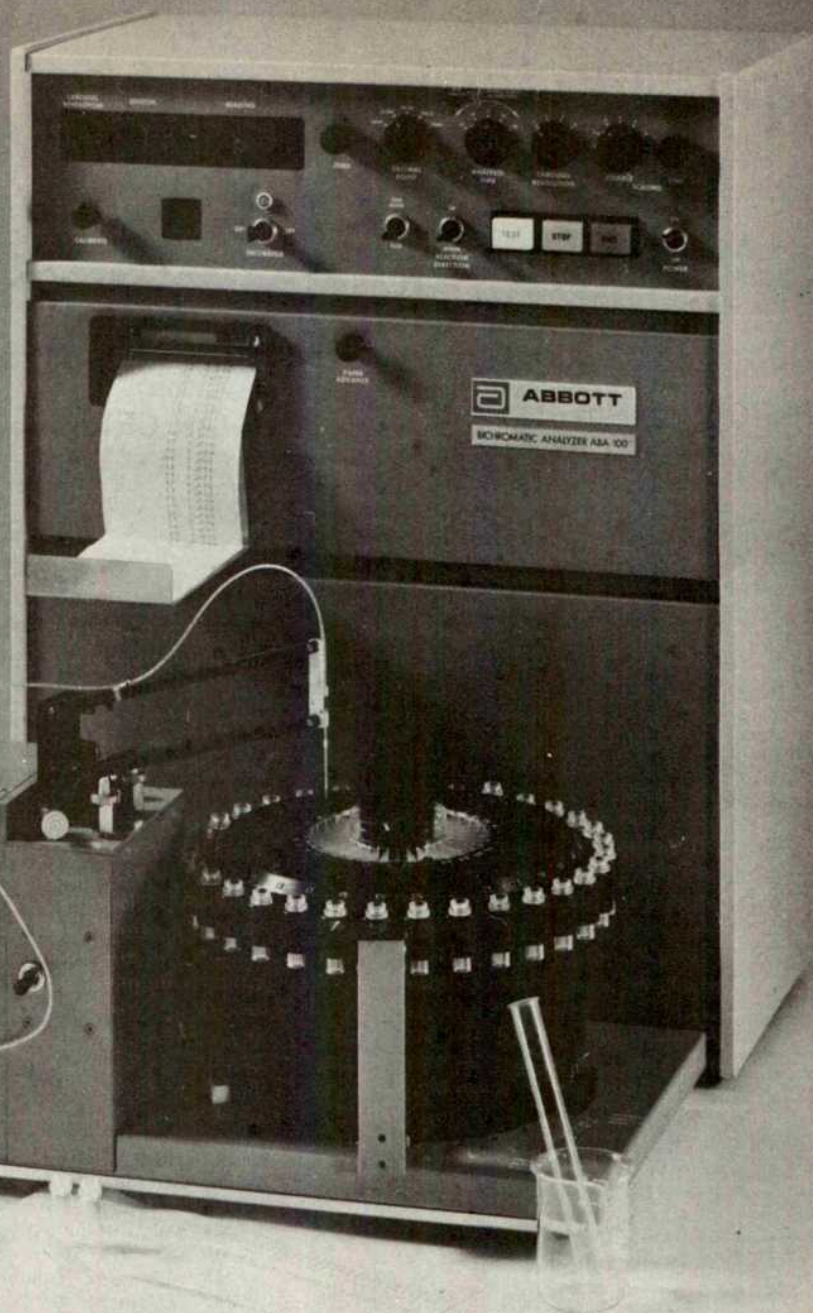
It will be seen that the relative efficacy of PGF_{2α} and the analogues at these later stages of pregnancy in rats is similar to their relative luteolytic potency in early pregnancy in hamsters and rats. Moreover PGE₂ which is a weak luteolytic agent³ but very potent smooth muscle stimulant (Table 2) has to be given in a very high dose (Table 1) to cause rats to litter prematurely. It is probably, therefore, by virtue of their luteolytic

Table 1 Doses of PGF_{2α}, its analogues, and PGE₂ required to interrupt pregnancy in rats.

Compound	MFED (µg kg ⁻¹ : total)			
	^a	^b	^c	
	Dosed day 5 (p.m.) + day 6 (a.m. and p.m.)	Dosed day 9 (a.m. and p.m.)	Dosed day 20 (1400 h)	
	s. c.	s. c.	s. c.	i. p.
PGF _{2α}	27,000	n.t.	n.t.	360
ICI 79,939	135	22	0.9	0.9
ICI 80,996	270	22	0.9	0.9
ICI 81,008	270	90	1.8	2.7
PGE ₂	n.t.	n.t.	n.t.	3,600

(n.t. = not tested).

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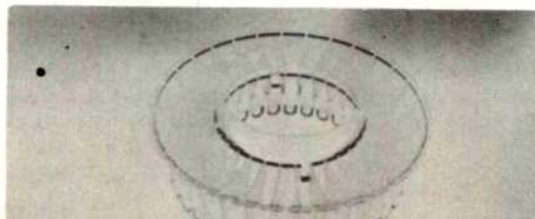
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activity that these compounds interrupt pregnancy at all stages in the rat.

The luteolytic potency of these agents is, however, in no way related to their capacity to stimulate smooth muscle. This was assessed *in vitro* from their potency in provoking isotonic contractions of the isolated uterus of dioestrous guinea-pigs and of segments of gerbil colon (Table 2).

As a smooth muscle stimulant, ICI 80,996 is similar in potency to PGF_{2α}, whereas ICI 79,939 is 10 times, but ICI 81,008 only 1/50 as potent.

In general, the severity of the side effects of these compounds parallels their potency as stimulants of smooth muscle. With ICI 79,939, early pregnancy can be terminated in hamsters and even in rats at doses that have no obvious side effects: these doses, however, are toxic to nonpregnant animals and sometimes lethal to young ones. In the doses needed to interrupt pregnancy in these species, ICI 80,996 and ICI 81,008 seem completely devoid of side effects in both pregnant and nonpregnant animals. Moreover, large multiples (× 20 or more) of these doses can be given without causing serious distress. With PGF_{2α}, side effects are sometimes seen at doses below the minimum required to terminate pregnancy. When seen, the side effects of the analogues are similar to those of PGF_{2α} and include watery diarrhoea, hypersalivation, gasping, abdominal 'squirming', ataxia, reduced motility, irritability and weight loss.

Table 2 Relative potencies of PGF_{2α}, its analogues and PGE₂ in stimulating contractions of smooth muscle *in vitro*

Compound	Guinea pig uterus	Gerbil colon
PGF _{2α}	1 (assigned)	1 (assigned)
ICI 79,939	10	9.5
ICI 80,996	1	0.6
ICI 81,008	0.02	0.036
PGE ₂	36	—

From our results it seems that, while the analogues described are similar in luteolytic potency and all are many times as potent as PGF_{2α}, they differ markedly as smooth muscle stimulants and in toxicity. By the criteria described above, ICI 79,939 is more toxic than PGF_{2α}, ICI 80,996 less toxic, and ICI 81,008 very much less toxic.

Reports have already appeared on the luteolytic activity of these analogues in farm animals^{4,5} and their potential for the control of oestrus and the treatment of infertility in cattle⁴ and horses^{5,6} and the induction of farrowing in pigs⁷. Results in pig-tail monkeys (*Macaca nemestrina*) suggest that ICI 80,996 and, more particularly, ICI 81,008 may also be of value as antifertility agents in women. A s.c. dose of 50 µg kg⁻¹ of ICI 80,996 or 100 µg kg⁻¹ of ICI 81,008 given on day 20 of the (30–31 d) cycle and on the next 4 to 5 d, causes menstrual bleeding 4 to 7 d before the expected time. The rapid fall in plasma progesterone during treatment and before the onset of bleeding indicates that this is due to luteolysis. Several of the monkeys given ICI 80,996 suffered transient side effects, chiefly diarrhoea, but with ICI 81,008 these were infrequent and mild.

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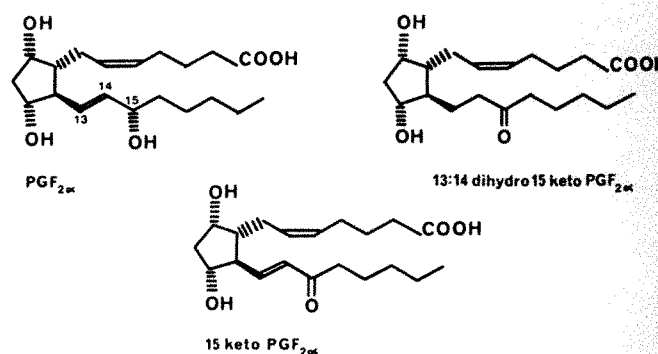
- ² Fuchs, A.-R., and Mok, E., *Fert. Steril.*, **24**, 275, (1973).
- ³ Labhsetwar, A. P., *Prostaglandins*, **2**, 23, (1972).
- ⁴ Tervit, H. R., Rowson, L. E. A., and Brand, A., *J. Reprod. Fert.*, **34**, 179, (1973).
- ⁵ Allen, W. R., and Rowson, L. E. A., *J. Reprod. Fert.*, **33**, 539, (1973).
- ⁶ Allen, W. R., and Rossdale, P. D., *Equine vet. J.*, **5**, 137, (1973).
- ⁷ Ash, R. W., and Heap, R. B., *J. agric. Sci., Camb.*, **81**, 365, (1973).

Potent bronchoconstrictor activity of 15-keto prostaglandin F_{2α}

THE effects of parent prostaglandins (PG) on bronchial muscle *in vitro*¹⁻³ and *in vivo*^{4,5} have been characterised, but the pharmacological properties of their metabolites are less well defined. The lung is an important site of PG metabolism and a better understanding of the effects of the various metabolites is necessary since PGs are now recognised as compounds released in asthma. Modification of PG metabolism itself could be responsible for the aetiology of the asthmatic bronchospasm, particularly since asthmatic subjects have been shown to be considerably more sensitive to inhaled PGF_{2α} than the normal population⁶. We have shown that 15-keto PGF_{2α} possesses very potent bronchoconstrictor activity and is probably important in asthma.

Two enzymes in the lung are responsible for metabolising PG: 15-hydroxy prostaglandin dehydrogenase, which converts, for example, PGF_{2α} to 15-keto PGF_{2α} and 13:14 prostaglandin reductase which reduces 15-keto PGF_{2α} to dihydro-15-keto PGF_{2α}. In lung from most species, these enzymes are closely related and the intermediate, 15-keto PGF_{2α} is not seen in the tissue or in the blood leaving the lung.

Dihydro-15-keto PGF_{2α}, the usual metabolite formed from PGF_{2α} has been shown to have little spasmogenic activity on many tissues⁷ but the data on 15-keto PGF_{2α} (ref. 8) are incomplete.

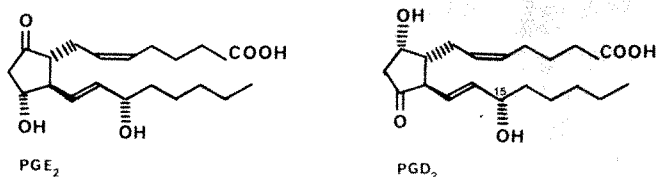


15-keto PGF_{2α} was synthesised from PGF_{2α} by oxidation with activated manganese dioxide⁹ and purified by column and plate chromatography; its structure was confirmed by nuclear magnetic resonance and mass spectrometry. Biosynthesis using homogenates of swine lung¹⁰ followed by similar purification gave the same compound.

15-keto PGF_{2α} was assayed biologically *in vitro* on guinea pig ileum, guinea pig tracheal chains, rat colon, stomach strip and uterus, rabbit aortic strips, gerbil colon and human bronchial muscle strips. Table 1 shows the relative potencies of PGF_{2α} and 15-keto PGF_{2α} on these tissues, indicating that 15-keto PGF_{2α} is more potent than PGF_{2α} on most preparations. Synthetic and biosynthetic 15-keto PGF_{2α} were equally active in each test.

PGD₂, an isomer of PGE₂ and 15-keto PGF_{2α}, was a potent bronchoconstrictor substance, having the same order of potency as PGF_{2α}. This suggests that the 9-hydroxyl group is necessary

¹ Binder, B., Bowler, J., Brown, E. D., Crossley, N. S., Hutton, J., Senior, M., Slater, L., Wilkinson, P., and Wright, N. C. A., *Prostaglandins*, **6**, 87, (1974).



for bronchoconstrictor activity but substitution in the 15 position is less critical. Uncharacteristic responses are, however, occasionally produced by most prostaglandins and although PGE₂ is usually bronchodilator, it sometimes causes bronchoconstriction (H. O. J. Collier and P. Gardiner, symposium on Bronchodilator Drugs, Royal College of Physicians, 1 October, 1973).

All three isomers have been isolated from bovine lung perfusates following immunological challenge. The contraction of both human bronchial muscle strips and guinea pig ileum produced by 15-keto PGF_{2α} is similar in character to that of slow-reacting substance in anaphylaxis (SRS-A), although the substances can be separated by chromatography.

Table 1 Effect of 15-keto PGF_{2α} relative to PGF_{2α} on various isolated smooth muscle preparations

Pharmacological isolated preparation	Relative potency of 15-keto PGF _{2α} (PGF _{2α} = 1)
Guinea pig ileum (11)	2–4
Gerbil colon (11)	5–10
Guinea pig trachea (12)	2–3
Human bronchial muscle (3)	1–2
Rat colon (11)	1–2
Rat stomach strip (11)	0–3*
Rat uterus (11)	0.1–6*
Rabbit aortic strip (11)	†

* Variable responses, with no apparent relationship to concentration.

† No response to either.

Figures in brackets indicate references to methods used.

The belief that prostaglandins are of importance in asthma³ is greatly strengthened by this work, which suggests a definitive involvement of PGs and their metabolites in the asthmatic bronchospasm. Further work to establish the role of 15-keto PGF_{2α} and other metabolites is in progress.

We thank Dr R. G. Harrison for the preparation of synthetic 15-keto PGF_{2α}, Mr J. R. Boot for PGD₂ and 15-keto PGF_{2α} and Mr J. W. Jackson and Sister J. DeMulder of Harefield Hospital, Middlesex for their help in obtaining specimens of human lung.

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- ¹ Änggård, E., and Bergström, S., *Acta Physiol. Scand.*, **58**, 1–12 (1963).
- ² Main, I. H. M., *Br. J. Pharmac.*, **22**, 511–519 (1964).
- ³ Sweatman, W. J. F., and Collier, H. O. J., *Nature*, **217**, 69 (1968).
- ⁴ Cuthbert, M. F., *Proc. R. Soc. Med.*, **64**, 15 (1971).
- ⁵ Kawakami, Y., Uchiyama, K., Irie, T., and Murao, M., *Eur. J. clin. Pharmac.*, **6**, 127–132 (1973).
- ⁶ Hedqvist, P., Holmgren, A., and Mathé, A. A., *Acta Physiol. Scand.*, **82**, 29A (1971).
- ⁷ Pike, J. E., Kupiecki, F. P., and Weeks, J. R., *Nobel Symposium 2: Prostaglandins* (edit. by Bergström, S., and Samuelsson, B., 161. (Almqvist and Wiksell, Stockholm, 1967).
- ⁸ Änggård, E., and Samuelsson, B., *Nobel Symposium 2: Prostaglandins* (edit. by Bergström, S., and Samuelsson, B., 97–105. Almqvist and Wiksell, Stockholm, 1967).
- ⁹ Granström, E., *Eur. J. Biochem.*, **20**, 451–458 (1971).
- ¹⁰ Änggård, E., and Samuelsson, B., *Arkiv Kemi*, **25**, 293–300 (1966).

¹¹ Vane, J. R., *Identification of Asthma* (edit. by Porter, R., and Birch, J.), 121–123. (Churchill Livingstone, Edinburgh and London, 1971).

¹² Constantine, J. W., *J. Pharm. Pharmac.*, **17**, 384–385 (1965).

Origin of asymmetry in biomolecules

ONE of the most exciting hypotheses put forward to explain the molecular asymmetry of living beings is based on a physical concept, the 'parity nonconservation law'¹. According to this, matter is intrinsically asymmetric (β particles are always spin polarised) and the molecular asymmetry on Earth is a reflection of the structure of matter itself^{2–5}. Though the theory is attractive, experimental results are controversial. Some people have reported differences in the radiation susceptibility of the two enantiomers (D and L forms) of a molecule if irradiated with β^- particles and/or their associated circularly polarised γ rays (bremsstrahlung)⁶, whereas others have failed to detect any differential radiolysis in similar experiments⁷. One possible explanation for the controversial data may be that the decomposition of molecules depends on many factors which may mask the expected differential interaction between β^- particles and optical isomers. To avoid these disturbing factors, we studied the annihilation of β^+ particles in optical isomers, exploiting the great advantage of this technique that it gives information about the interaction process itself rather than about the products of interaction.

The purity of the samples used throughout the experiments was checked by conventional methods. On paper chromatograms with different solvent systems each gave one spot. The absorption, CD and luminescence spectra of the enantiomers were the same between the limit of experimental errors. Sodium-22 was used as the positron emitter. Crystalline D and L amino acids were packed around the ²²Na source (activity 0.8 μ Ci) to a thickness of 6 to 7 mm, and it was placed between two scintillation counters of NE111 plastic phosphor coupled to AVP56 photomultipliers. Conventional electronic circuits selected coincidences between the 1.28-MeV γ radiation of the ²²Na and the 0.51-MeV annihilation radiation produced in the crystals of the two enantiomers. The positrons annihilate on free electrons with a short lifetime⁸ ($\tau \sim 2 \times 10^{-10}$ s) and may form positroniums in singlet ($\tau_s \sim 1.2 \times 10^{-10}$ s) and triplet states ($\tau_t \sim 2.7 \times 10^{-7}$ s) with bound electrons. The triplet positroniums do not, however, live as long in condensed matter, because they transform to the singlet state by the so-called 'pick-off' mechanism with a lifetime of $\tau_{t'}$ ~ 1 to 50×10^{-9} s and annihilate from this state into two photons giving 0.51-MeV γ rays. The time delay was measured by a time-to-amplitude converter and multichannel analyser.

Table 1 Triplet intensity in L and D isomers; data from positron annihilation time spectra

Material	No. of independent runs	L	D	D/L
Phenylalanine (Calbiochem)	8	11.0 (6)	13.4 (6)	0.83 (6)
Tyrosine (Sigma Chemical Corp.)	8	4.4 (2)	4.7 (2)	0.94 (6)
Dihydroxy-phenyl-alanine (Nutritional Biochemical Corp.)	4	1.6 (1)	2.2 (1)	0.72 (6)
Tryptophan ¹ (Nutritional Biochem. Corp.)	4	12.0 (1)	18.0 (1)	0.67 (7)
Tryptophan ² (Koch-Light Laboratories Ltd)	4	18.8 (10)	22.8 (10)	0.82 (5)

The results are summarised in Table 1. There are clearly significant differences in triplet annihilation intensity between L and D amino acids. In other words the experiments show that the D isomers of amino acids favour triplet states in case of forward polarised β^+ particles.

It seems likely from this evidence that β decay was the cause of an initial asymmetry in the racemic mixtures present on the primordial Earth. This tiny initial asymmetry was probably enough, in the course of chemical and biological evolution, to nudge the system into a highly asymmetric state by one or other of the mechanisms suggested by Wald⁹ and others¹⁰⁻¹².

For the physical interpretation of the effect we rely on the fact¹³ that the symmetry properties of optical isomers are compatible with the existence of second rank pseudotensors characterising the isomers. The correlation tensor $\langle v_\alpha \sigma_\beta \rangle$ between the velocity and the spin of the electrons of the sample is of this type. Therefore those components of the correlation tensor which are allowed by the symmetry may differ from zero. In a solution, for example, the correlation tensor has the form,

$$\langle v_\alpha \sigma_\beta \rangle = K \sigma_{\alpha\beta}$$

where the coefficient K is of different sign in L and D species. In other words, when $K \neq 0$ the electron spins are aligned predominantly parallel to the motion in one of the optical isomers, and antiparallel in the other.

If we suppose that the probability of positronium formation depends on the relative velocity of the positrons and the electrons, then since the positrons are polarised this assumption immediately leads to the consequence that when $K \neq 0$, the relative probabilities of *ortho*- and *para*-positronium formation differ for differential optical isomers. This difference is indeed observed in the experiment described above.

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- ¹ Lee, T. D., and Yang, C. N., *Phys. Rev.*, **104**, 254 (1956).
- ² Vester, F., Ulbricht, T. L. V., and Krauch, H., *Naturwissenschaften*, **46**, 68 (1959).
- ³ Ulbricht, T. L. V., *Q. Revs*, **18**, 48 (1959).
- ⁴ Ulbricht, T. L. V., and Vester, F., *Tetrahedron*, **18**, 629 (1962).
- ⁵ Vester, F. thesis, Univ. Saarlandes, Saarbrücken (1963).
- ⁶ Garay, A. S., *Nature*, **219**, 338 (1968).
- ⁷ Bernstein, W. J., Lemmon, R. M., and Calvin, M., *Molecular evolution* (edit. by Rohlfing, D. L., and Oparin, A. I.), 151 (Plenum, New York, 1972).
- ⁸ Goldanskii, V. I., *Positron annihilation* (edit. by Stewart, A. T., and Roelling, L. O.), 183 (Academic Press, New York, 1967).
- ⁹ Wald, G., *Ann. N.Y. Acad. Sci.*, **69**, 352 (1957).
- ¹⁰ Seelig, F. F., *J. theor. Biol.*, **31**, 355 (1971).
- ¹¹ Ageno, M., *J. theor. Biol.*, **37**, 187 (1972).
- ¹² Decker, P., *Nature new Biol.*, **241**, 72 (1973).
- ¹³ Landau, L. D., and Lifshitz, E. M., *Theoretical Physics*, **8**, 313 (Pergamon, Oxford, 1960).

Abnormality in tissue isoferritin distribution in idiopathic haemochromatosis

IDIOPATHIC haemochromatosis (IHC) is generally considered to result from an inherited abnormality of iron metabolism in which excess iron accumulates in tissues such as liver, heart and pancreas, causing structural and functional abnormalities¹. The clinical and pathological features of the disease have been well characterised^{1,2} although the mode of inheritance is uncertain because of the lack of a reliable genetic marker. In spite of several theories³⁻¹¹, the

metabolic abnormality leading to excess iron storage remains unknown.

Much of the excess iron is deposited in ferritin. Although the major function of this protein was thought to be the detoxification and storage of iron, it now seems to have a more extensive function. For example, it acts catalytically in the oxidation and storage of iron^{12,13}, and there is evidence¹⁴⁻¹⁶ for metabolically different ferritins. We found a structural heterogeneity in many tissue ferritins which may correlate with this functional heterogeneity. Most tissues from normal subjects contain several isoferritins, many of which are common to several tissues. The relative distribution and iron content of these isoferritins are characteristic of the tissue of origin (our unpublished results). Because of this evidence of multiple organ-specific forms and metabolically distinct pools of ferritin, and also the demonstration of an alteration in isoferritin profile in human hepatoma¹⁷, we have examined ferritin isolated from liver and other organs in patients with IHC and in control subjects. We found an apparent absence of the more acidic isoferritins that predominate in normal heart, pancreas and kidney ferritin. The haemochromatotic tissue ferritins all closely resembled normal liver ferritin in their isoferritin profile, iron content of the isoferritins and in amino acid composition. By contrast, tissue ferritins isolated from patients with alcoholic cirrhosis or secondary iron overload showed the normal organ distribution in isoferritin profile and in iron content.

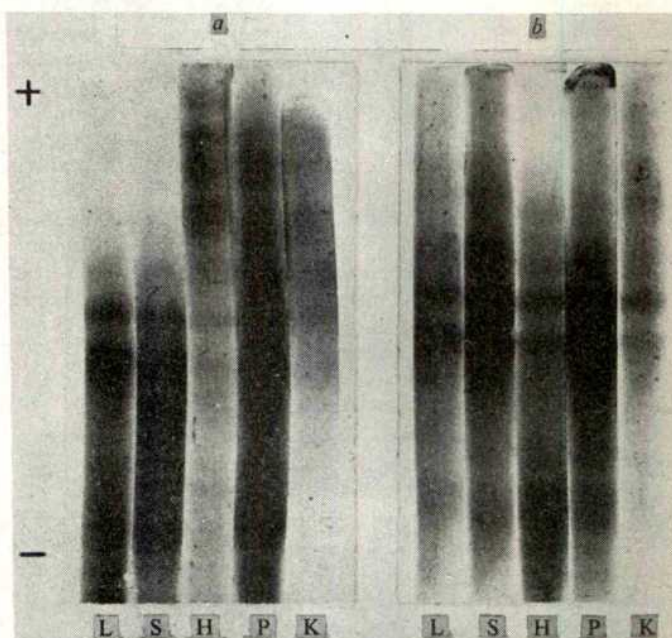


Fig. 1 Gel electrophoresis in 4% polyacrylamide using 2% ampholytes, pH range 5-7, of purified ferritin isolated from human liver (L), spleen (S), heart (H), pancreas (P) and kidney (K) from a normal subject (a) and a patient with idiopathic haemochromatosis (b). Between 25 and 35 μ g of protein was applied to all gels. After equilibrium was attained, the gels were stained for protein using Coomassie blue.

Fresh human tissues were obtained post-mortem from three patients with IHC (all untreated and diagnosed according to published criteria¹⁸), two with alcoholic cirrhosis, one with post-transfusional haemosiderosis and two control subjects. Ferritin was isolated by the method of Drysdale and Munro¹⁹ except that carboxymethyl cellulose chromatography was omitted to avoid possible selective loss of isoferritins of differing charge. In contrast to methods which include high speed centrifugation for purification, this procedure ensures the collection of both iron-laden ferritin and the lighter apoferritin, which is important in

Table 1 Amino acid composition of normal and haemochromatosis tissue ferritin

Amino acid	Normal			Haemochromatosis		
	Liver	Spleen	Kidney	Liver	Spleen	Kidney
Lys	13.1	13.8	9.15	12.3	13.3	12.5
Hist	6.6	7.4	4.9	7.1	7.1	6.3
Arg	8.6	9.8	7.2	10.3	9.6	8.2
Asp	20.1	19.5	19.7	21.1	21.4	21.0
Thre	8.5	9.3	9.15	7.3	7.7	7.7
Ser	10.0	10.3	10.45	8.2	6.9	9.6
Glu	22.9	18.3	22.5	22.8	20.6	23.9
Pro	7.1	7.6	10.3	5.3	6.3	5.1
Gly	13.1	13.0	16.2	12.5	13.9	12.9
Ala	15.2	15.4	15.1	16.1	16.2	15.8
Cyst*	2.1	1.7	4.9	2.6	2.3	2.0
Val	8.9	9.8	10.4	7.4	8.3	7.3
Meth†	3.2	1.8	3.5	3.5	2.2	3.9
Ileu	4.0	3.5	5.7	4.0	3.5	3.6
Leu	20.9	23.1	18.8	24.6	23.7	24.3
Tyr	5.6	7.2	5.2	6.2	7.0	6.7
Phe	7.6	8.3	7.0	8.1	7.9	8.7

Tissue ferritins were isolated and purified as described in the text. The results are expressed as residues 180 amino acid subunit and are means of at least two duplicate determinations on each sample.

* Determined as cysteic acid plus half cysteine.

† Includes methionine and methionine sulfoxide.

view of the variable iron content of unfractionated ferritin¹⁹ and its multiple molecular forms (our unpublished results). Each tissue ferritin preparation appeared to be pure by gel electrophoresis since all protein in each preparation also stained for iron with Prussian blue, a commonly accepted criterion for ferritin purity.

The tissue ferritin preparations were also analysed by gel electrofocusing (GEF) at 4° C in 4% polyacrylamide gel cylinders with 2% ampholytes, pH range 5–7, in apparatus from Medical Research Apparatus, Boston^{20,21}. The gels were stained for protein with Coomassie blue or for iron using potassium ferrocyanide. To establish that all protein bands obtained from each ferritin preparation by GEF represented isoferritins and not other contaminating proteins, duplicate gels were subjected to direct immunoprecipitation *in situ* with a specific rabbit anti-human-liver ferritin antiserum. This antiserum recognised all the tissue ferritins with a line of complete identity by Ouchterlony

analysis and, in addition, precipitated all the isoferritins previously demonstrated in the various normal tissues (our unpublished results). The resulting pattern of immunoprecipitates after GEF corresponded in position in each instance to the protein bands (our unpublished results), confirming that all protein bands were ferritins.

In agreement with our previous findings (unpublished), there were characteristic differences in electrophoretic mobilities of the ferritins from normal tissues. These differences were more evident at the higher resolution given by GEF. There were striking differences between the tissue ferritins in the isoferritin distribution and the iron content of the individual isoferritins (Figs 1 and 2). Thus, the number and relative amounts of various isoferritins, as well as their iron content varied with the tissue of origin. This organ-specific variation in isoferritin pattern and iron content was also demonstrable in ferritins isolated from the tissues of the two patients with cirrhosis and one with transfusional haemosiderosis. In contrast, ferritins isolated from each of the five tissues from three patients with IHC were remarkably uniform in electrophoretic mobility, isoferritin profile and the iron content of their isoferritins (Figs 1 and 2). These results were substantiated by amino acid analyses which showed that the composition of different tissue ferritins in IHC were very similar and closely resembled that of normal liver ferritin. This contrasts with the variation in amino acid compositions of normal tissue ferritins (our unpublished results). Immunodiffusion experiments using five animal antisera to heterologous ferritins revealed no immunological differences between normal liver ferritin and the ferritins isolated from haemochromatotic tissues when tested by double diffusion on Ouchterlony plates.

The electrophoretic mobilities and isoferritin profiles of tissue ferritins from a patient with post-transfusional haemosiderosis were normal. Alfrey obtained similar results by electrophoresis with two patients with post-transfusional haemosiderosis²³. Linder-Horowitz *et al.*¹⁶ demonstrated that experimental iron loading in rats did not alter the gene expression for ferritin. It is unlikely, therefore, that the abnormalities in tissue ferritins which we have demonstrated in patients with IHC are merely the result of increased iron concentration. Rather, the atypical isoferritin distribution seems to be specific for this disease.

There is increasing evidence that the structural heterogeneity in different ferritins from normal tissues reflects functional differences in these proteins in iron metabolism^{14,16,23}. Variations in isoferritin profile among normal tissue ferritins may be due either to the presence of different cell populations in the different tissues, or to different metabolic functions of the multiple isoferritins. Recent work²⁴ has suggested that much of the structural heterogeneity in tissue ferritins represents hybrid molecules consisting of different proportions of dissimilar subunits, somewhat analogous to other hybrid isoenzymes such as lactic dehydrogenase. The abnormally uniform distribution of tissue isoferritins which we have demonstrated in IHC could result from the generalised deposition of a catabolic or storage ferritin^{14,15} normally present predominantly in liver and spleen or from a decreased synthesis of the more acidic isoferritins present in heart, kidney and pancreas. This may represent a genetic defect in the synthesis of a subunit type found normally in these tissue ferritins. Whether the abnormality in tissue isoferritins in IHC is directly related to the primary genetic defect of this disease requires confirmation by further studies of asymptomatic relatives of patients and more detailed analysis of the primary structure of ferritin.

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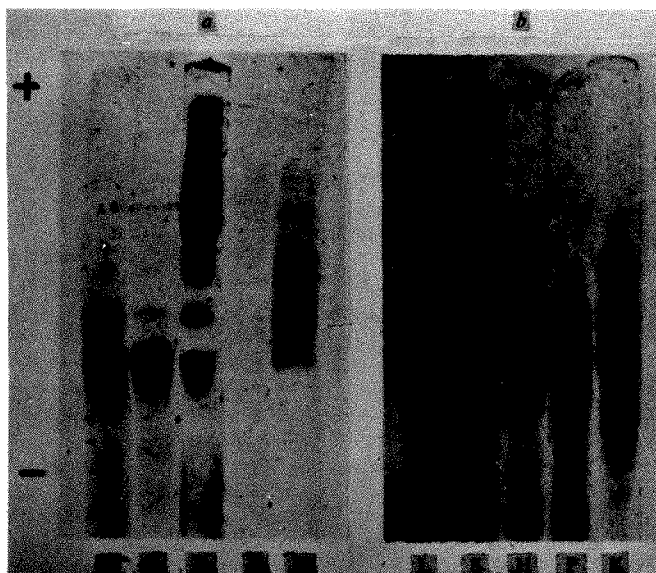


Fig. 2 Analytical gel electrofocusing was performed as described in Fig. 1. In this case, the gels were stained for iron using Prussian blue. The isoferritins in normal pancreas did not stain for iron, even when the gels were relatively overloaded with protein. *a*, Normal; *b*, haemochromatosis.

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- ¹ Polycove, M., in *The metabolic basis of inherited disease* (edit. by Stanbury, J. B., Wyngaarden, J. B., and Fredrickson, D. S.) (McGraw-Hill, New York, 1972).
- ² Powell, L. W., in *The molecular biology of liver disease* (edit. by Becker, F. F.) (Dekker, New York, in the press).
- ³ Charlton, R. W., Bothwell, T. H., in *Progress in hematology* (edit. by Brown, E. B., and Moore, C. V.), **5**, 298 (Grune and Stratton, New York, 1966).
- ⁴ Ross, J., Kochwa, S., and Wasserman, L. R., *Blood*, **24**, 850 (1964).
- ⁵ Bothwell, T. H., Cohen, H., Abrahams, O. L., and Perold, S. M., *Am. J. Med.*, **27**, 730 (1959).
- ⁶ Davis, A. E., *Lancet*, ii, 749 (1961).
- ⁷ Davis, A. E., and Badenoch, J., *Lancet*, ii, 6 (1962).
- ⁸ Biggs, J. C., and Davis, A. E., *Lancet*, ii, 814 (1963).
- ⁹ Davis, P. S., Luke, C. G., and Deller, D. J., *Lancet*, ii, 1431 (1966).
- ¹⁰ Conrad, M. E., and Crosby, W. H., *Blood*, **22**, 406 (1963).
- ¹¹ Powell, L. W., Campbell, C. B., and Axelsen, E., *Gut*, **11**, 727 (1970).
- ¹² Macara, I. G., Hoy, T. G., and Harrison, P. M., *Biochem. J.*, **126**, 151 (1972).
- ¹³ Bryce, C. F. A., and Chrichton, R. R., *Biochem. J.*, **133**, 301 (1973).
- ¹⁴ Gabuzda, T. G., and Pearson, J., *Biochim. biophys. Acta*, **194**, 50 (1969).
- ¹⁵ Shepp, M., Toff, H., Yamada, H., and Gabuzda, T. G., *Br. J. Haemat.*, **22**, 377 (1972).
- ¹⁶ Linder-Horowitz, M., Ruettinger, R. T., and Munro, H. N., *Biochim. biophys. Acta*, **200**, 442 (1970).
- ¹⁷ Alpert, E., Coston, R. L., and Drysdale, J. W., *Nature*, **65**, 471 (1973).
- ¹⁸ Powell, L. W., *Postgraduate Med. J.*, **46**, 200 (1970).
- ¹⁹ Drysdale, J. W., and Munro, H. M., *Biochem. J.*, **95**, 851 (1965).
- ²⁰ Drysdale, J. W., *Biochim. biophys. Acta*, **207**, 256 (1970).
- ²¹ Riechetti, P. G., and Drysdale, J. W., *Ann. N.Y. Acad. Sci.*, **209**, 163 (1973).
- ²² Alfrey, C. F., in *Proceedings of international conference on iron storage and transport proteins* (London, 1973).
- ²³ Alfrey, C. F., Lynch, E. C., and Whitley, C. E., *J. Lab. clin. Med.*, **70**, 419 (1967).
- ²⁴ Hazard, J. T., and Drysdale, J. W., in *Proceedings of international conference on iron storage and transport proteins* (London, 1973).

A phenylethylamine oxidising defect in migraine

For some two hundred years, certain foodstuffs have been known to trigger migraine attacks¹. In 1967, knowing that cheese is a common dietary trigger to migraine, Hanington² fed tyramine, which it is known to contain, to affected subjects and by this action was able to initiate headache episodes.

Even though cheese and tyramine-containing foods are frequently implicated as headache precipitants, by far the commonest of the dietary triggers is chocolate³ which does not contain tyramine according to a recent analysis performed by the British Food Manufacturing Industries Research Association. This analysis did however reveal the presence of large amounts of phenylethylamine, at least 3 mg per 2 ounce bar; of the other common dietary precipi-

Table 1 Effect of phenylethylamine in chocolate-sensitive migraine

	Lactose	Phenylethylamine
Headache	6	18
No headache	30	18

tants tested, a large number of cheeses, but not all, contained phenylethylamine, as do some red wines (M. J. Saxby, personal communication). It therefore seemed of interest to determine whether oral phenylethylamine alone is able to initiate an attack of migraine in susceptible subjects.

Forty-six migraine sufferers who claimed, in answer to a questionnaire, that their headache attacks were provoked by chocolate, volunteered to take part in the following sighting experiment, which was organised along similar lines to that reported earlier for tyramine².

A capsule containing lactose was sent to each, together with a further questionnaire to be completed and returned 24 h after it had been ingested. Those taking part were asked not to take the capsule within 48 h of a spontaneous migraine attack or when they thought one might be expected. After this questionnaire had been returned, a capsule similar to the earlier one but containing phenylethylamine (3 mg) was sent, together with another questionnaire and similar instructions for its return. Patients were not aware of the contents of either capsule.

Ten subjects failed to complete the trial after lactose ingestion only, which had been followed by headache in five of them. Thirty-six completed the trial, 3 men and 33 women. The results are shown in Table 1. Included in this table are two patients who developed a headache after both lactose and phenylethylamine. One had a mild generalised headache on both occasions but felt sick only after phenylethylamine; the other had a unilateral mild to moderate headache after both. Of the four subjects who had symptoms only after lactose one had no headache at all but saw patterns before her eyes, two had a moderately severe unilateral headache and the fourth a bilateral moderately severe headache.

There seems to be a *prima facie* case for the existence of a cause and effect relationship between phenylethylamine ingestion and migraine headache in certain patients.

One unexplained feature of the phenylethylamine-provoked headaches is that their onset was delayed for approximately 12 h after amine ingestion, a period of the same order as that observed clinically after chocolate

Table 2 Human platelet monoamine oxidase activity

Substrate	Migraine	Patient Control	Chocolate-sensitive migraine
Phenylethylamine	4.38 ± 0.51 (28)	10.66 ± 0.96 (26)	4.16 ± 0.36 (12)
	P < 0.001		P < 0.001
Tyramine	13.52 ± 1.60 (28)	28.48 ± 2.64 (26)	11.80 ± 1.26 (12)
	P < 0.001		P < 0.001
Dopamine	5.61 ± 0.51 (28)	8.29 ± 1.09 (26)	5.04 ± 0.44 (12)
	P < 0.05		P < 0.01
5-Hydroxytryptamine	14.54 ± 1.17 (16)	21.70 ± 1.82 (14)	15.84 ± 1.88 (7)
	P < 0.01		P < 0.05

Each value is expressed as nmol of substrate deaminated per 30 min incubation per mg protein at 37° C. Protein nitrogen was measured according to the method of Lowry *et al.*²⁴ using human serum albumin as standard. Figures in parentheses represent number of subjects.

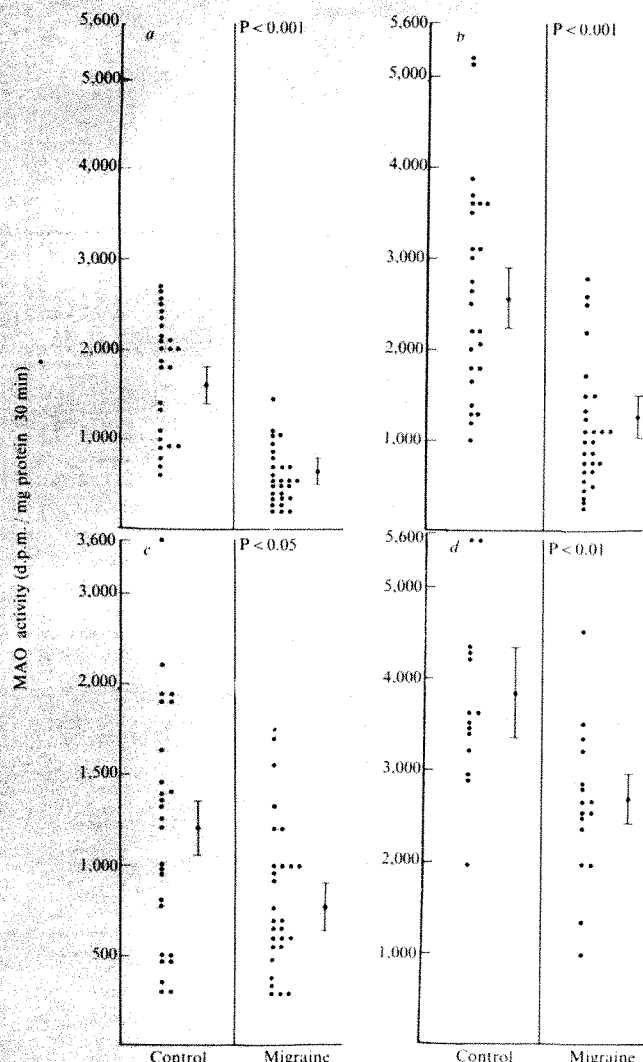


Fig. 1 Distribution of monoamine oxidase activity in blood platelets from migrainous and control subjects. Activity is expressed as d.p.m. radioactive deaminated metabolites formed per mg protein per 30 min using *a*, phenylethylamine; *b*, tyramine; *c*, dopamine; and *d*, 5-hydroxytryptamine as substrates.

ingestion in susceptible subjects. During the earlier experiment with tyramine² two groups of reactors were observed, with peak onset at 3 h and 12 h respectively. The dose of tyramine used then (100 mg) was considerably larger than that of phenylethylamine (3 mg) in the present trial. A 12 h lag period is puzzling and at first sight, seems to rule out simple trigger action such as that envisaged by one of us¹: taking as his starting point some published observations⁵ in which tryptamine and 5-hydroxytryptamine, when introduced into the afferent pulmonary circulation of an experimental animal, had been shown to cause a release of prostaglandins and other vasoactive agents into the systemic circulation, he suggested that migraine might, in a sense, be a pulmonary disease. A triggering agent arriving at the lungs from the gastrointestinal tract might, as in the perfused animal lung, provoke a secondary release of vasoactive substances to act on the cerebral vasculature. This interpretation has received a measure of experimental support⁶. Recently Y. S. Bakhle (personal communication) has found phenylethylamine to be an effective releaser of vasoactive substances in certain species, rather more so than tyramine⁷.

If Sandler's hypothesis⁴ were valid, one possible explanation to account for a facilitated release of vasoactive substances from the lungs into the systemic circulation would be the access of a higher concentration of monoamine into the afferent pulmonary vessels brought about by defective

inactivation. The predominant inactivation pathway of phenylethylamine involves oxidative deamination by monoamine oxidase (MAO)⁸. There is evidence of decreased platelet MAO activity during headache episodes in migrainous patients^{9,10}.

Johnson¹¹, working with enzymes from rat brain, obtained data which he interpreted in terms of the existence of two types of MAO, termed A and B, and similar conclusions have been drawn from findings on human neural tissue¹². It has been claimed that these forms are immunologically separable¹³. Type A is specific for 5-hydroxytryptamine¹¹ and noradrenaline¹⁴; tyramine¹¹ and dopamine¹⁵ are substrates for both A and B forms. Phenylethylamine is a preferred substrate for MAO B (ref. 16). It therefore seemed to us that, in migraine, we might be dealing with a specific defect of MAO B. To test this hypothesis, a study of platelet MAO activity, using several different substrates, was carried out in a further series of migrainous patients and in non-migrainous controls.

During a headache-free period, venous blood samples from the unselected group of 28 migrainous subjects (9 male, 19 female), age range 20 to 65 yr, were collected into plastic tubes containing lithium heparin. Nonmigrainous volunteers of a similar age range provided control samples. Platelets were collected¹⁷ and stored deep-frozen before assay. MAO activity was measured radiometrically¹⁸ using ¹⁴C-labelled phenylethylamine, tyramine, dopamine and 5-hydroxytryptamine as substrates.

The results are shown in Fig. 1 and Table 2. A highly significant decrease in phenylethylamine oxidising ability was observed in migraine compared with control subjects, but in addition, a difference in tyramine oxidation of a similar order was apparent between the two groups. A smaller but still significant decrease in activity towards dopamine was present. The deficit of MAO A may be less pronounced, for there was a small but significant decrease in 5-hydroxytryptamine-oxidising ability in the migrainous group. In retrospect the decrease in the normally high¹⁷ benzylamine-oxidising ability in platelets from migrainous subjects, observed by another group¹⁹, is compatible with our data on impaired phenylethylamine oxidation, for benzylamine seems to be another substrate of MAO B (ref. 11).

It seems likely, however, that the A and B classification is a gross over-simplification^{19,20}: one of the multiple forms of solubilised MAO which has been isolated electrophoretically²¹ seems to possess, for instance, an extremely high preference for dopamine as substrate. Nevertheless, whichever of the multiple forms of MAO turns out to be implicated in migraine, it seems quite possible that the deficit of phenylethylamine and tyramine-oxidising ability is of functional significance.

The migrainous patients under investigation were a mixed group, some with headache provoked by chocolate or other foodstuff and others without history of dietary precipitant. Platelet samples obtained from patients with dietary compared with non-dietary migraine variants, however, showed no significant difference in their ability to oxidise any of the substrates investigated (Table 2). Before firm conclusions can be drawn from data of this type, more information is needed showing to what extent any platelet MAO defect reflects a similar deficit in small intestinal or perhaps, pulmonary MAO. A pulmonary deficit, for instance, would make larger concentrations of amine available locally, thus helping the threshold to be reached for an 'all-or-none' release²² of vasoactive substances from the lung into the systemic circulation in accordance with Sandler's hypothesis⁴. Were this hypothesis to prove invalid, an alternative and simpler explanation might be that monoamines which escape destruction in the lungs²³ because of an MAO deficit exert some direct action on the cerebral vasculature. Preliminary observation (R. J. Stephens, personal communication), however, indicate that phenylethylamine has little or no direct effect

of this type, at least in the rat.

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¹ Fothergill, J., *Medical Observations and Enquiries*, 6, 103-137 (1784).

² Hanington, E., *Br. med. J.*, 2, 550-551 (1967).

³ Hanington, E., Horn, M., and Wilkinson, M., in *Background to Migraine: Third Migraine Symposium* (edit. by Cochrane, A. L.), 113-119 (Heinemann, London, 1970).

⁴ Sandler, M., *Lancet*, 1, 618-619 (1972).

⁵ Alabaster, V. A., and Bakhle, Y. S., *Br. J. Pharmac.*, 40, 582P-583P (1970).

⁶ Sandler, M., *Headache* (in the press).

⁷ Bakhle, Y. S., and Smith, T. W., *Br. J. Pharmac.*, 46, 543P-544P (1973).

⁸ Edwards, D. J., and Blau, K., *Biochem. J.*, 130, 495-503 (1972); Edwards, D. J., and Blau, K., *Biochem. J.*, 132, 95-100 (1973).

⁹ Sandler, M., Youdim, M. B. H., Southgate, J., and Hanington, E., in *Background to Migraine. Third Migraine Symposium* (edit. by Cochrane, A. L.), 103-112 (Heinemann, London, 1970).

¹⁰ Scuteri, F., Buffoni, F., Anselmi, B., and Del Bianco, P. L., *Res. Clin. Stud. Headache*, 3, 245-251 (1972).

¹¹ Johnson, J. P., *Biochem. Pharmac.*, 17, 1285-1297 (1968).

¹² Goridis, C., and Neff, N. H., *Proc. Soc. exp. Biol. Med.*, 140, 573-574 (1972).

¹³ McCauley, R., and Racker, E., *Molec. cell. Biochem.*, 1, 73-81 (1973).

¹⁴ Goridis, C., and Neff, N. H., *Br. J. Pharmac.*, 43, 814-818 (1971).

¹⁵ Neff, N. H., *Adv. biochem. Psychopharmac.* (in the press).

¹⁶ Yang, H.-Y. T., and Neff, N. H., *J. Pharmac. exp. Ther.*, 187, 365-371 (1973).

¹⁷ Collins, G. G. S., and Sandler, M., *Biochem. Pharmac.*, 20, 289-296 (1971).

¹⁸ Robinson, D. S., Lovenberg, W., Keiser, H., and Sjoerdsma, A., *Biochem. Pharmac.*, 17, 109-119 (1968).

¹⁹ Tipton, K. F., Houslay, M. D., and Garrett, N. J., *Nature new Biol.*, 246, 213-214 (1973).

²⁰ Youdim, M. B. H., Holzbauer, M., and Woods, H. F., *Adv. biochem. Psychopharmac.* (in the press).

²¹ Collins, G. G. S., Sandler, M., Williams, E. D., and Youdim, M. B. H., *Nature*, 225, 817-820 (1970).

²² Bakhle, Y. S., and Smith, T. W., *Br. J. Pharmac.* (in the press).

²³ Vane, J. R., *Br. J. Pharmac.*, 35, 209-242 (1969).

²⁴ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, 193, 265-275 (1951).

to inhibition of cell multiplication by serum limitation^{4,5}. In addition, different fractions of serum stimulate the growth of untransformed mouse 3T3 fibroblasts and 3T3 cells transformed by SV40 (SV3T3) in culture⁶ which suggests the involvement of different growth promoting molecules for normal and transformed cells. It has been shown that a new pituitary hormone, fibroblast growth factor⁷ (FGF), with a glucocorticoid, hydrocortisone, and with a nonspecific carrier protein could completely replace exogenously added serum for the induction of the complete mitogenic response in lines of BALB/c 3T3 cells⁷, whereas virtually no effects were observed on virally transformed cells (P. S. R., W. S. and D. G.; manuscript in preparation). In the presence of serum exhausted for its growth-initiating ability only FGF was required to initiate or increase DNA

Table 1 Additions to growing cultures

Additions	3T3		ts 3T3 Cl.1		Py3T3	
	a, c.p.m.	b, cells	a, c.p.m.	b, cells	a, c.p.m.	b, cells
32°C						
None	1.4	4.4	2.4	4.2	2.2	6.5
FGF	6.1	10.8	2.2	4.3	2.3	6.7
Serum	7.1	12.8	3.9	6.9	4.1	10.4
39°C						
None	1.6	4.0	2.0	4.1	6.5	2.1
FGF	7.6	6.6	9.4	6.6	5.6	2.0
Serum	6.0	7.1	9.7	6.7	9.3	2.9

BALB/c 3T3 cells¹³, polyoma transformed BALB/c cells (Py3T3), and temperature-sensitive transformed BALB/c 3T3 cells (ts 3T3 Cl.1) were plated at 10⁵ per 5 cm Petri dish and grown in Dulbecco's Modified Eagles Medium (5 ml) in 2% calf serum (Colorado) at 32° or 39° in a 10% CO₂ atmosphere. The CO₂ pressure was slightly varied to maintain a constant pH in the medium at the two temperatures. After 2 or 3 d when the final cell density was about 3 × 10⁵ per plate (3T3: 3.0 and 3.1; ts 3T3: 2.9, 3.0; and Py3T3: 1.6 and 4.5 × 10⁵ cells at 39°C or 32°C respectively) 50 ng ml⁻¹ of FGF or 10% serum (6 mg ml⁻¹) were added to the growing cultures. Autoradiography of control dishes without additions showed that more than 70% of all cell nuclei became radioactively labelled during the first 12 h after the time of addition of ³H-thymidine. Cultures were radioactively labelled with 3 µCi ml⁻¹ ³H-methyl thymidine at 3 µM from 7 until 30 h after additions, or the cells were counted in a Coulter Counter 48 h afterwards. The c.p.m. of ³H-thymidine incorporated into DNA (ref. 16) per 10 cells (a) or the number of cells per 5 cm Petri dish × 10⁻⁵ were recorded (b). FGF, a homogeneous polypeptide of molecular weight 13,400 was purified from bovine pituitary glands as described previously⁷.

synthesis and cell division; the hydrocortisone concentration present in serum was considered to be sufficiently high not to require further addition⁷. We have now investigated the ability of FGF to promote the growth of cell mutants of BALB/c 3T3 which show temperature dependent changes in many properties characteristic of the transformed state (W. E., manuscript in preparation). We found that FGF initiates DNA synthesis and cell multiplication only at the non-permissive temperatures ('normal' state) but fails to initiate or promote cell growth at permissive temperatures (transformed state).

Cell mutants with temperature-sensitive (ts) lesions in many properties characteristic of transformed cells have been described for SV3T3⁸ and baby hamster kidney⁹ (BHK) cells, similar to the earlier reports of temperature sensitive variants of BHK obtained by transformation with a thermosensitive mutant of polyoma virus¹⁰. Recently Eckhart has selected several different clones of BALB/c 3T3 cells which show temperature dependent properties. Properties of the three mutant clones used in this paper are stable and can be summarised. Cells from these clones show transformed morphology and ability to grow in agar at 32° C but normal morphology and lack of growth in agar at 39° C (W. E., unpublished observations). Growth rates are 1.8, 2.0 and 1.7 times as fast at 32° C as at 39° C in medium containing 10% serum. Their final cell densities in medium containing low serum concentrations are approximately 10 to 3-fold higher at 32° C than at 39° C (for example for ts 3T3 clone 1 in 0.25%, 0.5% or 2% serum

Cell transformation mutants are not susceptible to growth activation by fibroblast growth factor at permissive temperatures

CHEMICAL¹ or viral² transformation of fibroblasts in tissue culture results in cell variants with different properties from the original cells (see ref. 2 for review). These properties include morphological changes, surface alterations³ and an increased growth ability under various conditions. This changed pattern in growth control of many transformed cells is manifested *in vitro* by their ability to grow in agar, to attain higher saturation densities in monolayer cultures and to be much less responsive

Table 2 Stimulation of DNA synthesis in different cells

Additions	3T3	ts Cl.1	ts Cl.10	ts Cl.X	Py3T3
32° C					
None	1.1	0.5	13.8	8.0	46.0
FGF	110	0.8	15.8	10.2	42.3
39° C					
None	1.0	1.2	0.8	1.2	70.4
FGF	85.6	64.5	42.5	56.8	59.2

Abbreviations: 3T3; BALB/c 3T3; ts Cl.1, Cl.10, Cl.X: temperature sensitive transformed BALB/c 3T3 cell-lines; Py3T3: polyoma transformed BALB/c 3T3. The percentage of ^3H -thymidine incorporated into DNA compared with serum addition is shown. Cells were grown in DEM and 10% serum as described (Table 1) at either 32° C or 39° C until confluent (10^6 cells per 5 cm plate). Medium was then removed, cell monolayers were washed twice with DEM containing $250 \mu\text{g ml}^{-1}$ bovine serum albumin (BSA) and fresh DEM containing 0.2 ng ml^{-1} FGF, $0.5 \mu\text{g ml}^{-1}$ hydrocortisone (cortisol, Calbiochem) and $250 \mu\text{g ml}^{-1}$ BSA. After 3 d 50 ng ml^{-1} of FGF or 10% serum (6 mg ml^{-1}) was added and the cultures were radioactively labelled with ^3H -thymidine from 8 until 34 h after additions, the c.p.m. of ^3H -thymidine incorporated into DNA was recorded. Results for no addition or the addition of FGF are expressed as the percentage of the c.p.m. incorporated into DNA in the presence of 10% serum. Autoradiography of parallel cultures showed that the percentage of radioactivity labelled nuclei in the population with FGF addition were: for 3T3, 95 and 90; for ts 3T3 Cl.1, 1 and 60; for Cl.10, 10 and 40; for Cl.X, 7 and 51; for Py3T3, 40 and 53 at 32° C and 39° C respectively. Without addition of 50 ng ml^{-1} FGF the percentage of radioactively labelled nuclei was less than 1% for 3T3, ts 3T3 Cl.1 at 32° C and 39° C, Cl.10 and Cl.X at 39° C while for the remainder: Cl.10, 7%; Cl.X, 4% at 32° C; Py3T3, 41% at 32° C and 60% at 39° C. The percentages of the cell population which had divided by 48 h after addition of 50 ng ml^{-1} of FGF were for 3T3, 96 and 86; for Cl.1, 4 and 82; for Cl.10, 16 and 87; for Cl.X, 9 and 88; for Py3T3, 50 and 54 at 32° C and 39° C respectively.

the final cell densities were 19 and 2.2, 20 and 3, 32 and 11×10^6 cells per 5 cm dish at 32° C and 39° C respectively). At low serum concentrations (Table 2), ts 3T3 Cl.1 and to a lesser extent Cl.10 and Cl.X can be made to exhibit density-dependent inhibition of growth at 32° C similar to nontransformed 3T3 cells and unlike the virally transformed lines Py3T3 and SV3T3⁵. After addition of 20% serum to such quiescent cultures at 32° C they synchronously initiate DNA synthesis followed by cell division at times compatible with an original cell population arrested in the G_0 phase of the cell cycle (unpublished results).

Addition of FGF to cultures of BALB/c 3T3 growing slowly with 2% serum in the medium increased both rates of DNA synthesis and cell division but failed to increase growth rates of polyoma transformed 3T3 cells (Py3T3) (Table 1). For ts-3T3 clone 1 (ts-Cl.1) cells addition of FGF specifically increased cellular growth rates at 39° C but not at 32° C. 10% serum addition markedly increased DNA synthesis and cell division in all cultures (Table 1). The ability of FGF to initiate DNA synthesis in quiescent cultures was also checked. It was however impossible to maintain resting cultures of BALB/c 3T3 cells with Dulbecco's modified Eagle's medium (DEM) and only BSA, and hence low concentrations of FGF (0.2 ng ml^{-1}) and hydrocortisone were added to prevent cell deterioration¹¹. Addition of saturating amounts of FGF (50 ng ml^{-1}) to quiescent cultures of 3T3, ts Cl.1, Cl.10, Cl.X at 39° C induced a 40- to 80-fold increase in DNA synthesis but virtually no increase in resting cultures of ts Cl.1 at 32° C. It was more difficult to obtain completely quiescent cultures of ts Cl.10 and Cl.X at 32°, but FGF failed to increase the small residual amount of DNA synthesis (Table 2). Py3T3 cells continued synthesising DNA at half the rates of those in 10% serum but no additional stimulation was observed upon FGF addition (Table 2). Approximately twice the amount of FGF was added to the cultures in Tables 1 and 2 than was required for complete induction of DNA synthesis and cell division in resting cultures of BALB/c 3T3 cells (manuscript in preparation). Further increased additions of FGF up to five times the amounts added in Table 2 failed to stimulate DNA synthesis in mutant cultures maintained at 32° C,

whereas 10% serum addition induced more than 95% of the cells to synthesise DNA and divide.

The ability of various hormones to promote the initiation of DNA synthesis in resting cultures of ts Cl.1 at either 32° C or 39° C is shown in Table 3. Insulin at high, nonphysiological concentrations was reported to induce DNA synthesis and cell division in fibroblasts maintained in the presence of serum^{13,14}. But in the absence of serum, insulin at physiological (Table 3) (10^{-9} to 10^{-8} M) or nonphysiological (10^{-6} M) (unpublished results) concentrations failed to induce DNA synthesis in more than 5% of the cell populations at either temperature. FGF without hydrocortisone still induced DNA synthesis in a significant fraction (22%) of the mutant cells at 39° C but not at 32° C. With hydrocortisone the induction of DNA synthesis was comparable to that with serum at 39° C while only serum induces DNA synthesis at 32° C.

A comparative biochemical study of normal and transformed cells in tissue culture is complicated by the fact that often two very different cell types are being compared (for example,

Table 3 Induction of DNA synthesis by different hormones

Additions	^3H -DNA (c.p.m. $\times 10^{-6}$)		Labelled nuclei (%)	
	32° C	39° C	32° C	39° C
none	0.5	0.2	1.8	1.0
HC	0.7	0.2	2.6	1.2
FGF	0.7	5.8	2.8	22.7
insulin	0.4	0.8	2.1	3.0
HC + FGF	0.4	10.8	3.1	66.2
HC + insulin	0.3	0.3	2.0	1.6
HC + FGF + insulin	0.7	9.9	3.2	67.2
serum	12.0	11.5	92.5	71.7

Cell cultures of the temperature sensitive transformed BALB/c 3T3, clone 1 were plated and grown as described in Table 2. At confluency (10^6 cells per 5 cm dish) the medium was removed and the cell monolayers washed twice as described. Medium containing 0.2 ng ml^{-1} FGF and $250 \mu\text{g ml}^{-1}$ BSA was added back and the cultures incubated at their respective temperatures for another 3 d. To the resting cultures either $0.5 \mu\text{g ml}^{-1}$ of hydrocortisone (HC), 50 ng ml^{-1} fibroblast growth factor (FGF), 50 ng ml^{-1} insulin (bovine pancreas, Calbiochem) or 10% serum was added as indicated. Cultures were radioactively labelled with either $3 \mu\text{Ci ml}^{-1}$ ^3H -thymidine at $3 \mu\text{M}$ for DNA synthesis or at $1 \mu\text{M}$ for autoradiography, from 10 until 34 h after additions. The c.p.m. of ^3H -thymidine incorporated into DNA $\pm 10^{-6}$ per culture or the fraction of cells with radioactively labelled nuclei is shown for various hormone additions. Cell numbers were 9.2 and 9.3×10^6 cells per 5 cm dish at 32° C and 39° C respectively before the additions, and FGF + HC addition increased cell numbers to 9.4 and 17.0×10^6 at 32° C and 39° C respectively measured after 48 h.

chromosomal differences in SV3T3 and 3T3), and the observed differences could arise as a result of the transformation event itself or as a consequence of the selection for a particular stable transformant. With cells possessing thermosensitive lesions in the expression of the transformed phenotype, however, the latter difficulty is obviated. We now show that the growth-initiating properties of a mutant 3T3 cell can be controlled by a single purified polypeptide hormone at the nonpermissive temperature; this ability is lost at the permissive temperature when the cells exhibit many of the properties characteristic of the transformed state. Other components in serum can control the growth at this permissive temperature. The cellular site of action of many of the polypeptide hormones is the adenyl cyclase in the plasma membrane¹⁴. We have previously shown that FGF can directly activate the membrane-bound guanyl cyclase in 3T3 but not in SV3T3 cells (manuscript in preparation). This suggests that the temperature-sensitive lesion in the mutant cells is either wholly or in part caused by a membrane change. This change possibly prevents the polypeptide hormone from elevating intracellular cGMP concentrations to a level which may be responsible for the initiation of cell growth¹¹.

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- ¹ Mondal, S., and Heidelberger C., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 219–225 (1970).
- ² Dulbecco, R., *Science*, **166**, 962–968 (1969).
- ³ Burger, M., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 994–1001 (1969).
- ⁴ Holley, R. W., and Kiernan, J., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 300–304 (1968).
- ⁵ Dulbecco, R., *Nature*, **227**, 802–806 (1970).
- ⁶ Paul, D., Lipton, A., and Klinger, I., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 645–648 (1971).
- ⁷ Gospodarowicz, D., *Nature*, **240**, 123–127 (1974).
- ⁸ Renger, H. C., and Basilico, C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 109–114 (1972).
- ⁹ DiMayorca, G., Greenblatt, M., Trauthen, T., Soller, A., and Giordano, R., *Proc. natn. Acad. Sci. U.S.A.*, **40**, 46–49 (1973).
- ¹⁰ Dulbecco, R., and Eckhart, W., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1775–1781 (1970).
- ¹¹ Seifert, W., and Rudland, P. S., *Nature*, **248**, 138–140 (1974).
- ¹² Temin, H. M., *J. cell. Physiol.*, **69**, 377–384 (1967).
- ¹³ Vaheri, A., Ruoslahti, E., Hovi, T., and Nordling, S., *J. cell. Physiol.*, **81**, 355–364 (1973).
- ¹⁴ Robison, G. A., Butcher, R. W., and Sutherland, E. W., in *Cyclic AMP*, 22–29 (Academic Press, New York, 1971).
- ¹⁵ Aaronson, S. A., and Todaro, G. J., *J. cell. Physiol.*, **74**, 141–148 (1968).
- ¹⁶ Rudland, P. S., *Proc. natn. Acad. Sci. U.S.A.* **71**, 750–754 (1974).

Manipulation of sexual physiology by brain stimulation in insects

In insects the pars intercerebralis (PI) is a median protocerebral neurosecretory centre associated with postcerebral neurohaemal organs, the corpora cardiaca (CC). Numerous investigations based on ablations or implantations of the PI and CC have shown that the neurohormones released by the CC intervene in many physiological processes. *In vitro*, by means of electrical stimulations, it was possible to show that, as in vertebrates, there is a coupling between depolarisation and secretion¹. One may therefore contemplate the development of a new technique of investigation in neuro-endocrinology by electrical stimulation *in vivo* of neurosecretory cells. We were able to provoke by this way a precocious ovarian development in female locusts.

Experiments involving ablation and implantation showed that oocyte maturation and egg laying are under the endocrine control of the PI^{2–5} and a pair of endocrine glands, the corpora allata (CA)⁶. We tested two different situations by using the females of a species (*Locusta migratoria*) in which the oocytes develop immediately after metamorphosis (the first batch of eggs is laid after about 18 d) and the females of a species (*Anacridium aegyptium*) subject to an ovarian diapause of 6 months. The opening of a dorsal tegumentary cephalic window enables access to the PI which is superficial. The stimulatory electrode used is a stainless steel wire 30 μ m in diameter, insulated in an epoxy resin. Rectangular pulses of 30 μ A and 1 ms are applied at 5 s⁻¹ for 10 min between the stimulatory electrode

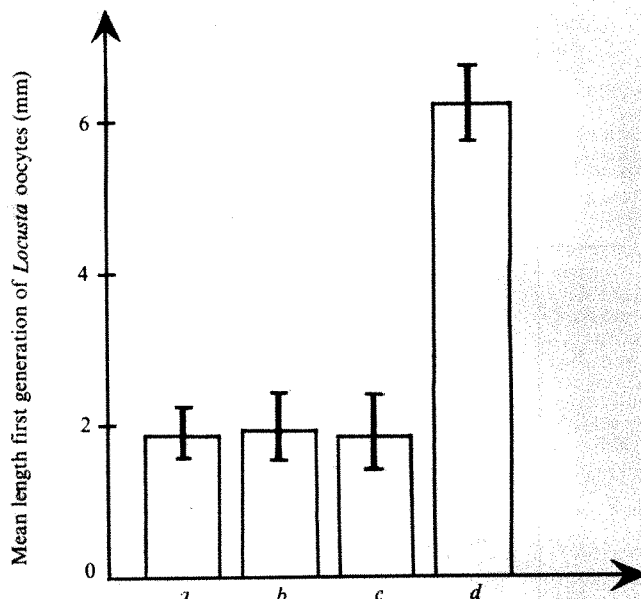


Fig. 1 Mean length (mm) of the first generation of oocytes in *Locusta*, on day 10 of imaginal life, after various electrical stimulations. *a*, Controls (electrode placed in the PI without passage of current; 16 animals, 1 died); *b*, stimulation of the ventral nerve cord; (16 animals, 2 died); *c*, stimulation of the deutocerebrum (16 animals, none died); *d*, stimulation of the PI (17 animals, 2 died, 2 laid one batch of eggs).

introduced into the PI and a thoracic indifferent electrode. After stimulation, the dorsal fin tegumentary window is closed. It is thus possible to achieve a stimulation every 48 h; each individual was subject to two stimulations (*Locusta*) or three (*Anacridium*). The results were compared with those obtained in the controls subjected to the introduction of an electrode without passage of current, the stimulation of the first abdominal ganglion, and stimulations of various regions of the brain (deutocerebrum in *Locusta*, tritocerebrum in *Anacridium*). The conditions were the same as for the operated animals. A parallel histological study enables verification of the placement of the electrode and the emptying of the cell bodies of the PI after stimulation. The results were expressed in terms of the length of the first oocyte generation 8 d (*Locusta*) (Fig. 1) or 28 d after the first stimulation (*Anacridium*) (Fig. 2).

In *Locusta*, stimulation of the PI is accompanied by a rapid growth of the oocytes. The first generation oocytes on day 10 have an average length of more than 6 mm (Fig. 1d); in all the controls (Fig. 1a, b and c), this length remains below 2 mm. This rapid growth even induced 2 of the 15 stimulated animals to lay eggs before day 10; in the usual breeding conditions egg laying occurs only about day 18.

In the case of *Anacridium*, the adult females used were about 2 month old and in natural conditions would have laid eggs only 6 months later. The triple stimulation of the PI provoked a partial emptying of the cell bodies of the PI and rupture of the ovarian diapause. All the animals laid eggs at least once in the 28 d following the first stimulation; one of them even laid twice. The first oocyte generation at this time had an average length close to 7 mm, so the eggs were mature (Fig. 2d). In all the controls (Fig. 2a, b and c) the average length of this same generation is less than 2 mm and storage of the protocerebral neurosecretion was observed^{4,5}.

Finally, it is possible to associate the stimulation method with that of ablations. Thus, in the case of *Anacridium*, stimulation of the PI of allatectomised animals results in almost zero growth of the oocytes (Fig. 2e): the gonadotrophic role of the CA is thus confirmed. Conversely, ani-

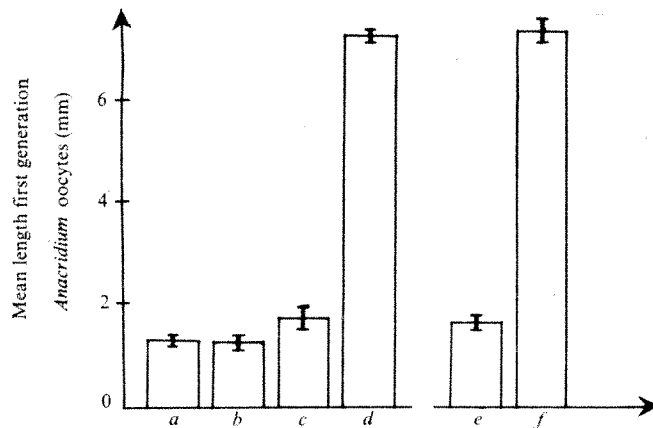


Fig. 2 Mean length (mm) of the first generation of oocytes in *Anacridium* 28 days after various electrical stimulations. *a*, Controls (electrode placed in the PI without passage of current; 14 animals, none died); *b*, stimulation of the ventral nerve cord (14 animals, 2 died); *c*, stimulation of the tritocerebrum (15 animals, 1 died); *d*, stimulation of the PI (13 animals, 2 died, all the survivors laid one batch of eggs, one animal laid two batches); *e*, stimulation of the PI after allatectomy (14 animals, 4 died); *f*, stimulation of the PI after section of the allatocardiac nerves (14 animals, 5 died, 8 animals laid one batch of eggs and 1 contained chorionated eggs ready to be laid).

imals in which the CA have been disconnected (by section of the allatocardiac nerves) experience, after stimulation of the PI, the same growth of the oocytes as that observed in animals in which the CA were not disconnected (Fig. 2*d* and *f*). The mode of action of the PI on the CA is therefore established as humoral rather than nervous.

Our results show unequivocally that the method used provokes a certain release of protocerebral neurosecretion followed by intense activation of oocyte maturation. In the case of *Locusta*, one may thus obtain precocious maturation and in the case of *Anacridium* rupture of the ovarian diapause. This method seems to be more efficacious—at least in the case studied—than the classic techniques of implantation. In effect, to obtain a simple maturation of the ovaries, in *Anacridium*, it is necessary to implant four active PI or four active CA³.

Certain general nonspecific stimulations, such as forced activity of the animals or the electrical stimulation of various regions of the brain, may provoke the start of a histologically detectable emptying of the cells of the PI and a very slight development of the oocytes². There is no doubt that the results obtained here are due to a specific stimulation of the PI and not to a general activation: the stimulation of a wide variety of regions of the central nervous system never provoked any maturation of the oocytes.

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¹ Hodgson, E. S., and Geldiay, S., *Biol. Bull. mar. biol. Lab., Woods Hole*, **117**, 275 (1959).

² Highnam, K. C., *Q. Jl microsc. Sci.*, **103**, 57 (1962).

³ Girardie, A., *Bull. Soc. zool. Fr.*, **91**, 423 (1966).

⁴ Geldiay, S., *J. Endocr.*, **37**, 63 (1967).

⁵ Girardie, A., and Granier, S., *J. Insect Physiol.*, **19**, 2341 (1973).

⁶ Joly, P., *Ann. biol.*, **34**, 97 (1958).

Properties of a calcium channel in snail neurones

In most cases where the action potential of nerve cells has been studied the initial inward current has been found to be carried by sodium ions. More recently calcium has been shown to be an important charge carrier in the inward current of vertebrate cardiac muscle, vertebrate smooth muscle and many nerve cell bodies¹. It is therefore of interest to know whether the properties of the calcium conductance mechanism are similar to those which have been described for sodium conductance. An important property of the sodium conductance mechanism is its reduction or 'inactivation' by a conditioning depolarisation prior to an action potential and its increase by conditioning hyperpolarisation.

This activation and inactivation property can be studied in voltage clamp experiments. The membrane potential is displaced from the holding potential (V) to a conditioning potential (V_c) and then to a fixed test potential (V_t) (see Fig. 2). The inward current (I_t) during the test potential is measured and I_t relative to the maximum inward current obtainable with that test pulse (I_t/I_{max}) is plotted against conditioning potential V_c . In squid giant axon Hodgkin and Huxley² showed that this inactivation behaviour was described by the expression:

$$I_t/I_{max} = [1 + \exp(V_h - V_c)/k_h]^{-1} \quad (1)$$

where V_h is the potential at which $I_t/I_{max} = 0.5$ k_h is a shape parameter.

In the *Aplysia* giant neurone Geduldig and Gruener³ found that in Ca-free saline containing Na the relationship between inward current and conditioning potential was similar to that in squid giant axon. In Na-free saline containing Ca ions, however, a hyperpolarising conditioning pulse reduced the inward current. Geduldig and Gruener³ have suggested that there is a fundamental difference between the activation mechanisms for Na and Ca ions in *Aplysia*. In *Helix pomatia* Neher⁴ found a similar reduction of inward current following a hyperpolarising conditioning pulse in normal saline containing both Na and Ca ions but proposed that it was due to activation of a fast transient outward potassium current which subtracted from the inward current. The experiments described here on *Helix aspersa* neurones suggest that the apparent hyperpolarising inactivation of Ca conductance is due to an early outward current which is sensitive to tetraethylammonium (TEA) and calcium ions.

The neurone used in each experiment was identified as Cell A⁵. This cell lies in the right parietal ganglion and shows an inward current in both Ca-free and Na-free salines in voltage clamp experiments. The cell was impaled with two 3 M KCl-filled microelectrodes of resistance 4–6 MΩ and 0.8–1.3 MΩ respectively. A voltage clamp circuit similar to that of Geduldig and Gruener³ was used, giving a voltage rise time of about 100 μs. Normal saline contained NaCl 75 mM, KCl 5 mM, MgCl₂ 15 mM, CaCl₂ 10 mM, Tris-Cl 5 mM, buffered to pH 7.5. In Na-free saline NaCl was replaced by 83mM Tris-Cl. In Ca-free saline CaCl₂ was replaced by MgCl₂. In (Na, Ca)-free saline both substitutions were performed.

In an attempt to eliminate the effect of early outward currents a current-separation procedure was used as follows. A clamping run consisting of a series of different conditioning potentials each followed by the same test potential was performed in the test solution (either Na-free or Ca-free saline). An identical run was then performed in (Na, Ca)-free saline, in which there is no inward current. (There may be an outward current carried by Na and Ca ions but because of their low intracellular concentrations this was small at the test potential used). The current in (Na, Ca)-free saline at a particular time after the beginning of the test pulse was then subtracted from that in the test solution to give the Na or Ca inward current alone. (All runs were interspersed with controls in normal saline.)

Figure 1a shows the results of such an experiment using Ca-free saline as the test solution. I_i/I_o is plotted against the potential of the conditioning pulse. I_o is the inward current with no conditioning pulse and is used instead of I_{max} as the cell is maximally activated at the holding potential. The conditioning potential was 300 ms long which was found to be long enough for maximum effect. The solid line is drawn to equation (1) with $V_h = -27$ mV and $k_h = -4$ (for squid $k_h = -7$). Thus the inactivation curve for sodium current is adequately described by the same equation as used for squid giant axon.

Figure 1b shows a similar experiment in Na-free saline (in which the inward current is carried by Ca ions). The solid line

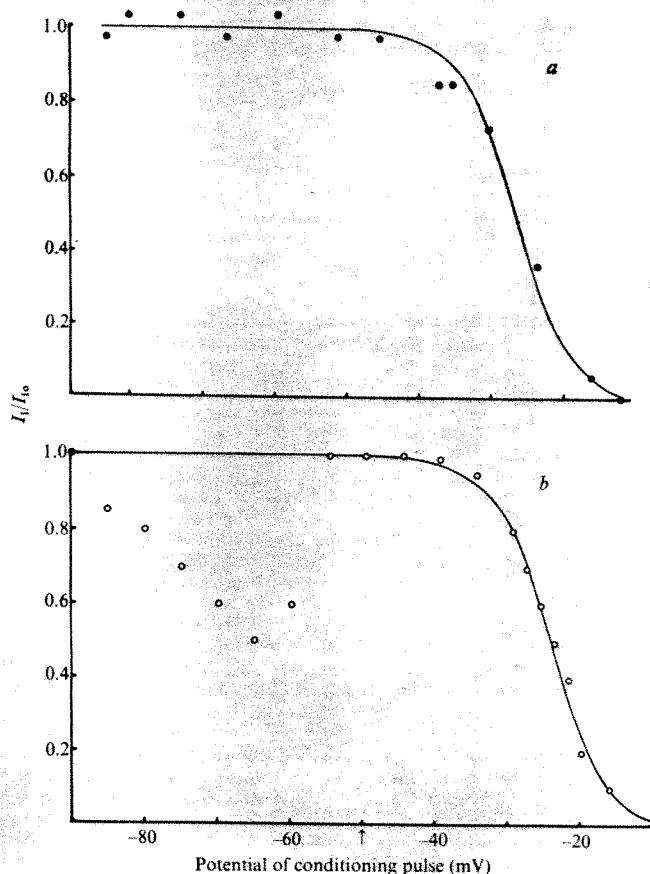


Fig. 1 *a*, Inactivation curve in Ca-free (Na-containing) saline. Ordinate: peak inward current relative to peak inward current with no conditioning pulse (I_i/I_o). Conditioning pulse length 300 ms. Holding potential (V) (arrowed) = -48 mV. Test pulse potential (V_e) = $+14$ mV. Cell 104. Diameter $180\text{ }\mu\text{m}$ 22.5°C . Solid line drawn to equation (1) with $V_h = -27$ mV; $k_h = -4$. *b*, Inactivation curve in Na-free (Ca-containing) saline. Conditioning pulse length 500 ms. $V_e = +10$ mV. Cell 139. Diameter $230\text{ }\mu\text{m}$ 20°C . Solid line drawn to equation (1) with $V_h = -24$ mV; $k_h = -4$.

is again drawn to equation (1). For depolarising conditioning pulses a good fit is obtained. But with increasing hyperpolarising pulses I_i/I_o falls steeply and then rises more slowly towards unity. Thus there is a considerable deviation from the behaviour described by equation (1). If these results arise from a property of the inactivation system for Ca conductance then this system must be both different from, and more complicated than, that for sodium conductance in *Helix* and squid giant axon. An alternative explanation is that the deviation may arise from the effect of an early outward current which was not eliminated by the current separation procedure. An early outward current activated by hyperpolarising conditioning pulses exists in many molluscs including *Aplysia*, *Helix pomatia* and *Helix aspersa*^{4,6}. If this early outward current was smaller in the (Na, Ca)-free saline than it was in the Na-free saline

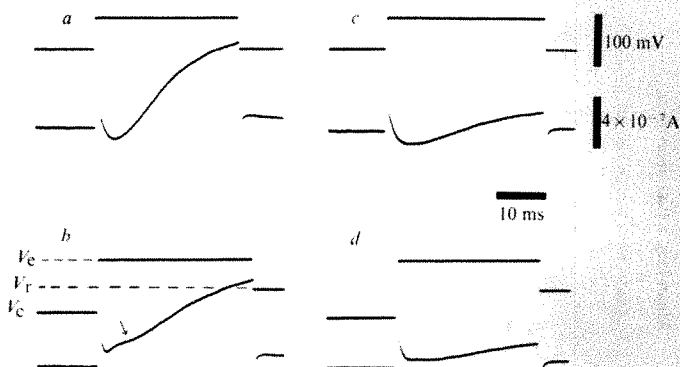


Fig. 2 Voltage clamp records showing effect of TEA. Upper traces voltage, lower traces current. Inward current is shown by a downward deflection. Holding Potential (V) = -48 mV; $V_e = +12$ mV. *a*, no prepulse in Na-free saline; *b*, 500 ms prepulse to -108 mV in Na-free saline (arrow shows early outward current); *c*, no prepulse Na-free saline with 50 mM TEA Cl; *d*, 500 ms prepulse to -108 mV Na-free saline with 50 mM TEA Cl. Cell 168. Diameter $205\text{ }\mu\text{m}$ 19°C .

then the amount of outward current subtracted would be too small. This could be, for example, if the early outward current was to some extent Ca-sensitive. There are two ways in which the absence of Ca in the external saline could affect the size of the early outward current. (1) The change in Ca concentration could shift the activation curve of the early outward current along the voltage axis as found for Na and K conductance in squid⁷. (2) The late outward potassium current in *H. aspersa* has a Ca dependent component so it is possible that the early outward current has a similar component⁸.

In order to test whether this deviation from equation (1) did indeed arise from a Ca-sensitive early outward potassium current the effect of 50 mM TEA was tested. This was a sufficient concentration to reduce late K currents. Figure 2 shows current records obtained under voltage clamp in Na-free saline with and without TEA. It can be seen that TEA reduces the late K current and also the early outward current seen with a hyperpolarising prepulse, but does not affect the Ca inward current. Figure 3 shows the effect of TEA on the dip in the inactivation curve in Na-free saline. Open circles are points obtained by subtraction of currents recorded in (Na, Ca)-free saline from currents in Na-free saline—that is, a similar experiment to that shown in Fig. 1b. Open triangles are results in which the same subtraction was performed when both salines contained 50 mM TEA-Cl substituted for Tris-Cl. The deviation from equation (1) is greatly reduced and in one experiment was abolished altogether. The TEA has no effect

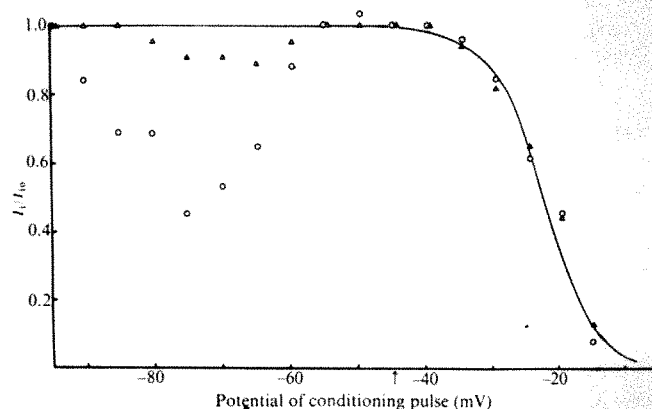


Fig. 3 Effect of TEA on inactivation curve in Na-free saline. \circ , Without TEA; \triangle , with 50 mM TEA Cl; V (arrowed) = -45 mV; $V_e = +15$ mV. Cell 178. $180\text{ }\mu\text{m}$ 21°C . Solid line drawn to equation (1) with $V_h = -23$ mV; $k_h = -4$.

on the normal inactivation by depolarising conditioning pulses.

From the results of these TEA experiments it seems likely that the deviation of Ca inactivation from equation (1) is due as Neher suggested to an early outward potassium current. The current subtraction method of compensating for this current is inadequate, probably because the current is sensitive to calcium. This cannot be shown directly but when K currents are reduced using TEA the deviation from equation (1) is also reduced or abolished. It has recently been shown that hyperpolarising inactivation of Ca current in guinea pig smooth muscle can be blocked by TEA ions⁹. The deviation is also abolished by large hyperpolarising conditioning pulses to potentials more negative than -90 mV probably because the early outward potassium current shifts in time relative to the inward Ca current with increasing prepulse potential.

Thus, it appears that the inactivation behaviour of Ca-conductance in *H. aspersa* is similar to that of the Na-conductance and can be described adequately by equation (1). It is unclear in *H. aspersa* whether the Na and Ca ions pass through the same channel or separate channels. But in vertebrate heart muscle the Ca inward current can be obtained relatively free from other currents in normal saline and does not show hyperpolarising inactivation¹.

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- ¹ Reuter, H., *Prog. Biophys. molec. Biol.*, **26**, 1-43 (1973).
- ² Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **116**, 497-506 (1952).
- ³ Geduldig, D., and Gruener, R., *J. Physiol., Lond.*, **211**, 217-244 (1970).
- ⁴ Neher, E., *J. gen. Physiol.*, **58**, 36-53 (1971).
- ⁵ Kerkut, G. A., and Meech, R. W., *Comp. Biochem. Physiol.*, **19**, 819-832 (1966).
- ⁶ Klee, M. R., Faber, D. S., and Heiss, W. D., *Science*, **179**, 1133-1135 (1973).
- ⁷ Frankenhauser, B., and Hodgkin, A. L., *J. Physiol., Lond.*, **137**, 218-244 (1957).
- ⁸ Meech, R. W., and Standen, N. B., *J. Physiol., Lond.*, (in the press).
- ⁹ Vassort, G., *J. Physiol., Lond.*, (in the press).

Resistance of mitotic B lymphocytes to cytotoxic effects of anti-Ig serum

THE use of antisera with specificity for cell surface antigens of T and B lymphocytes has become an increasingly important tool in cellular immunology, being extensively used both preparatively, and analytically^{1,2}. Very little is known, however, about the possibilities of variation in antibody-induced cytotoxicity during the various stages of the cell cycle of normal T and B lymphocytes. Studies mostly examining histocompatibility or virus-associated antigens on synchronised cell cultures of various tumour cell lines have yielded conflicting findings; in some cases cells seem to be most sensitive to antibody and complement during mitosis and perhaps early G₁³⁻⁵, whereas in others, the target cells have been judged to be insensitive in all phases except G₁^{6,7}.

If, depending on the nature of the lymphocyte and cell surface antigen, there is a similar variation to antibody-induced cytotoxicity during different phases of the cell cycle, this could have clear practical and theoretical implications. In particular, antisera are sometimes used to eliminate or assess cells in antigen-activated lymphoid cell populations which may contain a significant proportion of specifically activated cells, in various stages of the cell cycle, including mitosis. In this communication we

present evidence to show that although mouse B lymphocytes are uniformly killed by a heterologous anti-mouse immunoglobulin serum (anti-Ig) in the presence of complement, B cells in mitosis are resistant to such treatment.

Heterologous anti-Ig sera are being used with increasing frequency to kill B lymphocytes (see for example, refs 8-11); since B cells, in contrast to T cells, contain a dense coat of cell surface Ig (ref. 12), the cytotoxicity of anti-Ig can be selective for B cells after appropriate absorption. The preparation and cytotoxic properties of the anti-Ig used in these experiments is described in Table 1. In agreement with the other results^{9,10}, treatment of spleen cells with the anti-Ig, after it had been absorbed with thymocytes,

Table 1 Cytotoxic effects of rabbit anti-mouse Ig serum plus complement on mouse lymphocytes

Lymphoid cell source	Cytotoxic index
Thymus	2
Thoracic duct	12
Lymph node	21
Bone marrow	37
Spleen	48
Spleen	> 95
Spleen†	88
Spleen‡	14
Spleen§	12

The rabbit anti-mouse Ig serum used in these experiments was prepared as follows: 50 ml of neat serum from a large number of CBA mouse donors was precipitated in saturated ammonium sulphate to isolate gamma globulins²⁶. The precipitate was dissolved in saline and the process repeated twice. The final precipitate was dissolved in 4 ml of saline. Two millilitres of this was mixed with 2 ml of Freund's complete adjuvant (Burroughs Wellcome) and injected intramuscularly into a New Zealand White rabbit. The rabbit was boosted 2 and 4 week later with an intravenous injection of 1 ml of the saline solution containing the dissolved mouse γ globulins. Two weeks later the rabbit was bled, the serum collected and frozen at -20° C in 0.5 ml aliquots. The antiserum was first heat inactivated at 56° C for 30 min. Before use it was absorbed with CBA mouse thymocytes by incubating three volumes of antiserum with one volume of packed washed thymocytes for 60 min at 0° C with shaking every 5 min. The antiserum was tested for cytotoxicity using a two stage microcytotoxicity system¹⁰, in which the first stage was carried out at 0° C so as to prevent capping of cell surface Ig by the anti-Ig¹³. Centrifugations were carried in the cold. Assessment of dead cells was calculated by trypan blue exclusion testing. Normal rabbit serum was used as a control and the results expressed as a cytotoxic index, that is:

$$\frac{\% \text{ dead cells (anti-Ig)} - \% \text{ dead cells (NRS)}}{\% \text{ dead cells (NRS)} \times 10^{-2}}$$

*Anti-Ig not absorbed with thymocytes in this case.

†Cells obtained from adult thymectomised, lethally irradiated, bonemarrow reconstituted mice, 2 months after reconstitution.

‡Anti-Ig absorbed with mouse γ globulins (M γ G) as well as thymocytes. Neat anti-Ig (0.5 ml) was mixed with 0.5 ml of Sepharose beads coated with M γ G by cyanogen bromide activation. The incubation was carried out at 0° C for 1 h. Anti-Ig not absorbed with M γ G and diluted 1:4 as a control gave over 40% kill in this experiment.

§The primary stage of the assay was carried out at 37° C.

inhibited the *in vitro* response to *Escherichia coli* LPS but spared the PHA response (M. J. D. and R. S. K., unpublished observations). The % of cells killed from the various tissue sources is very close to the known corresponding percentage of B cells as assessed by other methods². If the primary stage of the assay was carried out at 37° C instead of 0° C, cytotoxicity was greatly diminished as found by Takahashi *et al.*¹³ which is presumably due to polarisation and loss of cell surface Ig induced by the anti-Ig ('capping') which occurs at 37° but not at 0° C (ref. 14). Absorption of the antiserum with mouse γ -globulin also markedly reduced the cytotoxic activity of the anti-Ig. Before thymus cell absorption, the anti-Ig killed almost all nucleated cells in the spleen and thymus, but after absorption, it was

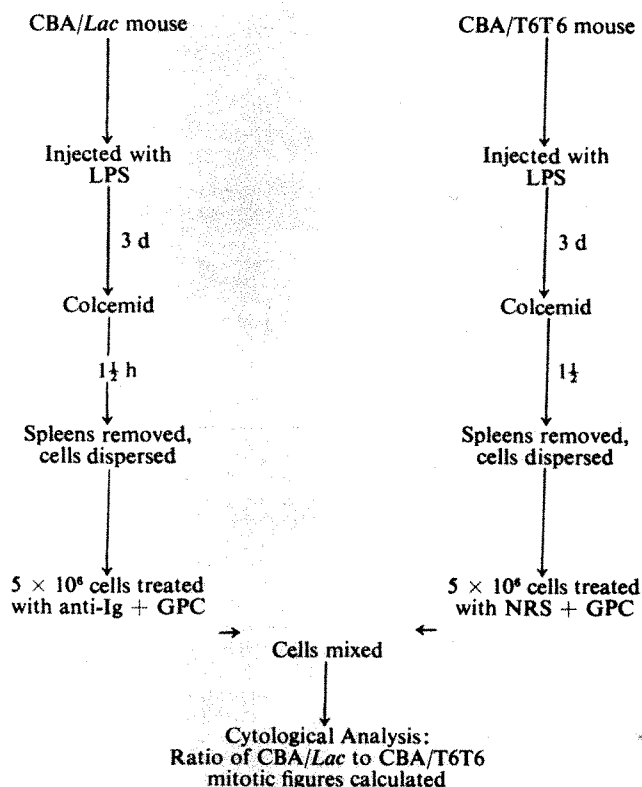


Fig. 1 The assay system¹⁶ used to treat and evaluate the effects of antisera (in this case, anti-Ig) plus GPC on mitotic B lymphocytes. Mitotic T cells are induced by stimulation with oxazolone and removing draining lymph nodes 3 d later¹⁶. Not shown above is the control in which both CBA/Lac and CBA/T6T6 cells are treated with NRS + GPC prior to mixture and cytological analysis. Since the cells are mixed in equal numbers, the ratio of mitotic figures should be roughly 50:50. The effect of the antiserum treatment on the CBA/Lac to CBA/T6T6 mitotic figures in the two different mixtures¹⁶. The percentage of mitotic cells killed was calculated by a cytotoxic index in which the ratio of CBA/Lac to CBA/T6T6 in the antiserum treated mixture is subtracted from the NRS-treated mixture and divided by the latter ratio. The figure is then multiplied by 100. CBA/Lac can be distinguished from CBA/T6T6 mitotic figures by virtue of the fact that the latter contain a tiny pair of marker chromosomes which can be easily visualised when the cells are arrested in metaphase¹⁷. The LPS used was *E. coli* 0:55 LPS (Difco) and the colcemid was purchased from Ciba Labs Ltd. (Horsham, Sussex).

devoid of cytotoxic activity when tested against thymocytes, though still able to kill almost 50% of spleen cells.

The anti-Ig was then examined for its ability to kill B lymphocytes arrested in mitosis. In addition, other antisera were tested such as anti- θ (ref. 1), anti-H2 and anti-MBLA¹⁵. The assay system used has been described in detail¹⁶; it uses chromosome marker techniques¹⁷ and is outlined diagrammatically in Fig. 1.

In Table 2 are recorded the results of experiments in which several types of antisera were tested for their ability to inhibit oxazolone-induced mitotic T cells¹⁶, or LPS-induced mitotic B cells. Anti- θ eliminated 100% of mitotic cells found in the draining lymph nodes of oxazolone-stimulated mice, as did anti-H2 serum. Conversely both anti-MBLA (a marker for B cells¹⁵) and anti-Ig were without such an effect. When LPS-induced mitotic cells were treated, anti-H2 and anti-MBLA were both found to be highly cytotoxic, whereas anti- θ was totally devoid of any such activity. But the anti-Ig was also found incapable of killing these LPS-induced mitotic cells in spite of the fact that 47% of the LPS-immune spleen cells could be killed in the same suspension as assessed by trypan blue cytotoxic testing.

In a parallel experiment, *E. coli* LPS was used as a mitogen to activate spleen B cells *in vitro*¹⁸ and PHA (Burroughs

Wellcome) was used to activate T cells¹⁹ in separate control cultures using methods described previously²⁰. Again anti-Ig was found to possess little ability to eliminate mitotic cells activated *in vitro* by LPS, whereas anti- θ eliminated about 90% of PHA-activated mitotic cells (Table 3).

It may be that the cell surface Ig molecules associated with B cells undergo a density or possibly distributional change²¹ during mitosis such that the cells are no longer capable of being lysed by anti-Ig and GPC. Antigen density on cells is known to play a critical role in complement-mediated cytolytic reactions²² as well as antigen distribution²¹. Buell and Fahey²³ tested Ig-bearing and Ig-producing human lymphoid culture cell lines during different phases of the cell cycle for the presence of Ig by direct immunofluorescence techniques. They found little Ig apparent immediately before, during, and immediately after mitosis; in contrast the G₁ and S phases were highly positive²³. Similar results were obtained by Takahashi *et al.*²⁴.

A factor which should not be overlooked in these experiments is the possible effect of colcemid itself on cell surface Ig: mitotic inhibitors such as colcemid are known to have an effect on lymphocyte receptor mobility²⁵. It should, however, be stressed that anti-Ig + GPC still killed almost 50% of spleen cells from LPS-injected mice which had been given colcemid *in vivo*, and where the assay was carried out in the presence of colcemid (Table 2).

We have also found that treatment of LPS-immune, colcemid treated spleen cells with rabbit anti-Ig followed by a sheep anti-rabbit immunoglobulin serum in the presence of complement was still incapable of killing cells arrested in mitosis (Table 3) (R. S. K., unpublished observations), which suggests that there may be loss of cell surface Ig on the mitotic cells. We are presently studying the cell surface Ig on mitotic B cells using peroxidase staining and electron microscopic

These results indicate that treatment of antigen-activated lymphoid cell populations with anti-Ig plus complement to kill B cells (for example, ref. 11) may spare a small number of specifically activated cells which could partially

Table 2 Cytotoxic effects of various antisera plus complement on non-dividing and on mitotic T and B lymphocytes

Stimulus	Lymphoid source	Antiserum	Cytotoxic index (trypan blue)	%Kill of mitotic lymphocytes
Oxazolone	lymph node	anti- θ *	68	100
Oxazolone	lymph node	anti-H2†	>95	100
Oxazolone	lymph node	anti-MBLA‡	23	0
Oxazolone	lymph node	anti-Ig	20	0
<i>E. coli</i> LPS	spleen	anti- θ	28	0
<i>E. coli</i> LPS	spleen	anti-H2	>95	98
<i>E. coli</i> LPS	spleen	anti-MBLA	32	84
<i>E. coli</i> LPS	spleen	anti-Ig	47	4

The assessment of cytotoxic activity on mitotic lymphocytes of the various antisera was carried out as described previously¹⁶ and shown in Fig. 1. Just before mixing the antiserum-treated CBA/Lac cells with the control treated CBA/T6T6 cells, a small aliquot was withdrawn and assayed for the % cells killed by the dye exclusion method. Control treated CBA/Lac cells were also tested and the cytotoxic index calculated. An equivalent aliquot was withdrawn from the CBA/T6T6 cell suspensions to control for cell loss before mixing and cytological analysis. Draining axillary lymph nodes or spleens were removed 3 d after stimulation of the mice. In all cases 250 μ l of neat antiserum was used to treat 5×10^6 nucleated cells except in the case of anti-MBLA, where only 100 μ l was used. This gave a lower cytotoxic index than is usually obtained and may account for only 84% of the LPS activated mitotic cells being killed.

*The anti- θ_{C3H} serum which is specific for T cells¹ was prepared as described previously¹⁶, and its properties have been described elsewhere¹⁶.

†The anti-H2 serum was made by injecting Balb/C (H2^a) with 20×10^6 spleen cells from CBA (H2^b) donors at weekly intervals for 6 weeks and bleeding the mice 1 week after the last injection.

‡The anti-MBLA serum which is specific for B cells after absorption was made according to the method of Raff *et al.*¹⁵ and absorbed with M γ G as well as thymocytes.

Table 3 Cytotoxic effects of anti- θ serum or anti-immunoglobulin serum plus GPC on mitotic lymphocytes activated *in vitro* by PHA or *E. coli* LPS

Experiment	Mitogen	Serum treatment of spleen cells from:		Cytological analysis		Ratio of Lac/T6T6	% Kill by antiserum
		CBA/Lac cultures	CBA/T6T6 cultures	CBA/Lac	CBA/T6T6		
1	PHA	a anti- θ	NMS	6	94	0.064	90.4
		b NMS	NMS	40	60	0.667	
2	PHA	a anti-Ig	NRS	35	65	0.538	— 9.1
		b NRS	NRS	35	67	0.493	
3	LPS	a anti- θ	NMS	33	67	0.493	21.8
		b NMS	NMS	39	61	0.639	
4	LPS	a anti-Ig	NRS	39	61	0.639	8.1
		b NRS	NRS	41	59	0.695	

On day 0, CBA/Lac or CBA/T6T6 spleen cells were incubated with either PHA or LPS in a manner as previously described (1×10^6 cells per culture)²⁰. On the evening of day 2 colcemid was added to each of the cultures and they were collected 16 h later. Using experiment 1 as an example, 5×10^6 PHA activated CBA/Lac spleen cells were incubated with 0.25 ml of neat anti- θ , followed by 0.5 ml of 1:8 GPC. The surviving cells were mixed with 5×10^6 PHA activated CBA/T6T6 spleen cells which had been treated with NMS+GPC and the mixture was then subjected to cytological analysis. In 1a, only six of the mitotic figures counted were CBA/Lac in origin. A control mixture (1b) in which both the CBA/Lac and CBA/T6T6 cells were treated with

NMS+GPC, showed that 40 of 100 mitotic figures were CBA/Lac in origin. The % kill by anti- θ was calculated by subtracting the ratio of CBA/Lac to CBA/T6T6 figures in the anti- θ treated mixture from the ratio found in the NMS mixture, dividing by the ratio found in the NMS mixture

and multiplying by 100, that is $\frac{0.667-0.064}{0.667} \times 100 = 90.4\%$ kill

of PHA activated CBA/Lac mitotic cells by anti- θ +GPC treatment. A similar procedure was done where anti-Ig was used. In these cases 5×10^6 PHA or LPS activated spleen cells were incubated with 0.25 ml of anti-Ig at 0°C for 1 h before the GPC was added.

compromise the use of such antisera in such cases. This in turn suggests a means by which it may be possible to 'negatively' select for (and subsequently isolate) a small number of specifically activated B cells. Treatment of a population of antigen-activated, colcemid-treated, lymphoid cells by anti-Ig plus complement should kill all B cells except those arrested in metaphase, and the majority of these mitotic cells should have specificity for the activating antigen.

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Note added in proof: Preliminary electron microscope studies using immunoperoxidase staining have revealed that mitotic B cells do in fact contain large amounts of cell surface Ig molecules, similar to that found on resting B cells.

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- ¹ Raff, M. C., *Transplant Rev.*, **6**, 52 (1971).
- ² Greaves, M. F., Owen, J. J. T., and Raff, M. C., in *T and B Lymphocytes: Origins, Properties and Roles in Immune Responses* (Excerpta Medica and American Elsevier, Amsterdam, 1973).
- ³ Pasternak, C. A., Warmley, A. M. H., and Thomas, B. D., *J. cell Biol.*, **50**, 562 (1971).
- ⁴ Graham, J. M., Sumner, M. C. B., Curtis, D. H., and Pasternak, A., *Nature*, **246**, 291 (1973).
- ⁵ Sumner, M. C. B., Collin, R. C. L. S., and Pasternak, C. A., *Tissue Antigens*, **3**, 477 (1973).
- ⁶ Cikes, M., and Friberg, S., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 566 (1971).
- ⁷ Lerner, R. A., Oldstone, M. B. A., and Cooper, N. R., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2584 (1971).
- ⁸ Takahashi, T., Mand, J. J., Carswell, E. A., and Thorbecke, G. J., *J. Immun.*, **107**, 1520 (1971).
- ⁹ Forman, J., and Möller, G., *Transplant Rev.*, **17**, 108 (1973).
- ¹⁰ Gorczynski, R., and Rittenberg, M. B., *J. Immun.*, **112**, 47 (1974).
- ¹¹ Rabinowitz, S. G., and Adler, W. H., *J. Immun.*, **110**, 1344 (1973).

- ¹² Raff, M. C., *Immunology*, **19**, 637 (1970).
- ¹³ Takahashi, T., Old, L. J., McIntire, K. R., and Boyse, E. A., *J. exp. Med.*, **134**, 815 (1971).
- ¹⁴ Taylor, R. B., Duffus, N. P. H., Raff, M. C., and DePetris, S., *Nature new Biol.*, **233**, 225 (1971).
- ¹⁵ Raff, M. C., Nase, S., and Mitchison, N. A., *Nature*, **230**, 50 (1971).
- ¹⁶ Kerbel, R. S., Elliott, E. V., and Wallis, V. J., *Cell Immun.*, **11**, 146 (1974).
- ¹⁷ Ford, C. E., in *Tissue Grafting and Irradiation* (edit. by Micklem, H. S., and Loutit, J. F., 197 (Academic Press, New York, 1966).
- ¹⁸ Greaves, M. F., and Janossy, G., *Transplant Rev.*, **11**, 87 (1972).
- ¹⁹ Doenhoff, M. J., Davies, A. J. S., Leuchars, E., and Wallis, V., *Proc. R. Soc. Lond.*, **B176**, 69 (1970).
- ²⁰ Doenhoff, M. J., Janossy, G., Greaves, M. F., Gomer, K. J., and Snajdr, N., *Clin. exp. Immun.*, (in the press).
- ²¹ Bernoco, D., Cullen, S., Schudeller, G., Trinchieri, G., and Ceppellini, R., in *Histocompatibility Testing*, 527 (Munksgaard, Copenhagen, 1972).
- ²² Winn, H. J., *Ann. N.Y. Acad. Sci.*, **101**, 23 (1962).
- ²³ Buell, D. N., and Fahey, J. L., *Science*, **164**, 1524 (1969).
- ²⁴ Takahashi, M., Yagi, Y., Moore, G. E., and Pressman, D., *J. Immun.*, **103**, 834 (1969).
- ²⁵ Yahara, I., and Edelman, G. M., *Nature*, **246**, 152 (1973).
- ²⁶ Campbell, D. H., Garvey, J. S., Cremer, N. E., and Sussdorf, D. H., in *Methods in Immunology*, 189 (Benjamin, New York, 1970).

Yeast-mycelial conversion induced by N-acetyl-D-glucosamine in *Candida albicans*

DIMORPHISM in fungi is generally defined as a reversible transition from a yeast habit of growth (Y) to a mycelial one (M)¹. In *Candida albicans* Y→M transition can occur rapidly in serum^{2,3}, serum substitutes and other natural⁴⁻⁶ and synthetic media⁷. In a few hours the yeast cell or blastospore forms a germ tube which grows as a true mycelium^{8,9}. Since the cellular form depends on wall construction¹⁰, marked modifications in the organisation of wall components are expected to, and in fact do, occur during morphogenesis. Nevertheless, there is chemical¹¹, cytochemical and ultrastructural evidence^{12,13} that the differences between Y and M walls of *C. albicans* are essentially quantitative, strongly suggesting that hyphal conversion in this yeast is controlled by the modulation of pre-existing enzymatic activities rather than by any new factors.

In view of the usefulness and reliability of germ-tube formation in *C. albicans* as a model of hyphal morphogenesis, it is unfortunate that most of the media so far used are too chemically complex to enable an understanding of the biochemical basis of this phenomenon. We are therefore investigating the minimal requirement for germ-tube formation and show here that acetylglucosamine meets this requirement. We shall also discuss a working hypothesis for a possible role of chitin synthetase in the mycelial conversion of *C. albicans*.

Strain A_{IF} of *C. albicans* (Robin) Berkhout was grown as described elsewhere¹³. A standard inoculum of 0.8×10^8 cells (essentially pure blastospores from the late logarithmic phase of growth) was given to a mixture containing the test substances, at the desired concentrations, dissolved in H₂O or in buffer imidazole-HCl 0.01 M, pH 6, 7 (standard), in a

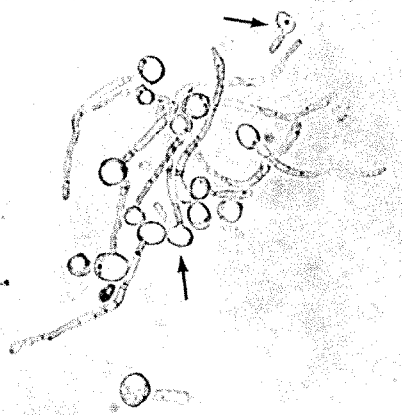


Fig. 1 Germ tubes from blastospores of *C. albicans* after 5 h of incubation in imidazole-HCl buffer, 0.01 M, pH 6.6 containing acetylglucosamine 2.5 mM and MnCl₂ 0.1 mM. The arrows point to cells with two germ tubes ($\times 500$).

final volume of 1 ml. After temperature equilibration, reagents were added and the mixture kept under slight but constant agitation at 37° C. At fixed time intervals, a drop of cell suspension was examined under the light microscope for germ-tube formation, this being recorded as the percentage of elements having at least one recognisable germ tube per 200 elements, regardless of the length of the tube. N-acetyl-D-glucosamine (standard *purissimum*) was obtained from Fluka, Switzerland. All other substances employed were of standard reagent grade.

Owing to the heterogeneity of the initial blastospore suspension, the process of germ-tube formation was essentially asynchronous but in appropriate inducing conditions (see below) after 5 h more than 90% of the cells showed a germ tube of variable length, with many germ

Table 1 Potentiation or inhibition of acetylglucosamine-induced germ-tube formation in *Candida albicans*

Inducer (2.5 mM)	Medium and ions	Other substances	Germination (%)
absent	H ₂ O; Mg ²⁺ or Mn ²⁺ (0.1 mM)	—	3–5
absent	imidazole-HCl Mn ²⁺ (0.1 mM)	—	6–8
present	H ₂ O	—	40–42
present	imidazole-HCl	—	44–47
present	H ₂ O; Mn ²⁺ (0.1 mM)	—	60–65
present	imidazole-HCl Mn ²⁺ (0.1 mM)	—	74–78
absent	imidazole-HCl	cysteine (1 mM)	0
present	imidazole-HCl	cysteine (1 mM)	0

Germination values were taken from two independent determinations, after 4 h incubation at 37° C. Imidazole-HCl buffer was used at pH 6.7 and acetylglucosamine-water measured approximately pH 6.65.

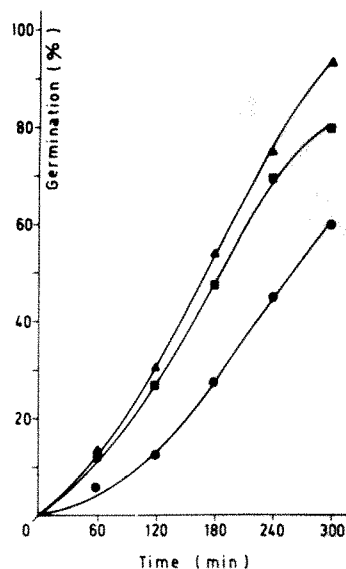


Fig. 2 Time course of germ-tube formation induced by acetylglucosamine 2.5 mM, alone (—●—●—●—), plus MgSO₄ 0.1 mM (—■—■—■—), or plus MnCl₂ 0.1 mM (—▲—▲—▲—). Tests were carried out in imidazole-HCl buffer 0.01 M, pH 6.6, at a temperature of 37° C. Values are means of two independent determinations.

tubes appearing as true hyphae (Fig. 1). The time course of germ-tube formation in various conditions of stimulation with acetylglucosamine is shown in Fig. 2. The germination curve took a sigmoid shape, the maximum rate being recorded between the second and the fourth hour of incubation. The addition of 0.1 mM MgSO₄ or MnCl₂ greatly enhanced the germinating effect of acetylglucosamine (slightly more evident with Mn²⁺). But higher doses of these ions caused no activation but instead a variable degree of inhibition, which is maximal (germination completely abolished) in the presence of 20 mM Mg²⁺ or 25 mM Mn²⁺. Germination did not occur in phosphate Na/K buffer 0.1 M even at high doses of inducer (50 mM) and the same imidazole-HCl buffer was somewhat inhibitory when used at a concentration (0.5 M) higher than that of the standard reaction mixture. Therefore, considering the lack of specificity of these ionic inhibitors, it seems likely that germ-tube formation is sensitive to a high ionic strength. Indeed, high levels of germination can be induced by acetylglucosamine in plain water. Both in water and in imidazole-HCl buffer, the presence of 0.1 mM cysteine completely suppressed the germination (Table 1).

Acetylglucosamine behaves as a strong and rather specific inducer. At 20 μ M it causes, in the presence of 0.1 mM Mn²⁺, 20 to 25% germination after 2 h at 37° C; in this case, however, the phenomenon does not reach completion, even if the mixture is incubated for long periods (12 h). The minimum necessary concentration to achieve full germination falls in the range 0.8–1 mM. Of the analogues tested for their effect on germination (glucose, glucosamine, galactosamine, fructose, glucose-1 phosphate and acetyl-galactosamine) none had any effect until a concentration of 20 mM was reached; at 50 mM, glucosamine and acetyl-galactosamine showed a very slight effect (5 to 8% of germination) of the same order of magnitude as that recorded by incubating blastospores in Mn²⁺-enriched imidazole buffer in the absence of acetylglucosamine (Table 1).

As shown in Fig. 3, the useful ranges of temperature and pH for acetylglucosamine-induced germination in *C. albicans* are rather narrow, the optima falling at around 37° C at pH 6.6. In particular, the germination was markedly low or absent at alkaline pH, at variance with what occurs in serum or other media^{3,6}.

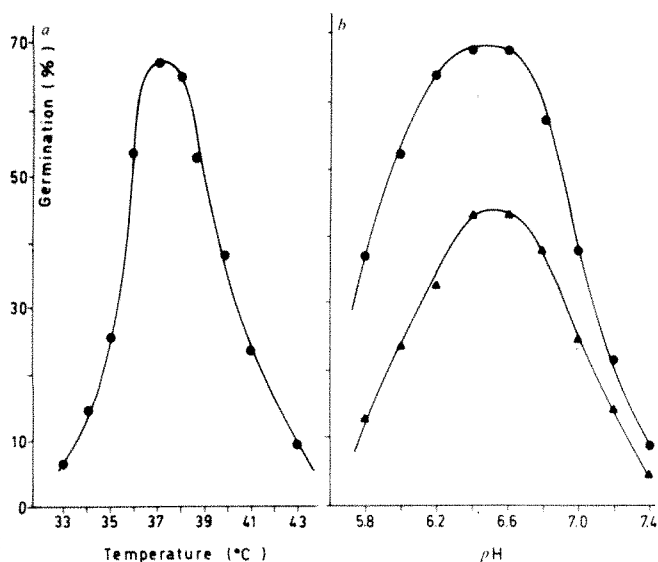


Fig. 3a Temperature dependence of *C. albicans* blastospore germinations induced by acetylglucosamine 2.25 mM in imidazole-HCl 0.01 M, pH 6.6 containing Mn²⁺ 0.1 mM. Effect of pH on germ-tube formation by acetylglucosamine 2.25 mM in imidazole-HCl buffer with (—●—●—●—) or without (—▲—▲—▲—) Mn²⁺ 0.1 mM. The tests were performed at 37°C. Values are means of two independent determinations.

Thus even at very low doses, acetylglucosamine, unlike related carbohydrates, can induce significant germination in the absence of other carbon and nitrogen sources. This, together with consideration of the experimental conditions under which acetylglucosamine induces germ-tube formation in *C. albicans* suggests a specific activating role for acetylglucosamine.

While appropriate experiments must still be performed before an understanding of the mechanism of acetylglucosamine action is reached, we shall consider the possibility that acetylglucosamine directly triggers some key reaction in the dimorphism in *C. albicans*. An obvious candidate for such a role is the chitin synthetase because N-acetyl-D-glucosamine is both the monomer and a specific activator of such an enzyme in yeasts (and other fungi)¹⁴⁻¹⁷.

A controlled activation of chitin synthesis could be a simple way to meet the requirement for increased chitin content of the emerging germ tube and of the hyphal wall¹¹⁻¹³. In *Mucor rouxii*, acetylglucosamine is incorporated, through its UDP derivative, into chitin and it has been observed that chitin synthetase activity is highest at hyphal tip¹⁵. The experimental conditions under which acetylglucosamine induces germ-tube formation in *C. albicans* are comparable to those reported as characteristic of a chitin synthetase assay *in vitro* (pH range, activation by Mg²⁺ and Mn²⁺, specificity)¹⁴. Certainly, as shown in studies on septum formation, the cellular control over chitin synthesis may be highly sophisticated¹⁸ but it is attractive, in line with the results reported above, to think that the levels of free acetylglucosamine in the cell may have a part in controlling overall chitin synthesis and, consequently, germ-tube formation in *C. albicans*. It is also worthwhile to note, in view of the classical concepts on the control of morphogenesis by Nickerson and Falcone^{19,20}, that acetylglucosamine-induced mycelial conversion cannot occur in the presence of an adequate amount of SH compound such as cysteine.

Regardless of these speculations, we have here described an experimental system which is very simple and quite amenable to direct biochemical investigations aimed at an understanding of the biochemical basis of mycelial conversion in *C. albicans*. These investigations may support or

rule out a role for chitin synthetase in this phenomenon.

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- Romano, A. H., in *The Fungi* (edit. by Ainsworth, G. C., and Sussman, A. S.), 2, 181 (Academic Press, New York and London, 1966).
- Reynolds, R., and Braude, A., *Clin. Res. Proc.*, 4, 40 (1956).
- Taschdjian, C. L., Burchall, J. J., and Kozinn, P. J., *J. Dis. Child.*, 99, 212 (1960).
- Esterly, N. B., Brammer, S. R., and Crounse, R. C., *J. invest. Derm.*, 49, 437 (1967).
- Buckley, H. R., and Van Huden, N., *Sabouraudia*, 2, 205 (1963).
- Simonetti, N., and Cassone, A., *Atti XV Congr. Soc. ital. Microbiol.*, 2, 150 (1969).
- Mardon, D. N., Hurst, S. K., and Balish, E., *Can. J. Microbiol.*, 17, 851 (1971).
- Mackenzie, D. W. R., *Sabouraudia*, 3, 225 (1964).
- Davies, R. R., and Dennings, T. J. V., *Sabouraudia*, 10, 180 (1972).
- Bartnicki-Garcia, S., *A. Rev. Microbiol.*, 22, 87 (1968).
- Chattaway, F. W., Holmes, M. R., and Barlow, A. J. E., *J. gen. Microbiol.*, 51, 367 (1968).
- Cassone, A., Simonetti, N., and Strippoli, V., *Atti XVI Congr. Soc. ital. Microbiol.*, 2, 227 (1972).
- Cassone, A., Simonetti, N., and Strippoli, V., *J. gen. Microbiol.*, 77, 417 (1973).
- Keller, A. F., and Cabib, E., *J. biol. Chem.*, 246, 160 (1971).
- McMurrough, I., Flores-Carreon, A., and Bartnicki-Garcia, S., *J. biol. Chem.*, 246, 3999 (1971).
- Glaser, L., and Brown, D. H., *J. biol. Chem.*, 228, 729 (1957).
- Porter, C. A., and Jaworski, E. G., *Biochemistry*, 5, 1149 (1966).
- Cabib, E., and Farkas, V., *Proc. natn. Acad. Sci. U.S.A.*, 68, 2052 (1971).
- Nickerson, W. J., and Falcone, G., *Science*, 124, 722 (1956).
- Falcone, G., and Nickerson, W. J., in *Biochemistry of morphogenesis* (edit. by Nickerson, W. J.), 65 (Pergamon Press, London, 1959).

Early visual adaptation in goldfish retinal ganglion cells

THE human visual system responds with transient losses of sensitivity when background light levels are suddenly increased or decreased. Crawford¹ measured the early threshold changes in humans by means of a small circular test flash presented before, during and after presentation of a larger diameter adapting light. He found that threshold increased just before the onset of the adapting light, reached a peak shortly after onset, and then decreased during the subsequent 0.5 s. Immediately before the offset of the adapting light, threshold increased slightly, peaked at offset and then returned to its original level. Other investigators have reported essentially the same results under various conditions²⁻⁴. The increase in visual sensitivity during the first few seconds of light adaptation and the peaked loss of sensitivity at the offset of the adapting light are in the wrong direction as predicted by bleaching of the photopigments. Consequently, Baker⁵ suggested that neural factors account for the early change in visual adaptation. Unfortunately, the question of mechanisms involved to account for these rapid changes in visual sensitivity cannot be

answered from psychophysical experiments since the response range cannot be assessed during these periods.

We examined the transient losses of sensitivity at onset and offset of an adapting light in thirteen isolated retinal ganglion cells of the goldfish *Carassius auratus* by extracellular recordings with tungsten microelectrodes. The preparation was an open eye intact procedure developed by Adams⁶. The apparatus, recording procedure and methods have been described previously^{7,8}. All ganglion cells were opponent types; the centre of each cell's receptive field received an inhibitory input from receptors containing a photopigment whose maximum absorption was at 625 nm and excitatory input from receptors containing a photopigment whose λ_{\max} absorption was at 530 nm (refs 6, 9). All cells had a central receptive field area of diameter about 1 mm on the retina and a surround larger than 3 mm. The tests and adapting stimuli were focused directly on to the centre of the cell's receptive field under steady photopic background conditions. A 480 nm test light was chosen to stimulate the $\lambda_{\max} = 530$ nm excitatory cone system, while a 570 nm adapting light was chosen because there is an equal quantal absorption at this wavelength by the two goldfish cone photopigments¹⁰.

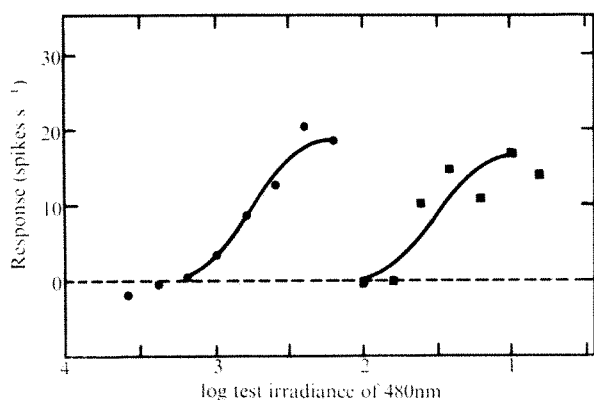


Fig. 1 Intensity response function of the excitatory mechanism of a light adapted opponent, (-R/+G) cell before and 500 ms after onset of an 0.5 mm adapting light of 570 nm containing 3.6×10^{15} quanta $\text{s}^{-1} \text{cm}^{-2}$ (abscissa represents log test irradiance of 480 nm, 0 corresponds to $7.2 \times 10^{14} \text{s}^{-1} \text{cm}^{-2}$). The mean of 10 incremental responses measured in spikes s^{-1} above the spontaneous level during the 0.5 s presentation of the test spot is plotted on the ordinate (dotted line 0 corresponds to the normalised spontaneous level in both figures). ●, Measured at steady photopic levels ($2.2 \mu\text{W cm}^{-2}$), spontaneous level being 23 ± 5 spikes s^{-1} . ■, Response function at 0.5 s after onset of the 570 nm adapting light presented at the same photopic background; spontaneous level at this time being $31 \pm$ spikes s^{-1} .

The sensitivity of the excitatory mechanism at various times before, during and after the presentation of adapting lights parallels those seen in human psychophysical experiments, although the time course is longer in this cold blooded vertebrate retina^{7,8}. We measured the intensity response function during the periods when these cells showed a transient loss of sensitivity (Figs 1 and 2). The effect of the onset of the adapting light on a cell's intensity response function is shown in Fig. 1. A smooth curve drawn through the intensity response function at a steady background level illustrates the range of about 1 log unit from threshold to the peak response (in Fig. 1). We refer to threshold-to-peak and not saturation because at higher an intensity level than the peak response the inhibitory 625 nm cone system input reduces the peak response. Adapting light of wavelength longer than 570 nm selectively depresses the sensitivity of the $\lambda_{\max} = 625$ nm cone system and increases the threshold to peak response range⁸. The curve is shifted to overlap the intensity response function measured at 500 ms after the

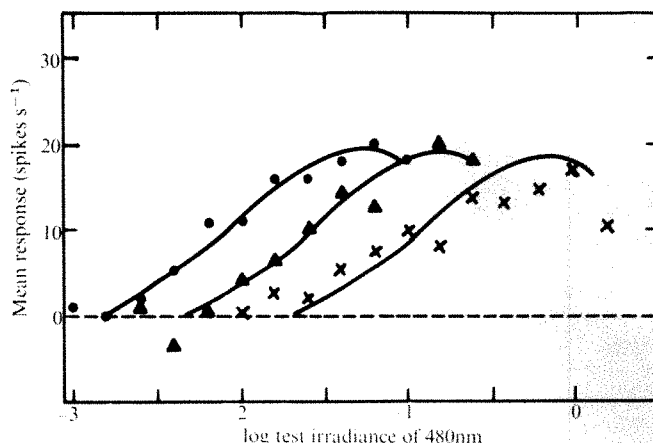


Fig. 2 Intensity response function of the excitatory mechanism of a light adapted opponent (-R/+G) cell. ●, Response function at photopic levels of $2.2 \mu\text{W cm}^{-2}$, spontaneous level being 9 ± 3 spikes s^{-1} . ▲, Response function at the same photopic level when a 0.5 mm adapting light of 570 nm containing 3.6×10^{15} quanta $\text{s}^{-1} \text{cm}^{-2}$ has been on for at least 5 min, spontaneous level is 16 ± 4 spikes s^{-1} at this time. X, Response function at photopic levels 0.75 s after the offset of the 0.5 mm adapting spot of 570 nm light, spontaneous level at this time is 30 ± 5 spikes s^{-1} . (Abscissa represents log test irradiance of 480 nm, O corresponds to $7.2 \times 10^{14} \text{s}^{-1} \text{cm}^{-2}$.)

onset of the adapting light. The intensity response function has shifted to the right on the intensity axis, about 1.5 log units, but the range from threshold-to-peak is unaltered. A similar shift in the intensity response function occurs at the offset of the adapting light as illustrated on a different cell in Fig. 2. The intensity response function at a steady background level is shown for comparison. The steady 570 nm adapting light decreased the sensitivity by about 0.5 log units as illustrated by the shift of the intensity response function to the right on the intensity scale. At the offset of the 570 nm adapting light, the sensitivity was further depressed and the range of response is further shifted to the right on the intensity axis. Although it is not shown in Fig. 2, after 3–5 min the response function shifted back to its pre-adapted position. The important generality expressed in these specific examples is that under all conditions of adaptation, transient and steady state, the range of incremental response from threshold to peak remained the same.

The early loss of sensitivity at onset and offset of an adapting light in these experiments was not due to a compression of the response function or a saturation effect at the ganglion cell. The range of light intensity necessary to elicit a threshold to peak response was the same immediately after onset or offset of the adapting list as in steady state conditions. The response function shifted when plotted on a log intensity axis and maintained its general shape and slope. The results show that in these ganglion cells, the operating range shifts across the intensity axis, consistent with a gain change.

The knowledge that gain changes occur in the visual system is neither new nor unexpected, and has been shown in many physiological experiments involving both field and bleaching adaptation^{11–15}. Previously, however, the gain changes, as measured by a cell's response function, were assessed at steady stages of light and dark adaptation where photochemical events are monotonically related to sensitivity. The early threshold events of light and dark adaptation in man are not monotonically related to photochemical events and have been presumed to be limited by neural events. However, in the ganglion cell of the goldfish retina, we have found that the early adaptation events are best described as gain changes and are similar to the change in gain which occurs in the later stages of light adaptation. The signals

from amacrine and horizontal cells show a transient change in the resting potential with the onset and offset of light consistent with the observed shift in the ganglion cell's response function¹⁶. The signal from either of these cells may be involved in the rapid translation and return of the ganglion cell response function across the intensity axis.

Since the early visual sensitivity changes in the human closely parallel those seen in the ganglion cell of the goldfish retina, gain changes may also be responsible for the early transient sensitivity changes observed in humans.

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- ¹ Crawford, B. H., *Proc. R. Soc.*, **B132**, 283-302 (1947).
- ² Baker, H. D., *J. opt. Soc. Am.*, **39**, 172-179 (1948).
- ³ Bush, W. R., *J. opt. Soc. Am.*, **45**, 1047-1057 (1955).
- ⁴ Boynton, R. M., and Miller, N. D., *Illumin. Engineer*, **58**, 541-550 (1963).
- ⁵ Baker, H. D., *J. opt. Soc. Am.*, **53**, 98-103 (1963).
- ⁶ Adams, A. J., dissertation, Indiana Univ. (1970).
- ⁷ Adams, A. J., and Afanador, A. J., *Am. J. Optom. Arch. Am. Acad. Optom.*, **48**, 889-894 (1971).
- ⁸ Afanador, A. J., dissertation, Univ. California, Berkeley (1972).
- ⁹ Wagner, H. G., MacNichol, E. F., Wolbarsht, M. L., *J. gen. Physiol.*, **18**, 43, 45-62 (1960).
- ¹⁰ Marks, W. B., *J. Physiol., Lond.*, **178**, 14-32 (1965).
- ¹¹ Jacobs, G. H., *J. opt. Soc. Am.*, **55**, 1535-1540 (1965).
- ¹² Naka, K. T., and Rushton, W. A. H., *J. Physiol., Lond.*, **194**, 259-269 (1968).
- ¹³ Sakmann, B., and Cruetzfeld, O. D., *Pflügers Arch.*, **313**, 168-185 (1969).
- ¹⁴ Werblin, F. S., *J. Neurophysiol.*, **34**, 228-241 (1971).
- ¹⁵ Dowling, J. E., and Ripps, H., *J. gen. Physiol.*, **58**, 163-189 (1971).
- ¹⁶ Werblin, F. S., *J. Neurophysiol.*, **34**, 228-241 (1971).

Carcinogenicity of bracken and shikimic acid

SINCE our initial reports of the carcinogenic potential of bracken for rats and mice^{1,2}, part of our work has been directed towards the isolation of the causative factor(s). The assays used have included acute toxicity testing in mice (orally and intraperitoneally (i.p.)) and mutagen trials with *Drosophila* and mice, as well as long term cancer induction in rats and mice. The former suffer from the disadvantage of being nonspecific for carcinogens and the latter from the delay of a year or 15 months before results can be assessed. A fraction was isolated, however, which proved positive for acute toxicity, mutagenicity and carcinogenicity in mice and the preliminary molecular formula of $C_7H_8O_4$ was given for the main constituent^{3,4}.

We have now carried the chemical characterisation further and after correction by a low temperature mass spectrum (the addition of a molecule of water to bring the molecular weight to 174 ($C_7H_{10}O_5$)), the full analysis gave clear evidence that the isolated compound was shikimic acid (3,4,5 trihydroxy-1-cyclohexene-1-carboxylic acid). The detailed report on the chemical elucidation will be given elsewhere.

At first sight, shikimic acid does not seem to be a likely candidate for a carcinogen, it is widespread in plants⁵, being on the biosynthetic pathway of aromatic ring compounds, and it does not fall readily into the well known groups of chemical

Table 1 Mouse tumours following administration of shikimic acid

Sex	Dose (mg)	Route*	Age at death (weeks)	Neoplasms
M	1	i.p.	70	Glandular stomach advanced grade II
M	5	i.p.	70	Glandular stomach grade II
M	10	i.p.	62	Glandular stomach grade I
M	10	i.p.	62	Glandular stomach early grade I
M	15	i.p.	62	Glandular stomach grade II
M	20	i.p.	58	Reticulum cell B leukaemia
F	20	i.p.	58	Reticulum cell A leukaemia
				I Pulmonary adenoma
M	20	i.p.	38	Lymphocytic leukaemia
M	100	ST	34	Reticulum cell B leukaemia
				Glandular stomach early grade I
M	10	i.p.	78	—
M	10	i.p.	70	—
M	15	i.p.	62	—
F	20	i.p.	62	—
F	30	i.p.	70	—

* i.p., intraperitoneal; ST, stomach tube.

substances which can induce cancer in experimental animals. Considerable effort was therefore devoted to the minor constituents of the fraction as revealed by chromatography, but fortunately shikimic acid was not entirely neglected and single administrations of the commercially produced acid of tested purity (BDH Ltd) were given to mice. We now have to report the results, to date, of this experiment which are shown in Table 1.

TF1 strain mice (Tucks), averaging 10 weeks of age, were given single i.p. injections of aqueous shikimic acid. Later groups have been dosed orally by stomach tube and the first fatality among these is listed in the table. Out of 14 animals, 9 had cancerous and precancerous lesions. A total of 57 control mice were treated in the same way with various inert materials in aqueous solution and killed after comparable periods of time. It is important that none of these animals had any neoplasms at all, as it indicates that for this strain of mice the spontaneous tumours of old age are not encountered at ages of 15 months or less.

The leukaemias are classified according to the system described by Dunn⁶, and display the variety of classic features including widespread dispersal through the peritoneal and pleural cavities and involvement of organs such as lymph nodes, spleen, liver, and kidney. The reticulum cell A leukaemia was extremely disseminated, with the elongated and round cell types equally represented. One of the reticulum cell B leukaemias had binucleate giant cells and also a greater mixture of plasma cells with the lymphocytes than usual. Grade I neoplasms of the glandular gastric mucosa include those changes which are limited to the mucosa itself, whereas in grade II invasion through the muscularis mucosae into the submucosa has taken place to give the typical 'carcinoma in situ'. In two of the latter, intestinalisation of the tumour epithelium had also taken place.

In the light of these results a mouse test for the production of dominant lethal mutations was conducted according to the method of Bateman⁷, five mature male mice (TF1 strain, averaging 10 weeks old with weights 25-30 g) were used in each test group and ten injected with 0.5 ml of physiological saline for control figures. One group was injected (i.p. with 25 mg of shikimic acid in 0.5 ml of water, which is approximately the LD₅₀ dose (1000 mg kg⁻¹), and a second group had an oral dose administered by stomach tube of 80 mg in 0.2 ml of water. For the 8 successive weeks of the spermatogenic cycle, the males were mated with four virgin females. These were removed at the end of each week and kept until the 12th day from the midweek of their mating, when they were killed, the uterus removed and scored for total implantations, early deaths indicated by deciduomata, and late deaths.

The results are plotted as the percentage of dead implants against weeks from injection (Fig. 1). The percentage, which has

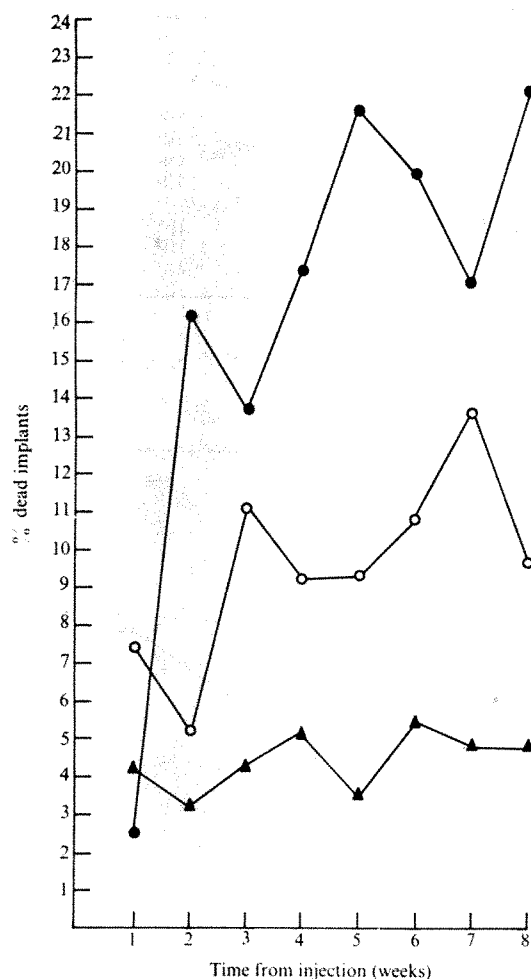


Fig. 1 Dominant lethal mutations induced in mice by commercial shikimic acid. ●, 25 mg (intraperitoneal injection); ○, 80 mg (stomach tube); ▲, control.

also been termed the Mutagenic Index, is derived from the formula:

$$(\text{deciduomata} + \text{late deaths}) / \text{total implants} (\times 100).$$

Each weekly figure is based on the results from 20 females and the numbers not pregnant will decrease the accuracy. It is important that total implants should exceed 100, and in the 16 shikimic groups they averaged 163 (104–210), while the controls averaged 171 (115–237). Taken over the whole 8 weeks, the average control figure was 4.4% (3.2–5.4), whereas the oral shikimic group reached a peak of 13.6 and those injected i.p. were consistently high from the second week onwards, reaching a final maximum of 22.1%.

It is interesting to compare these results with those of Epstein and Shafner⁸ investigating the mutagenic effects in mice of a variety of environmental pollutants, including carcinogens, with an almost identical experimental design. The main difference is that their control mice had an average dominant lethal rate of 1.0% (0–3) while our corresponding figure was 4.4%.

On the other hand, the dosage was of the same order, approximating to LD₅₀ in both cases. On this basis, of the carcinogens listed, only aflatoxin and benzo-a-pyrene reach a level of dead implants of 11% and of all the groups of compounds only METEPA (ref. 8) and related pharmaceuticals reach into the 20% range.

The long term results from previous experiments are now showing clearly that there is a second and different fraction derived from bracken which contains a strong carcinogen and is also capable of inducing the syndrome of acute bovine bracken poisoning (I. A. E., R. S. Jones, D. L. Jones and W. C. Evans, unpublished). That this agent is a more powerful tumour inducer

in mice than shikimic acid is indicated by the fact that single administrations (i.p. and orally) will produce malignant squamous carcinoma of the forestomach as well as the glandular gastric tumours. As well as investigating this fraction, the trials on shikimic acid are being extended to include its effects on other experimental animals and daily small oral dosage as opposed to the single administration tested so far.

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- Evans, I. A., and Mason, J., *Nature*, **208**, 913 (1965).
- Evans, I. A., and Widdop, B., *B.E.C.C. A. Rep.*, 377 (1966).
- Evans, I. A., Barber, G. D., Jones, R. S., and Leach, H., *B.E.C.C. A. Rep.*, 466 (1969).
- Leach, H., Barber, G. D., Evans, I. A., and Evans, W. C., *Biochem. J.*, **124**, 13 (1971).
- Bohm, B. A., *Chem. Rev.*, **65**, 435 (1965).
- Dunn, T. B., *J. natn. Cancer Inst.*, **14**, 1281 (1954).
- Partington, M., and Bateman, A. J., *Heredity Lond.*, **19**, 191 (1964).
- Epstein, S. S., and Shafner, H., *Nature*, **219**, 385 (1968).

Phylogenetic origin of xenogeneic recognition

SINCE Metchnikoff (quoted in ref. 1) showed that amoebocytes, the circulating cells in invertebrates, are capable of phagocytosis¹, it has become increasingly apparent that they have various immunological functions. For example, amoebocytes mediate resistance to infection^{2,3} and rejection of xenogeneic grafts^{4,5}.

We have described recognition at the cellular level in the northern sea star, *Asterias vulgaris* (Echinodermata). These animals distinguish between autologous, allogeneic and xenogeneic cell challenge, as evidenced by the development of clumping, and a corresponding decrease in the number of circulating single cells, a phenomenon occurring only in the xenogeneically challenged host. Xenogeneic cells could also be identified in these large multi-cellular clumps⁶.

In addition to host clumping, which is a mechanism of trapping foreign cells, we were interested in determining whether there was a second component to the response, for example, a humoral as well as cellular reaction to foreign antigen. We have now found that a soluble factor which mediates *in vivo* cell aggregation is released by the host in response to xenogeneic but not allogeneic cell challenge.

Common sea stars (*Asterias forbesi*) collected near the Marine Biological Laboratory, Woods Hole, were maintained in tanks containing circulating seawater. Variation in the concentration of unclumped amoebocytes in coelomic fluid fluctuated very little over 60 min (Fig. 1).

Thirty sea stars were inoculated with various cell preparations, both of allogeneic and xenogeneic origin. All 15 xenogeneically challenged animals had decreases in circulation cell counts of greater than 90%. In addition, eight sea stars were evaluated in their initial response: 5 min after challenge when clumping was maximal and the circulating cell count had reached its nadir, the tips of two arms were cut, and the coelomic fluid containing the

Table 1 Effect of cell-free transfer on circulating amoebocyte counts in sea stars

Inoculum	Preinoculum (A) ⁺	Postinoculum (B) [*]	B/A × 100	Time 0 (A) ⁺	Time 5 (B) ⁺	B/A × 100
Seawater	1.3 × 10 ⁶	1.0 × 10 ⁶	77	1.3 × 10 ⁶	1.1 × 10 ⁶	85
Carbon particles	2.3 × 10 ⁶	1.1 × 10 ⁶	48	1.9 × 10 ⁶	4.5 × 10 ⁵	24
Sea star	7.0 × 10 ⁵	4.0 × 10 ⁵	57	1.3 × 10 ⁶	9.5 × 10 ⁵	73
Sea star	1.9 × 10 ⁶	1.7 × 10 ⁶	89	6.0 × 10 ⁵	6.5 × 10 ⁵	108
Sea urchin	2.4 × 10 ⁶	9.0 × 10 ⁴	<1	1.7 × 10 ⁶	3.0 × 10 ⁴	2
Horseshoe crab	1.3 × 10 ⁶	3.0 × 10 ⁴	2	1.3 × 10 ⁶	4.0 × 10 ⁴	3
Horseshoe crab	3.5 × 10 ⁷	1.3 × 10 ⁵	<1	1.7 × 10 ⁶	3.5 × 10 ⁵	21
Human RBC	7.5 × 10 ⁵	2.0 × 10 ⁵	27	1.8 × 10 ⁶	4.0 × 10 ⁴	2

Sea stars were inoculated with 3.0 ml seawater, 1×10^8 carbon particles, 1×10^6 sea urchin amoebocytes, 1×10^6 horseshoe crab amoebocytes, or 1×10^8 human red blood cells. Stars were counted just before inoculation (time 0 (A)) and 5 min after inoculation (time 5 (B)). Percentages of single cells (B/A × 100) were determined. Precounted naive animals were then inoculated with 1.0 ml cell-free supernatant from the challenged sea stars, 5 min counts were obtained, and percentages (B/A × 100) determined.

* Numbers represent single amoebocyte count per ml coelomic fluid.

cells was collected. It was then centrifuged for 20 min at 280g and 1.0 ml of cell-free supernatants (as verified by microscopy) inoculated into a secondary 'naive' recipient. The concentrations of single amoebocytes in the recipient was determined before and at intervals after inoculation.

As Table 1 shows, when either seawater or sea star 'allogeneic' cells were used as challenge inocula, clumping did not occur either in the challenged animal or the passively transferred recipient. Inoculations of sea urchin, horseshoe crab or human peripheral blood cells resulted in a sharp decline in the circulating cell count. Supernatants from positive responders in turn initiated the same response in naive recipients. An example of the response of sea stars treated with cell-free supernatants from either non-reactive or reactive animals is shown in Fig. 2. Only when clumping occurred in the xenogeneically challenged animal was there a corresponding decline in single cell concentration of the passively transferred recipient. Carbon particles induced a moderate clumping response which could be transmitted in the supernatant, indicating that host responsiveness rather than lysis of inoculum was primarily responsible for subsequent *in vivo* clumping.

Prendergast⁷ has isolated a protein (SSF) with a molecular weight of 32,000 from the amoebocytes of the sea star which he has shown mediates clumping in sea stars treated with SSF. It is possible that the factor mediating *in vivo* amoebocyte clumping when transformed to secondary recipients in our system, may cross react with SSF.

The data suggest that recognition in echinoderms is char-

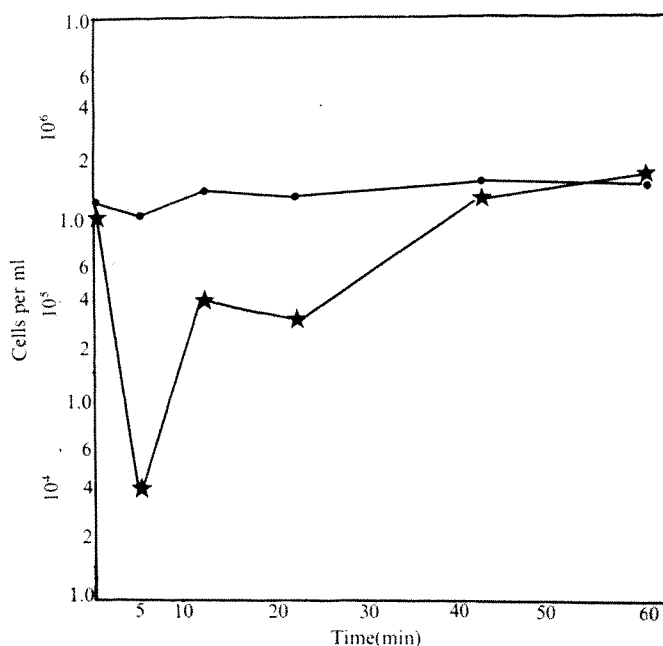


Fig. 2 Sea stars were inoculated with 1.0 ml cell-free supernatants from animals given either 3.0 ml seawater (●) or 1.0×10^6 horseshoe crab amoebocytes (★). Circulating single cell counts were determined over 1 h.

acterised by the following *in vivo* sequence: (1) distinction of xenogeneic antigen; (2) release of activator; (3) clumping, and (4) trapping of xenogeneic cells. The mechanism of host distinction between allogeneic and xenogeneic antigen is unclear. It is becoming apparent, however, that at this stage of phylogeny xenogeneic rather than allogeneic recognition is an intrinsic property of the relatively undifferentiated amoebocyte in contrast to vertebrate and mammalian lymphocytes which respond vigorously to alloantigens.

This work was supported in part by a grant from the National Cancer Institute. Technical assistance by H. Cantor is gratefully acknowledged.

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Received March 13; revised April 25, 1974.

¹ Lectures on the comparative pathology of inflammation, (Trench, Tribner and Co., London, 1883).

² Bang, F. B., and Lemma, A., *J. Insect Path.*, **4**, 401 (1962).

³ Bang, F. B., *J. Reticuloendothelial Soc.*, **7**, 161 (1970).

⁴ Hostetter, R., and Cooper, E., *J. Immun.*, **9**, 384 (1973).

⁵ Ghiradella, H. T., *Bio. Bull.*, **128**, 77 (1965).

⁶ Reinisch, C. L., and Bang, F. B., *Cell. Immun.*, **2**, 496 (1971).

⁷ Prendergast, R. A., Cole, G. A., and Henney, C. S., *Ann. N.Y. Acad. Sci.* (in the press).

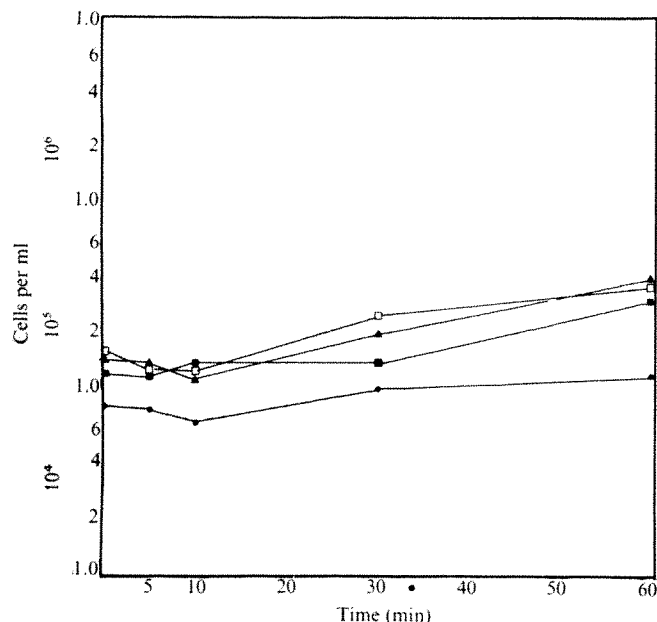


Fig. 1 Single amoebocyte counts per ml coelomic fluid were determined over time in four normal animals.

matters arising

Turtle drift

SIR,—It occurs to me that the origin of the peculiar migration pattern of the green turtle, *Chelonia mydas* may be otherwise than suggested by Carr and Coleman¹. They argued that since the early Cretaceous certain turtles have inherited a tendency to swim a particular WNW–ESE path from Brazil to the Ascension Islands, swimming against the prevailing current for about eight weeks. They note, however, that *Chelonia*, which is a herbivorous, frequently seagrass ('turtle grass') eating form, is not recorded before the Miocene. This concurs with my observations² on *Thalassia* (turtle grass) and its associated biota which did not succeed in reaching the tropical Americas until the early Miocene. It seems likely that these seagrasses came from the Indo-Pacific through the southern tip of Africa, and drifted passively across to Brazil from West Africa on the equatorial surface current. Therefore seagrass feeding grounds at least, were situated on the eastern side of the Atlantic first. One might infer from this that the herbivorous turtles followed the seagrass biota westwards across the Atlantic (through the mid-oceanic islands) but returned for the breeding season. If this is so, then the early ancestors of *Chelonia mydas* should be found in Africa or further east.

A second consideration is that the behavioural pattern suggested by Carr and Coleman seems to make little ecological or evolutionary sense (which they have almost admitted). They imply that the home breeding grounds around South America became inexplicably untenable (presumably many millions of years after turtles had become established) so favouring a change in behaviour in these otherwise ultraconservative creatures. The progeny produced by this 'freakish' behaviour were then lucky enough to drift back to the old feeding grounds on the current. Bearing in mind the extremely conservative breeding behaviour of past and present amphibians and primitive reptiles I feel this postulation will need a more thorough explanation.

Would it not be more in keeping with the evidence to postulate the passive drifting of juveniles westwards across the Atlantic (where, after the Palaeogene they would have found plentiful seagrass) followed at breeding

time by a homing instinct that leads them eastwards to the midoceanic islands? The seafloor spreading part of their hypothesis would work equally well.

Yours faithfully,
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¹ Carr, A., and Coleman, P. J., *Nature*, **249**, 128 (1974).

² Brasier, M. D., *Nature*, **243**, 342 (1973).

No radioactive silver detectable in silver–uranium ore

SIR, — Lindner *et al.*¹ have reported finding the radioactive silver isotopes ^{108m}Ag and ^{110m}Ag in silver bars of eastern European origin, and have conjectured that the silver may have been mined using nuclear explosives. Boyle² has suggested that it was more probable that the radioactive silver had been produced by neutrons from (α , n) reactions and spontaneous fission reactions of uranium in the silver ores. In January 1974, I was able to secure a sample of uriferous silver ore from a mine at Contact Lake, North West Territories, near Port Radium on Great Bear Lake. The 70 g sample was crushed and ground. The resulting powder was assayed using X-ray fluorescence spectroscopy and was found to contain 6.8% Ag and 0.97% U. Fifty grams of the powder was roasted, treated with HF and H₂SO₄, leached with HNO₃ and the extracted silver was precipitated as the chloride. Four grams of AgCl, corresponding to 3 g of Ag, was counted in a defined geometry on a large, high resolution, low background Ge(Li) detector for 25 h. No silver gamma peaks were detected.

The 25 h background of the detector in the region of the 658 keV ^{110m}Ag γ ray was 110 counts. Using the detection limit³ of $4.65\sigma_B$, where σ_B is the standard deviation of the background for paired observations, gives a detection limit of 50 counts in the peak.

Lindner *et al.*¹ found that the silver bullion contained 84 pCi of ^{110m}Ag per gram of silver. If this figure is used to calculate the expected number of counts in the same peak, using a 3 g sample of

silver, a 25 h counting time, 94% peak abundance (ref. 4), 48% isotopic abundance of the parent isotope, ¹⁰⁹Ag (ref. 4), and a measured absolute efficiency of the detector of 2.8% for that energy, then the peak would contain 1.1×10^4 counts. The ratio of the ^{110m}Ag found in the bullion to our detection limit for radiosilver from the Canadian ore is $1.1 \times 10^4/50$, or 220. Therefore it seems that Boyle's suggestion that the radioactive silver found in the bullion by Lindner *et al.* was of natural origin is incorrect.

I thank R. R. Wallace for obtaining the ore sample and G. J. Jarbo for the silver and uranium analyses.

Yours faithfully,
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¹ Lindner, L., Brinkman, G. A., and Schimmel, A., *Nature*, **240**, 463 (1972).

² Boyle, R. W., *Nature*, **243**, 461 (1973).

³ Adams, F., and Dams, R., *Applied Gamma-Ray Spectrometry*, 233 (Pergamon Press, Oxford, 1970).

⁴ *Radioactive Decay Gamma-Ray Compilation DECYGAM*, Oak Ridge National Laboratory, Report No. DLC-19 (1972).

ABO matching in kidney graft survival

SIR,—Joysey *et al.*¹ reported on kidney graft survival in Cambridge with particular reference to the ABO group of the recipient. The data from Birmingham confirm these findings and allow a further breakdown by ABO status of the donor.

A system of computer documentation of transplantation and serological data is being used, which includes the display of survival on a graphical plotter. The data are presented in a direct form, showing the fraction of grafts surviving, rather than as 'actuarial' curves. The graphs, therefore, may show irregularities instead of a smooth descending curve. The advantages and disadvantages of various methods of presenting survival data will be discussed elsewhere.

Excluding second and third grafts, 161 patients were transplanted between May 1968 and January 1974 with cadaveric kidneys. Figure 1a shows the survival information in intervals of 3

months over a 4-yr period. All graft failures are included. Survival in group O and non-group O recipients shows the same difference as in the Cambridge data, that is that grafts survive better in group O patients. At 1 yr a 2×2 test shows formal significance, $\chi^2 = 6.49$, $P = 0.02-0.01$; however this test was only made after looking at the graphical output. The group A patients were divided according to whether they received group O or group A donor kidneys, Fig. 1b. There seems to be no initial difference in survival, but the numbers are small.

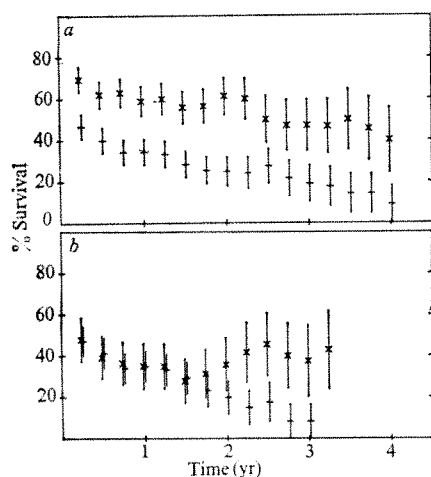


Fig. 1 Survival of kidney grafts in Birmingham. *a*, Survival in group O (x) and group A (+) recipients. *b*, Subdivision of group A recipients according to whether they received group O (x) or group A (+) kidneys. There were 62 group O recipients; the 75 group A recipients received 51 group A and 23 group O kidneys, and one of unknown blood group. Vertical bars represent standard error.

Only group O recipients are guaranteed an identical ABO match. If a mismatch for the product of the O gene is not acceptable, using the genotype frequencies for southern England from Race and Sanger², this will only account for 14.4% of A to A transplants and 17.4% of O to A transplants because the majority of group As will be heterozygous AO. Is this, together with an allowance for A₁ and A₂ mismatching, enough to account for the observed discrepancy in graft survival between group O and group A recipients? If ABO genotype is important, then without A₁ and A₂ grouping and matching, only 44% of A to A grafts will be genotypically identical.

I am indebted to Professor J. H. Edwards and Mrs Karen Glenn for developing the system of computer documentation.

I thank Mr A. D. Barnes for making the survival information available and the Kidney Research Fund for financial

assistance with the analysis.

Yours faithfully,

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¹ Joysey, V. C., Roger, J. H., Evans, D. B., and Herbertson, B. M., *Nature*, **246**, 163 (1973).

² Race, R. R., and Sanger, R., *Blood Groups in Man* (Blackwell Scientific, Oxford and Edinburgh, 1968).

Spittlebug morph mimics avian excrement

SIR,—Most colour forms^{1,2} of the spittlebug, *Philaenus spumarius*, are cryptic, but the *marginella* morph has a black body outlined by white. Thompson³ has proposed that this phenotype represents an example of escape warning coloration, suggesting that experienced predators ignore *marginella* individuals because they remember previous "frustrating encounters" with *marginella* more vividly than similar encounters with cryptic morphs. The logic of this argument is tenuous. It seems equally likely that predators should chase *marginella* more actively because of an equally vivid recollection of successful encounters. Moreover, if the phenotype was a warning pattern its fitness should increase with increased frequency, for a greater proportion of experienced predators would be encountered. Both cryptic and *marginella* forms must be present for such conditioning, but this is assured even when the *marginella* allele is fixed, for only the females are brightly coloured, males are cryptic². On this basis fixation of the *marginella* allele should occur, yet in natural populations its frequency remains low.

Patterns of black and white have functions other than predator warning; among small arthropods such patterns are frequently associated with excrement mimicry⁴. Indeed the *marginella* pattern, coupled with the elongate shape of *P. spumarius*, produces a convincing resemblance to a bird dropping. Ignoring possible complications, such as the occurrence of other excrement mimics or changes in the grouped abundance of the cryptic morphs, *marginella* frequency should be intimately related to model abundance. A uniform distribution of bird droppings could, for instance, be the cause of the relatively constant frequency of the *marginella* morph in the Great Lakes area of North America².

The frequency-dependent fitness of mimetic forms can lead to sex limitation when the timing of predation differs between sexes. For example, if predation is postreproductive in males, but pre-reproductive in females, suppression of male mimicry allows population size to

increase while not affecting male fitness. This situation is closely approximated in *P. spumarius* as mating occurs in the early summer while egg deposition is delayed until autumn⁵. It is not surprising then, that modifiers have accumulated to restrict the *marginella* pattern to females.

In conclusion there seems little reason to suspect that the *marginella* phenotype has any warning significance; rather it mimics avian excrement in a situation analogous to classical Batesian mimicry.

Yours faithfully,

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¹ Weaver, C. R., and King, D. R., *Ohio Agr. Exp. Sta. Res. Bull.* **741**, 1 (1944).

² Thompson, V., and Halkka, O., *Am. Midl. Nat.*, **89**, 348 (1973).

³ Thompson, V., *Nature*, **242**, 126 (1973).

⁴ Cott, H. B., *Adaptive Colouration in Animals* (Methuen, London, 1940).

⁵ Wiegert, R. G., *Ecol. Monogr.*, **34**, 217 (1964).

MR THOMPSON REPLIES: I believe these objections to my proposal are not well founded. Cryptic coloration of all forms is not necessary for the maintenance of an apostatic polymorphism. This can be demonstrated quantitatively by a simple extension of the model of Clarke and O'Donald¹ for frequency-dependent selection at one locus with dominance. Let *A* represent a dominant allele for conspicuous coloration with frequency $1-q$ and *A*¹ a recessive allele for more cryptic coloration with frequency q . Let *t* and *t*₁ represent factors relating the frequency of each phenotype to its selective value, and let *I* be a standard fitness.

Genotypes	<i>AA</i> and <i>AA</i> ¹	<i>A</i> ¹ <i>A</i> ¹
Frequency after random mating	$1-q^2$	q^2
Selective value:		
Case 1	$I-t(1-q^2)$	$I-t_1q^2$
Case 2	$I-t(1-q^2)+w(1-q^2)$	$I-t_1q^2$

At equilibrium the selective values of the two phenotypes must be equal and for this example (Case 1) it can be shown that there is a non trivial stable equilibrium for the two alleles at $\hat{q} = \sqrt{[t/(t+t_1)]}$ provided that *t* and *t*₁ are both positive (each colour form becomes less fit as it becomes more common). Both forms will be maintained in the population with the more conspicuous form at lower frequency.

Furthermore, an allele determining a conspicuous pattern which is also a warning pattern will not necessarily increase to fixation, even though the warning pattern *per se* confers greater fitness as its frequency increases. Let *w* represent the relation between the frequency of the conspicuous phenotype and that part of its selective value determined by its function as a warning

pattern, with t and t_1 again representing apostatic relations (Case 2). In this case there is a non trivial stable equilibrium at $\hat{q} = \sqrt{[(t-w)/(t-w+t_1)]}$ so that when $t > w$ the tendency of the conspicuous warning pattern to increase will be held in check by apostatic selection. Though this example probably underestimates the complexity of the predator-prey relations in question it does demonstrate that there is no *a priori* reason to reject the hypothesis that *marginella* is a warning pattern maintained in polymorphic condition with more cryptic forms by apostatic selection.

Dr Hebert's counter proposal is difficult to accept. The *marginella* pattern of black on white is too sharp and the black on most specimens too unbroken to reasonably suggest a resemblance to bird excreta. But, as Hutchinson² has noted, the colour form *trilineata* may vaguely resemble grass seeds and I do not rule out protective resemblance as a factor in *Philaenus spumarius* polymorphism.

I thank Dr L. Van Valen for comments.
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¹ Clarke, B., and O'Donald, P., *Heredity*, **19**, 201 (1964).

² Hutchinson, G. E., *Entomologist's mon. Mag.*, **99**, 175 (1963).

Genetic control of natural resistance to *Leishmania donovani*

SIR,—In studies of infections in mice with *Leishmania donovani*, an intracellular parasite, (in preparation) several observations are relevant to the recent letter¹ on mouse susceptibility to *Salmonella* showing that seven mouse strains fell into two resistance categories.

Colleagues and I have found that the growth rate of *L. donovani* (Ethiopian strain L82) populations in mice varied greatly and examined the acute phase, the first 2 weeks after infection, in detail. Amastigotes from hamster spleen, cleaned by differential centrifugation, were injected intravenously into three mice from each of 25 inbred strains and five F_1

hybrids. Parasite numbers at day 15 in the liver were estimated by Stauber's method^{2,3}. The ratio of parasites to liver cells was determined on liver imprints and multiplied by the liver weight in mg to give a reproducible index of parasite load in LDU (Leishman-Donovan units).

The mean liver parasite load on day 1 of 30.5 LDU did not differ between strains but at day 15 inbred mice fell into two distinct categories which did not overlap. Twelve highly susceptible strains showed about a hundred-fold multiplication whereas the remaining 13 strains were resistant with less than eight-fold increase. No intermediates were seen except for an F_1 cross between a susceptible and a resistant strain which was of intermediate susceptibility. The observation suggested a rather simple genetic control of resistance. Our series included the seven strains studied by Plant and Glynn¹ and the results are in Table 1. There is precise correspondence between resistance to *S. typhimurium* and to *L. donovani*.

In breeding experiments resistant C3H mice were crossed with susceptible NMRI. The F_1 progeny were both selfed and back-crossed to each parental strain. Mice were infected as before. Liver counts from mice killed after 2 weeks are summarised in Fig. 1. Susceptible mice reappear as a distinct group clearly separable from a wider group corresponding to resistant and F_1 categories. The proportion of susceptible mice does not differ significantly from one quarter in the F_2 and one half in the back cross to susceptible parents. Also, the observed distribution of other counts fits closely to the predicted distribution on Mendelian expectations using independent estimates of the means and variances of counts in F_1 and resistant mice.

This is strong evidence for control of *L. donovani* growth in the mouse liver by a single gene or tight linkage group. The coincidence of response pattern to *L. donovani* and *S. typhimurium* has a 1:64 probability of having arisen by chance as there are concordant results for six separate mouse strains (C57BL and B10.D2 are genetically very similar). It is therefore possible that we may be

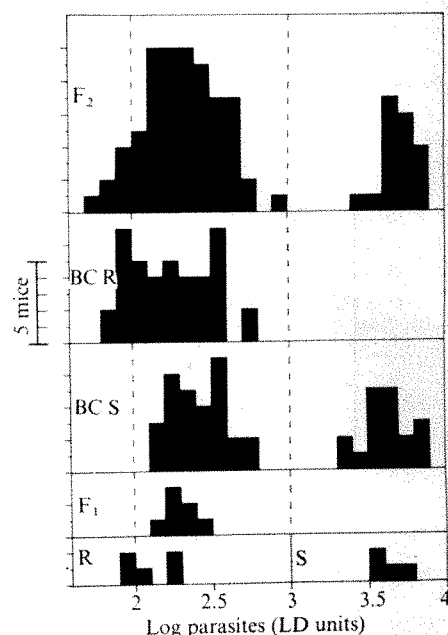


Fig. 1 Histogram of liver parasite burdens, 14–16 d after infection with 10^7 *L. donovani*, from 16th passage, October 1972. Mice derived from crossing susceptible[†](S) NMRI and resistant (R) C3H strains. Results from F_2 back-crosses (BC) to S and to R, F_1 , and parental strains are shown. Parasite counts have been logarithmically transformed.

dealing with the same genetic mechanism.

There is no evidence from our 25 strains that any $H-2$ alleles correspond with acute phase resistance or susceptibility, nor have attempts to map the gene (provisionally name *Lsh*, leishmaniasis resistance) given support for any linkage to *Ir* or $H-2$ loci (D. J. B. and B. A. Taylor, in preparation). Results so far do not suggest any direct relation of acute leishmaniasis susceptibility to *Ir*1, $H-2$ or the ability to mount an acquired immunological response.

I thank Jean Kirkley for technical assistance and the Royal Society and Wellcome Trust for financial support.

Yours faithfully,

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Table 1 Parasite numbers in mouse livers

Mouse strain	Mean count (LDU)	Mean log count* (\pm s.e.)	Parasite increase (day 15 count / day 1 count)	Susceptibility	
				<i>Leishmania</i>	<i>Salmonella</i> [†]
BALB/c	3,438	3.534 ± 0.030	115.1	S	S
C57BL	3,697	3.565 ± 0.035	123.3	S	S
B10.D2 new	6,410	3.793 ± 0.078	208.4	S	S
DBA/2	94	1.967 ± 0.044	3.1	R	R
C3H/He	165	2.216 ± 0.030	5.5	R	R
A	149	2.170 ± 0.030	5.0	R	R
CBA/Ce	125	2.095 ± 0.040	4.2	R	R

* Here the counts in LDU have been logarithmically transformed which normalises the distribution and renders the variance independent of the mean.

[†] *Salmonella typhimurium* susceptibility from ref. 1 for comparison.

Parasite numbers were measured 15 d after intravenous infection with 10^7 amastigotes of *Leishmania donovani* derived from hamster spleen, 14th passage, May 1972. Three mice of each strain used.

DR PLANT AND PROFESSOR GLYNN REPLY: Bradley's observation that in strains of inbred mice resistance to infection with *Leishmania donovani* is either very high or very low and corresponds to the level of resistance to *Salmonella typhimurium* found by us in six of the same strains reinforces the idea that resistance is controlled by only one or a few closely linked genes.

Like him, we do not find in further breeding experiments that resistance is invariably associated with a particular *H*-2 type. But because of our results with delayed hypersensitivity reactions as well as on general grounds we do believe that some sort of enhanced immune response is involved. Both *S. typhimurium* and *L. donovani* are intracellular parasites and cellular immunity is the most important defence mechanism in both. It is unlikely though not impossible that there is a significant protective antigen common to *S. typhimurium* and *L. donovani*, though it is also unlikely that anyone has actually looked for one. An immune response gene controlling responses to several unrelated antigens would explain the results.

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¹ Plant, J., and Glynn, A. A., *Nature*, **248**, 345-347 (1974).

² Stauber, L. A., *Rice Institute Pamphlet*, **45**, 80-96 (1958).

³ Goodwin, L. G., *Trans. R. Soc. trop. Med. Hyg.*, **38**, 151-160 (1944).

Area postrema and blood pressure

SIR,—Ylitalo *et al.*¹ based their suggestion that the area postrema is a control centre of blood pressure on experiments in which a maintained rise in the level of blood pressure, which was also more labile than normal, followed destruction of the area postrema in rats. As it is well known, however, that such effects are produced by section of the buffer nerves², the possibility must be considered that the baroreceptor pathway could have been interrupted where the sinus and aortic nerves terminate in the medulla in the nucleus of the tractus solitarius^{3,4}, which lies immediately adjacent to the area postrema.

We suggest, therefore, that a simpler explanation of the results¹ is that the damage caused by thermocoagulation of the area postrema had spread the fraction of a millimetre necessary to involve the nucleus of the tractus solitarius. In the absence of evidence that the baroreceptor pathway had been spared, it is not justified to put forward the suggestion that

the area postrema acts as a special blood pressure regulating centre.

Yours faithfully,

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R. M. McALLEN
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¹ Ylitalo, P., Karppanen, H., and Paasonen, M. K., *Nature*, **247**, 58-59 (1974).

² Ferrario, C. M., McCubbin, J. W., and Page, I. H., *Circ. Res.*, **24**, 911-922 (1969).

³ Torvik, A., *J. comp. Neurol.*, **106**, 51-141 (1956).

⁴ Humphrey, D. R., in *Baroreceptors and Hypertension*, (edit. by Kezdi, P.) (Pergamon, 1967).

On fighting strategies in animal combat

SIR,—The article¹ by Maynard Smith and Price is unfortunately based on a number of unwarranted assumptions, and on an inadequate literature research. It perpetuates an old ethological myth that animals fight so as not to injure each other, or refuse to strike 'foul blows' and, presumably, kill each other. The authors assumed that there were such categories as 'conventional' and 'dangerous' in animal conflict, that opponents in combat retreat when injured, and that opponents retain no memory of past contests. None of these assumptions can be regarded as valid. They were not aware of the published field studies primarily of large mammals which have shown not only how dangerous combat is, but, more importantly, have also led to new theories of explaining aggressive behaviour on the basis of individual selection²⁻¹⁰.

Two authors at least^{2,3,11} have developed the concept that combat can be understood as an interplay of defensive and offensive behaviour. This is a simple point but one missed previously, and one that leads to the conclusion that animals need not rely on altruistic impulses in their opponents to escape injury, but rely on their abilities to block, evade or frustrate attacks. First, the inhibition against engagement in overt aggression in species with excellent weapons but poor morphological or behavioural defences can be explained by the principle of retaliation^{2,3}. This explanation assumes that an animal will attack a conspecific if it experiences severe pain, an assumption amply verified^{12,13}. Second, it assumes that even dangerously armed species (excepting humans) usually cannot kill an opponent outright and thus escape retaliation. This second assumption is entirely in line with data

from diverse field studies on carnivores and ungulates^{2-9,14}.

The authors also become victims of one study of mule deer which can be faulted for inadequate observations. Linsdale and Tomich¹⁵ in their work apparently failed to see a fight between mule deer bucks and confused the common sparring matches with fighting. Sparring matches are performed by bucks of unequal size or dominance rank; they are initiated by the subordinate buck and terminated by him; they are long lasting with many engagements and have antler wrestling as their principal behavioural component. Fights are exceedingly rare, occur between matched bucks, and differ strikingly in their execution from sparring matches; moreover the victor chases and attempts to gore the vanquished. There are no 'winners' or 'losers' in sparring matches. Severe wounding does occur in mule deer, usually on smaller bucks unable to withdraw from onrushing dominants which guard females. Flanks, shoulders, haunches and faces are pierced; the rate of visible wounding is about 10% yr⁻¹ among bucks exceeding 1.5 yr of age. I shall report in detail on this in the near future.

Yours faithfully,

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¹ Maynard Smith, J., and Price, G. R., *Nature*, **246**, 15 (1973).

² Geist, V., *Behaviour*, **27**, 175 (1966).

³ Geist, V., *Mountain sheep: A Study in Behaviour and Evolution*, chapter 7 (University of Chicago Press, Chicago and London, 1971).

⁴ Bannikov, A. G., Ahimov, L. V., Lebedeva, L. S., and Fandee, A. A., *Biology of the Saiga*. (US Department of Commerce, Springfield, Virginia, 1961).

⁵ Kurt, F., *Das Sozialverhalten des Rehes Capreolus capreolus*, *L. Mammalia depicta*. (Parey, Berlin, 1968).

⁶ McHugh, T., *Zoologica*, **43**, 1 (1958).

⁷ Frädich, H., *Handbuch der Zoologie*, **8**, 10, 16 (De Gruyter, Berlin, 1967).

⁸ Schaller, G. B., *Natn. geogr. Mag.*, **135**, 494 (1969).

⁹ Schaller, G. B., *The Serengeti Lion*. (University of Chicago Press, Chicago and London, 1972).

¹⁰ Schenkel, R., *Am. Zool.*, **7**, 319 (1967).

¹¹ Heptner, W. G., Nasimovitch, A. A., and Bannikov, A. G., *Die Säugetiere der Sowjetunion*. (Fischer-Verlag, Jena, 1961).

¹² Ulrich, R. E., and Azrin, N. H., *J. exp. Analysis Behav.*, **5**, 511 (1962).

¹³ Azrin, N. H., Hutchinson, R. R., and McLaughlin, R., *J. exp. Analysis Behav.*, **8**, 171 (1965).

¹⁴ *The Behaviour of Ungulates and its Relation to Management* (edit. by Geist, V., and Walther, F.) (International Union for the Conservation of Nature, Morges, in the press).

¹⁵ Linsdale, J. M., and Tomich, P. Q., *A Herd of Mule Deer* (University of California Press, Berkeley and Los Angeles, 1953).

book reviews

Physicist on the pyramids

The Riddle of the Pyramids. By Kurt Mendelssohn. Pp. 224. (Thames and Hudson, May 1974.) £3.50.

DR KURT MENDELSSOHN, the noted cryogenist, has in this book turned his attention to the unrelated subject of Egyptology, in which he confesses an intense amateur interest, and in particular to the riddle presented by the pyramids. Students of the subject may well heave a sigh at the addition of yet another title to the immense literature on Egyptian pyramids which has been written since the days of Herodotus. They may also view with misgiving the entry of a physicist into this unfamiliar field, bearing in mind the intervention of an earlier scientist, Piazzi Smith, the Astronomer Royal for Scotland, who in 1877 produced in his work *Our Inheritance in the Great Pyramid* a source of endless inspiration to the obscurantists who caper on the fringes of the subject. Such fears may immediately be set at rest: Dr Mendelssohn has a unique contribution to make which deserves very careful consideration by the experts as well as the attention of the general reader to whom this book is primarily addressed.

What the author sets out to do is to produce some rational explanation of why such an immense effort was made in raising the great stone pyramids of Egypt over four and a half thousand years ago. The usual explanation advanced by Egyptologists is that such edifices were the tombs of kings who were regarded as gods incarnate, and who according to the religious ideas of the age had to be buried in or under them to ensure the prosperous continuation of the divine government of Egypt. Dr Mendelssohn finds this an inadequate explanation. Some at least of the royal tombs of the Pyramid Age were cenotaphs and what he has to say about this aspect of the problem (pages 74-77) is well worth pondering. He admits that the pyramids served as royal mausolea, not necessarily tombs, but he considers that their funerary function was not the only purpose of their construction, or indeed even the main one.

The point of departure of his enquiry is a discussion of the constructional problems presented by the large stone pyramids at Meidum, Dahshur and Giza. He has already formulated most of his ideas in recent papers in the scientific journals, but in this book

he is able to expand them significantly. The step pyramid which raises its impressive tower above extensive mounds of debris on the desert verges at Meidum was evidently completed by Sneferu, the first king of the fourth Dynasty in about 2600 BC, by adding a cladding of fine limestone, thus converting it to the first true pyramid in Egypt. All the archaeologists who have excavated this monument have agreed that the loss of the outer casing was due to the subsequent operation of stone thieves who used the pyramid as a quarry. Dr Mendelssohn will not accept this finding and has produced impressive testimony to show that the Meidum pyramid collapsed during its final building because its structure had a number of inherent weaknesses. He draws a comparison with the Aberfan disaster; and certainly the aerial view of the pyramid given in his plate 23 clearly shows the plastic flow of the ruined mantle around its nucleus. The shearing-off of this outer covering by such a catastrophe also explains satisfactorily certain architectural features evident in the remaining core and the extraordinarily high mounds of rubble at its base. There are few Egyptologists who will wish to challenge these arguments of Dr Mendelssohn, and pending any final discoveries that may be made by the complete excavation of the pyramid, we may regard his explanation as the most plausible that exists.

The author then proceeds to discuss the effect of this disaster on the design of subsequent pyramids and tries to show that it was responsible for the abrupt change of angle in the Bent Pyramid at Dahshur and for the character of its mantle. He also argues that it promoted improvements in the design of the North Pyramid at Dahshur and the three giants at Giza. If his arguments be accepted, some revision of our views about pyramid building is required since it is clear that two or more great pyramids were being built at the same time during the reign of Sneferu at least, and the implications of all this form the second and more provocative and perhaps more interesting part of the book.

In essence the author's argument is that the consecutive construction of pyramids, one during each reign, is an economic and organisational impossibility. Owing to their immense size the building of pyramids on the scale undertaken in the Fourth Dynasty had become an activity in its own right

demanding its own economic rules. It was the pyramid and not the pharaoh that ruled Egypt; and new pyramids had to be raised whether a pharaoh was ready for burial or not. The erection by the genius of Imhotep of the first great stone building in Egypt, the Step Pyramid of King Djoser in about 2680 BC, began a movement which escalated into a self-sustaining process that affected the seasonal employment of a vast army of workers whose communal feeding, clothing and upkeep for several months during each year must have completely revolutionised the pattern of life for the whole country. A central administration became responsible for the livelihood of a great proportion of the rural and artisan population in place of local councils and village elders. In short, the immense communal activity of building the pyramids created the cohesive Egyptian state with its cohorts of civil servants, and could not be abandoned though it was subsequently modified.

This brief analysis may not do full justice to the author's thesis which is persuasively argued with analogies to the later Mexican pyramids and modern state enterprises. Of course not all the interpretations will command the approval of Egyptologists who form a notoriously sceptical body of opinion; nevertheless we should be grateful to Dr Mendelssohn for obliging us to re-think the received view of the Pyramid Age. One of his readers, at least, will be unable to look again at the monuments of Meidum, Dahshur, Sakkarah and Giza in quite the same old way.

CYRIL ALDRED

Making electricity

Thermionic Energy Conversion. Vol. 1: Processes and Devices. By G. N. Hatsopoulos and E. P. Gyftopoulos. Pp. xi+265. (MIT: Cambridge, Mass. and London, 1973.) \$17.95; £9.00.

THE increasing interest in alternate energy systems makes the appearance of this book apposite; unfortunately like *The Times* it is wise after the event. The method of converting heat to electrical energy described requires thermal sink temperatures high by comparison to room temperature thus making the method suitable in principle for space use. The buildup of the US space programme thus corresponded to a time of intense activity in this field: the rundown of space activity has led to the demise of thermionic energy



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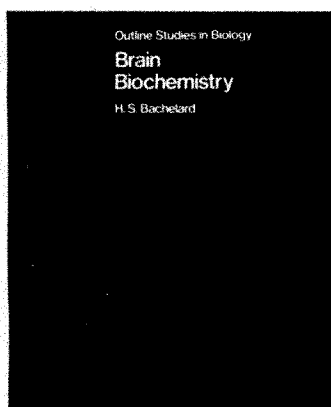
E. DUFFEY, M. G. MORRIS, J. SHEAIL,
L. K. WARD, D. A. WELLS and T. C. E. WELLS

July 1974: 0 412 12290 1: 304 pages:
tone and line illustrations: hardback: £5.40

This book describes the distribution and ecology of lowland grasslands in Britain with special reference to their flora and fauna, history, and management for wildlife conservation. The maintenance and manipulation of grasslands for agricultural, scientific, conservation and recreational purposes requires an extensive knowledge of the responses of plants and animals to different treatments and disturbance factors. The book examines these in relation to the range of variation in lowland grassland ecosystems and to the known land-use history.

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July 1974: 0 412 12760 1: 72 pages:
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illustrated: paperback: 95p

Further details of these and related titles, together with a list of stockists, are available from the publishers on request.

conversion. It is therefore difficult to see for whom this book was written; without device requirements it will be of little use to the engineer; it will infuriate the physicist.

Volume 1 is intended to summarise the field and present ideas which will not be changed by subsequent developments. Unfortunately the analogues and the presentation of physical principles do not bring out the excitement of understanding and therefore make it worth undergraduate study; the continued reliance on the statement "a more detailed presentation appears in volume 2" means that the physicist without volume 2 will give up in disgust.

The four chapters of this book present an introduction to thermionic converters, an analysis of ideal performance, a description of vacuum converters and finally a chapter on vapour filled thermionic converters. In what is clearly intended to be a definitive book the brevity of the historical review in chapter 1 is surprising, but most of the various types of converter prior to 1966 are well illustrated. The book has, however, clearly been some time in preparation and converters developed after 1966 are not mentioned. The book is lavishly illustrated, so that the relevant text is often several pages away; it is, however, reasonably indexed, gives a large number of references and contains a useful set of tables and curves. The latter when used in conjunction with the step-by-step instructions given in chapters 3 and 4 give reasonable approximations to observed device characteristics and thus form one of the most useful parts of this volume.

In view of the coherence of presentation suggested by the authors for the complete book, it is perhaps unfair to judge this volume in isolation; however without volume 2 it does not seem worth £9.00.

A. W. PENN

Antibody diversity

The Variation and Adaptive Expression of Antibodies. By George P. Smith. Pp. xii+219. (Harvard University: Cambridge, 1973.) \$12.

THE comparative analysis of amino acid sequences of immunoglobulins has been a major preoccupation of immunologists and molecular biologists for a number of years. The mass of data and the conclusions drawn from this have had a fundamental influence on present ideas on the genetic bases of antibody diversity and the evolution of protein structure. This book presents an exhaustive computer analysis of the data available to the author at the time, in an attempt to define evolutionary relationships.

The book starts with a short survey

of fundamental concepts, intended for readers who are unfamiliar with immunology, and goes on to present tables of all the amino acid sequences that are the subject of subsequent analysis. Unfortunately, since the tables were completed a very considerable amount of important data has been published. In this respect the book is already out of date and does not include an interesting variety of sequences of human, mouse or rabbit κ chains. The analysis of heavy chains fares worst as the book does not adequately cover human μ , α and ϵ chains or mouse γ chains.

A very useful chapter is then devoted to the procedures used for the reconstruction of protein evolution. This provides the reader with a good introduction to the intricacies of the computer programs used. This is followed by an elaborate study of the evolution of C regions. The analysis, although merely confirming accepted ideas, does provide a very balanced view on the reliability of the proposed evolutionary patterns.

The following two chapters are devoted to an exposition of different theories on the origin of antibody diversity and the contrast between the expectations of each theory and the evolutionary patterns derived from the data. The exposition of the theoretical expectations is clear and detailed. The arguments in favour of the germ line theories are presented at their best. This is not the case in the presentation of the opposed arguments. (For instance, the critical discussion on linked parallel mutations (page 103) is only marginally touched on and does not include the possible requirements of tertiary structure.) This is to be expected since the author presents himself as a convinced germ liner.

Three appendices cover references for all the proteins listed, a discussion on allotypes and on ribosomal RNA genes as a model of multigene evolution.

Altogether this is a very readable book which transmits the intellectual excitement of the intricacies of the significance of every possible evolutionary clue. But the problem of antibody diversity is no longer the exclusive domain of protein chemists and the impact of the book is likely to be affected by the success of other approaches. Such new avenues are not properly presented. I was particularly struck by the author's remarks on DNA-RNA hybridisation (page 112): "Conclusive results from this type of experiment depend on the purification of immunoglobulin mRNA . . . a difficult technical feat". Alas, such a "technical feat" was being performed in several laboratories and in fact relevant papers were published at the same time as this book.

C. MILSTEIN

Change in New Guinea

The Chimbu: A Study in the New Guinea Highlands. By Paula Brown. Pp. ix+151. (International Library of Anthropology.) (Routledge and Kegan Paul: London, July 1973.) £3.25.

DR BROWN has, during a period of 15 years, observed the effects of social change in the population of the New Guinea Highlands. This group of people was first discovered by an Australian prospecting expedition in 1933 and many observations were made by administration officials, missionaries and an occasional anthropologist. These records were available to Dr Brown and with her own observations from 1958 onwards, she has been able to make a careful study of the effects of social changes. There is probably no other country in the world where it has been possible to observe profound changes which have occurred over a relatively short time. New Guinea has consequently become a haven for anthropologists but Dr Brown has made one of the most complete studies.

The Chimbu are usually regarded as among the most intelligent and energetic people in New Guinea and as Dr Brown describes, after an initial turbulent period, they seem to be adopting European culture with apparent enthusiasm. The introduction of medical services in recent years has reduced the infant mortality and this, in turn, has caused overpopulation. A few years ago the severe disease kwashiorkor, due to under-nutrition, was prevalent amongst Chimbu infants but this no longer occurs. Local government councils, the introduction of the internal combustion engine for transport and for agricultural purposes, the provision of schools, trading stores, cash crops, missions, European dress, roads, the development of law enforcement and political debating are all part of the new European culture. It is a fascinating story related accurately and entertainingly by Dr Brown.

The 19 chapters of the book are clearly divisible into three parts. The first describes the historic features of the life of the Chimbu people, the second is concerned with the dynamics of tribal life and the third describes many of the effects of change.

A biologist perhaps might be pardoned if he regrets that the biological changes, especially those affecting the growth and development and general health standards of infants and children in the area, were not studied at the same time as the social changes. It is known that such changes have occurred and their correlation with the cultural and social study of Dr Brown would have been interesting. An adjacent area

was the scene of the recent study during the International Biological Programme. Regrettably the latter did not include a study of the effects of social change such as that undertaken by Dr Brown in Chimbu. The problems of communication between different groups of research workers are often as great in New Guinea as in the more advanced countries, and opportunities are lost.

R. J. WALSH

Soviet geology

Geology of the U.S.S.R. By D. V. Nalivkin. Translated from the Russian by N. Rast. Edited by N. Rast and T. S. Westoll. Pp. xviii+855. (Oliver and Boyd (Longman): Edinburgh, November 1973.) £25.

APART from a very slim volume, published in translation in 1960, this is the first and only comprehensive work on the geology of the Soviet Union in English and so, whatever its quality, 855 pages of close type must be taken very seriously. But in relation to the immense geoscientific output from the Soviet Union, with so many multi-volume series on systematic aspects of Soviet geology, this work also is possibly unique in covering so large a field within a single volume.

It suffers a little from age. The interval between 1962 when published in the Soviet Union and late 1972 leaves a time of very great progress in Soviet geology unrecorded. But the value of the work to the English reader is to set the whole range of Soviet geology in perspective in a single framework, which in broad outline at least is hardly affected by recent work. So this volume serves as an introduction for western geologists as well as Soviet students to that vast territory.

The scheme of the book is distinctive, and as in so many general works in Russian science there is a rather explicit philosophy, reflecting scientific as well as political fashion (more than ten years old). In this case the philosophy is made to work throughout the volume. In short it is that geological history can best be understood in terms of geosynclinal development taken in a somewhat comprehensive, genetic and extensive way so as to include many aspects of tectonics including orogeny.

In consequence the book is classified thus: part 1, introduction; part 2, Precambrian geosynclines (Russian platform; Siberian platform; Western Siberian lowlands); part 3, Palaeozoic geosynclines (Uralian geosynclines; Western Arctic geosynclines; Argara geosynclines; Middle Asiatic geosynclines); and part 4, Meso-Cainozoic geosynclines (Mediterranean geosynclines; Pacific Ocean geosynclines).

There is indeed considerable attention paid to these controlling concepts both in the introduction with its historical review of Russian geology and a treatment of such principles, as well as in the translator's introduction. This treatment is indeed quite effective because geosynclines are strata, and the book adopts a thorough and explicit stratigraphical approach to tectonics, magmatism, and useful minerals in each chapter. This coherence is its strength in that a great amount of detail is well organised; it is also well referenced and well indexed.

Because such a unified approach is, with increasing knowledge and specialisation in the Soviet Union, likely to become less and less possible, this work will probably stand without competition as one of the classic regional descriptions, in the line of Heim's *Geologie der Schweiz*, David's *Geology of Australia*, and the recent multi-author Geological Survey work on Canada. Indeed national pride harnessed in this way is most welcome.

Professor Rast is an experienced translator and the work reads well in English. The many photographic illustrations give a sample of varied field conditions in that vast territory directed towards the student population about to explore it. There are a number of attractive sections but as with many Russian works the maps leave much to the imagination. The work is interspersed with innumerable fold-out correlation charts from which are seen, at a glance, both the regional variations and the contemporary Soviet view of stratigraphic classification.

W. B. HARLAND

Genetics practical

Practical Genetics. Edited by P. M. Sheppard. Pp. xii+337. (Blackwell Scientific: Oxford and London, 1973.) £8.50.

FIRST experiences of practical classes in biology are usually traumatic but rewarding for students and teachers alike, for what they observe for themselves often differs from what they were led to expect by plausible lecturers and the pedantry of textbook figures. This is particularly so in genetics practicals where the main technique of crossing and progeny analysis rarely provides the same goodness of fit to expectation which blessed Mendel's results. A working knowledge of statistics goes hand in hand with practical work in genetics and this, coupled with problems of obtaining suitable material, has given rise to the misleading impression that successful practicals are too difficult to organise.

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and the final year of degree courses will be grateful to Professor Sheppard and the other contributors for producing this excellent hybrid between text book and practical manual. The seven chapters include full descriptions of the techniques of cytogenetics and the culture methods for a wide range of organisms with detailed practical guides to exercises in basic genetics using *Drosophila*, fungi, bacteria and bacteriophage. Other chapters outline exercises in population, ecological and quantitative genetics and provide clearer accounts of biometrical genetics and elementary statistics than are found in most textbooks. Perhaps the chapter of most general value is the one concerned with higher organisms where a mine of information on the use and availability of teaching material emphasises that practicals in genetics need not be restricted to the few organisms commonly used in research.

Some of the suggested exercises can each be carried out by individuals in a single practical session, whereas others require the cooperative efforts of a number of students over a full term. All of the authors have provided a useful list of altruistic university departments and commercial suppliers that can provide suitable materials.

The editing is lenient, allowing some useful repetition, but the legend to plate 4.7 underlines the role of practicals in encouraging students in critical observation: those who follow the instructions and prepare slides of meiosis in locust species, might question whether the plate depicts diakinesis in *Schistocerca gregaria* or diplotene in some other species. JOHN GIBSON

Protein in stereo

Atlas of Molecular Structures in Biology. 1, Ribonuclease-S. Prepared by F. M. Richards and H. W. Wyckoff; edited by D. C. Phillips and F. M. Richards; figured by J. L. Mouning and J. W. Schilling. Pp ix+75. (Clarendon: Oxford; Oxford University; London, 1973.) £3.50.

PRESENTING the structure of a protein molecule is perhaps as immense a problem as solving the structure. In this volume on ribonuclease-S the editors and authors present a workable format for this task. It is the intention of the series to present structure unencumbered by interpretation. A short description of what the enzyme does, giving appropriate references to more detailed reviews and primary source material, starts the pleasantly brief text. After all, it is the pictures which are important in this book. Following is a series of tables and figures giving such items as physical properties, amino acid

sequences, Cartesian coordinates, hydrogen and ionic bonds, and various contact and angular properties of the chain. Some of the measured coordinates and angles are presented in several different forms, thus eliminating some conversion by the reader and making the work more usable.

The heart of the volume is the computer-generated drawings of the molecule. Forty-three red-green stereo drawings depict the main chain and all non-hydrogen side chain atoms from three mutually perpendicular directions. Four of these are specially devoted to the environment around the UpcA inhibitor complex. Many of the others are taken in ordered slices through the molecule. The publishers did an excellent job in the alignment of the stereo pairs but there was a slight shadow effect caused by an incorrect match of the green ink with the green filter in the stereo glasses provided. This effect became more pronounced as one moved closer to the diagram to read the small residue labels. From normal viewing distance the three-dimensional effect was quite good.

The last section contains the electron density maps for the apo-protein. This section should give the non-crystallographer the opportunity to see how a complete electron density map looks. The electron density of each section of the map is printed in green, and superimposed on it are all the atoms involved in that section with their Van der Waals radii drawn in red. By closing one eye and using the stereo glasses, one can see either the map or model. This section is excellent in its concept and execution. Unfortunately there is no index as to which sections on the map a residue will appear in, so the co-ordinate list must be used.

In general, the volume provides an excellent source for the structure of ribonuclease-S. Distances between residues of interest can easily be calculated from the coordinate lists and environment relationships can be found by consulting the many stereo diagrams. The major fault of the series is not in this volume, but perhaps in future volumes. Ribonuclease-S is approximately one-fifteenth the size of the larger multi-component proteins now being studied. The inclusion of the necessary diagrams to fully depict the subunit structure and the important subunit-interactions of one of these large proteins would not only make the volumes larger, but more expensive to produce and to purchase. Unfortunately, there seems to be no simple solution to the problem of disseminating all the structural information of a molecule as large as a protein, and this volume presents a good compromise solution. STEVEN J. STEINDEL

Muscle and silk

Conformation in Fibrous Proteins and Related Synthetic Polypeptides. By R. D. B. Fraser and T. P. MacRae. Pp. xviii+628. (Molecular Biology: An International Series of Monographs and Textbooks.) (Academic: New York and London, December 1973.) \$45; £19.50.

THE fibrous proteins form an important class among the complex structures found in living organisms, and the investigation of their structure poses special problems because they cannot, in general, be obtained in the form of single crystals suitable for X-ray investigation. The first part of this book describes the major techniques in current use and their application to the problems. They include X-ray, electron and optical diffraction, electron microscopy, infrared spectroscopy (using polarised radiation) and the important if less direct techniques based on the use of known atomic dimensions and interaction energies which may be described as model building. There is also a chapter on methods for obtaining the optimum structure and another on several techniques of more limited application.

The second part describes in considerable detail the several well-characterised conformations found in synthetic polypeptides (α -helix, β -conformation and so on) which have been basic to an understanding of the structure of fibrous proteins, and there are separate chapters on the silks (of which many kinds are known), collagens, muscle proteins and the keratins. There is a chapter on fibrin, fibrinogen, flagellin, elastin and resilin.

The writing is lucid and critical. The many line diagrams are excellent and there is a good selection of half-tone reproductions, chiefly of diffraction patterns and electron micrographs. The list of references is very comprehensive and it is evident that care has been taken with the indexes. The authors (who have made major contributions to our understanding of fibrous protein structure) are to be congratulated on the publication of such a thorough and useful work.

A. ELLIOTT

Drifting in the Pacific

The Settlement of Polynesia: A Computer Simulation. By M. Levison, R. Gerard Ward and J. W. Webb. Pp. vi+137. (University of Minnesota Press: Minneapolis, 1973.) £5.50.

THE question of how the scattered islands of Polynesia were settled during a 2,000-year period ending about a thousand years ago has been a perplexing one for European colonisers

whose own methods of navigation only allowed them to arrive there less than 300 years ago. One rather loud voice in the debate in recent times has been that of Andrew Sharp. He simply applied to the problem his hard-headed common sense, Occam's razor, and a due cynicism about far-fetched or fantastical tales of intrepid ancient Polynesian seamen with sophisticated navigational skills. After all, Polynesians were both illiterate and living in the Stone Age. Sharp's thesis is that island discovery took place by unnavigated one-way voyages either of exile, or accidental drift, and that there is no reason to suppose that deliberately navigated journeys of exploration or long voyages with return to the starting place occurred. In the preface to his 1964 book, Sharp states, "It is six years since my previous book . . . During those years I have gathered upwards of a hundred published notices . . . and have been involved in 2,191 oral discussions of the book's themes. I have yet to hear of a fact or read an argument which impugns the basic contentions of the former book". The present book by Levison, Ward and Webb, contains both facts and arguments which do impugn Sharp's contentions.

What Levison, Ward and Webb have done is to take the recorded data of frequencies of different winds and currents throughout the Pacific, and simulate by computer one-way drift voyages from a variety of starting points.

All the assumptions of the simulation seem remarkably sensible. On each day a particular wind and a current direction are chosen randomly from the available recorded frequencies of winds and currents for the particular month in the particular $5^\circ \times 5^\circ$ (approximately 300 nautical mile) square, and the vessel is moved an appropriate distance and direction. A craft makes a landfall if it passes within 10 miles of a low island or 20 miles of higher ones. There is a sigmoid curve of increasing tendency of a voyage to end due to factors like death of the crew (50% of voyages terminate in this way by 10 weeks). But there is also a 0.5 probability of foundering if a gale of force 9 or above occurs.

The authors have simulated more than 100,000 drift voyages, and 8,000 deliberately guided ones. The drift voyages included a main series of 46,000 from 61 starting points starting every day of the year, 11,000 under shifts of climatic conditions of the kind that might have occurred in the last 2,000 years or so, 32,000 starting from specially advantageous points at specific months of the year, and 10,000 in reverse from destinations towards possible starting points.

Their conclusion was that drifting produced a zero probability of reaching either Hawaii or Easter Island from any inhabited part of Polynesia, and less than 0.1% chance of reaching New Zealand. Thus drifting alone does not seem to be able to account for the colonisation. Among other things they also show that Kon Tiki would never have reached Easter Island, or anywhere else in Polynesia unless it had been very deliberately steered westward (which it was). Indeed, in simulated voyages with an attempt to hold a particular course, and with a limitation of only being able to lay as close as 90° to the wind, the chances of reaching the outlying parts of Polynesia become quite reasonable.

Levison *et al.* are judicious on the subject of relevant archaeological data and the various theories that have been put forward. They also are scrupulous about the technical part of their simulation. Both a complete listing of their ALGOL program and a large selection of their results in the form of maps of scattered destinations of voyages from particular starting places appear in appendices which occupy about half the book. But the non-technical should not be deterred. The text though detailed is well written, and the whole is a substantial contribution. Put together with the recent publications by Gladwin and Lewis of sophisticated navigational techniques still practiced in the Caroline Islands, this work finally disposes of the idea that Polynesians were savages who just happened to have been blown to accidental discovery of the vast reaches of the Pacific which they came to inhabit.

KEITH OATLEY

Relativity with reprints

General Theory of Relativity. By C. W. Kilmister. Pp. ix+365. (Commonwealth and International Library: Selected Readings in Physics.) (Pergamon: Oxford and New York, November 1973.) £3.50 boards; £2 paper.

ACCORDING to the publisher's note on the back cover, this book meets "the increasing need for an undergraduate text on the general theory of relativity". It will be an unusual undergraduate work which absorbs more than a portion of it but there is a need for a textbook for all students with ambitions to research into relativity, cosmology and astrophysics (and pure differential geometry). This should ideally not only present the facts about relativity they need, but also assist the transition from didactically helpful lectures to the scientific literature.

This book is important because it has both these aims. Before venturing

to carp at unimportant idiosyncracies I should say that it is wholly recommended to such students because it largely fulfills them. The first hundred pages contain the author's exposition of the principle of equivalence, of Einstein's theory and of more recent developments. The rest of the book consists of eleven reprinted papers.

The author's account of the motivation for the theory is largely from the equivalence principle. Mach's principle is dealt with summarily; Einstein is said (page 12) to have hoped that his theory would incorporate it but the grounds for this hope are not made clear. Eddington's demonstration that relativity diminishes the number of constraints that "laws of nature" impose on the world is scarcely mentioned though for many students it is the most stimulating feature of the elementary part of the theory. Cosmology is dealt with quite briefly; the Robertson-Walker models are not derived and the book is not advanced enough for global properties or singularity theorems. Gravitational radiation is taken no further than the classic 1962 paper of Bondi, Burg and Metzner which is reprinted with explanation in the text; and the spinor calculus no further than Penrose's 1960 paper reprinted without.

The book is therefore not quite an introduction to current research, but an advanced textbook, and a very good one.

P. E. ROE

Teaching mathematics

Developments in Mathematical Education. Edited by A. G. Howson. Pp. ix+318. Proceedings of the Second International Congress on Mathematical Education, Exeter, UK, August 1972.) (Cambridge University: London, 1973.) £4.80; \$14.50.

THE conference reported by this book consisted of thirty-eight working groups which dealt with the subject, on a broad basis, from kindergarten to the university.

The report appears in three parts. The first consists of a survey of the congress and covers a wide range of topics, including the psychology of mathematics learning; mathematics and language; mathematics at primary and secondary level and its link with other subjects, applications, history and assessment of mathematics; professional training of teachers and the use of teaching aids; and mathematics in developing countries.

The second part of the report consists of invited papers whose authors include G. Pólya, J. Piaget and Sir James Lighthill. There is also a paper by S. L. Sobolev on "Some Questions

of Mathematical Education in the U.S.S.R.". The third part consists of a selection of congress papers. These cover problem solving; intuition, structure and heuristic methods; geometry as a gateway to mathematics; and the work of Piaget in the training of students to teach primary mathematics.

My impression of the present state mathematical education is that too much is going on at the same time on too many fronts. Confusion is evident in the clash of differing philosophies, and it will probably be a matter of decades before a settled place is found for mathematics as a field of human endeavour. There seems to be far too much experiment, much of which is badly planned, or, worse still, introduced on impulse. But mathematical concepts, as a way of thinking, are recognised as being of greater importance than in any past period of history. Mathematics is no longer a matter only of number and numeracy. Structure, including concepts and their relationships, is now consciously identified as a fundamental aspect of human thinking. One of the main problems of mathematical education in the coming years will be to determine the place of structure in the thinking and behaviour of the mass of mankind.

L. S. GODDARD

Air and ocean

The Physics of Air-Sea Interaction. By S. A. Kitaigorodskii. Translated from the Russian. Pp.v+237. (Israel Program for Scientific Translations: Jerusalem, 1973.) n.p.

THIS book will undoubtedly become an essential work for the ever increasing circle of physicists, meteorologists and oceanographers for whom the interaction of the air with sea has become an important problem. Although there are omissions it will provide an up-to-date text upon which to build some of the newest theories of air-sea interaction.

The book is written in three parts. The first part, "Dynamics of the Marine Surface Mixing Layer of the Atmosphere", is devoted to the physics of the layer of the atmosphere immediately adjacent to the water surface. From the starting point of the Monin-Obukhov form for the generalisation of a logarithmic boundary layer to a temperature-stratified medium, the author examines the usual turbulent fluxes of momentum, heat and water vapour before continuing with a study of the aerodynamic drag of the sea surface and its relation to the characteristics of the turbulent atmosphere and of wind-generated waves. Several chapters are devoted to the analysis of results from field measurements of atmospheric

turbulence over waves in the Mediterranean and show some of the procedures used for evaluating the results from this complex problem. The last chapter in part 1 is concerned with the possible influence of high humidity on the vertical density stratification over oceans and collates and analyses data from many sources on gradient measurements and standard seaborne observations.

Part 2, "Wind Waves in Deep Sea" is a description of some fairly general similarity hypotheses concerning wind waves, and of tests of these on experimental data. The author attempts to reduce the complexity of the problem by obtaining some universal dimensionless parameters to describe the relationship between the turbulent wind and the spectrum of the wind waves. The approach and content is very different to that found in Kinsman's *Wind Waves* and these two texts complement each other well. Again there is much use made of experimental data with prominence given to the Soviet Mediterranean expeditions in 1965. Particularly interesting are the results from a wide-band string wave recorder used to obtain extensive measurements of open-sea wave characteristics in the high frequency region (up to 7.5 Hz). No mention is made, other than a statement confirming its omission, of the non-linear interaction between components in the wave spectrum.

In the third part, "Dynamics of Vertical Mixing Processes in the Upper Ocean", the author turns from his extensive use of experimental data and considers some of the many physical hypotheses underlying the characteristics of sea turbulence and the ways in which they effect the vertical mixing processes. Several models are used to make predictions about the energy balance of dynamic small scale turbulence in the wind mixing layer. The final chapter considers the seasonal variations in the active layer of the ocean. It seems a little out of place in a book concerned mainly with the physics of small scale interactions. Perhaps the author is right at this point to expand the field of view and to introduce some long term variations.

The text is also valuable for the attention it pays to some of the less well known expedition results of the Institutes of Atmospheric Physics and Oceanology of the Academy of Sciences of the Soviet Union and for the comparisons made between these data and those of the more usually quoted sources. Overall, an excellent book; my only criticisms are the minute size of type used for the equations and the confusing choice of symbols on a few diagrams.

C. E. VINCENT

science on television

Human face of Watson and Crick

by Peter Newmark

Back in the balmy days of the early 1950s, before the national press was heavy with doom and the scientific press with molecular biology, small groups of scientists in London, Cambridge and California were working away on the academic problem of the structure of DNA. And then it happened. The day after Mr Churchill became Sir Winston, Francis Crick and James Watson published their proposed structure for DNA in these pages and "The Race for the Double Helix" was over. The Horizon programme of that title dealt with the last few months leading up to this discovery.

Treading much the same ground as, and in a similar vein to, Watson's book *The Double Helix*, the programme dealt with the people involved much more than with the science. Indeed the only real bit of experimentation shown, R. G. Gosling trying to stretch a DNA fibre, failed (intentionally?) in front of the camera. What was shown, and very successfully, was the way in which the course of the race was affected by the personalities involved.

The story was narrated by Sir Michael Swann and divided into segments each introduced, silent movie style, with a still caption—such as "The Race Is On". But anyone expecting matching melodrama, villains and chivalrous heroes would have been disappointed. The characters were no larger or better behaved than life. There were Maurice Wilkins and his group studiously improving their X-ray data and moving towards the answer, Linus Pauling applying his expertise to other X-ray photographs and Watson and Crick building models *ad infinitum*.

All these people and more appeared to tell their tale. Of the main characters only Rosalind Franklin is no longer alive. It was her involvement that provided the most human touch to the whole story. Employed by Wilkins for her knowledge of crystallography, it soon became evident that the two were incompatible. This, or so it was related in the programme, resulted in a degree of isolation for Franklin that probably cost her the first place in the race. Nor were relations between her and Watson much better. He needed her data but

could offer little in return. Meetings were tense and even degenerated to fisticuffs on one occasion. Pauling was equally human. Although expecting to discover a double helix, he made a crucial misinterpretation of some data and opted for a triple helix. His son passed a pre-publication copy of the paper describing this to Watson and Crick. Their disbelief in the proposed structure acted as a spur for their own activities.

From their own account, surely a bit exaggerated by the passing of years, theirs was casual labour. Between coffee and tennis, Watson would play with bits of cardboard whilst Crick, trying to complete a mature doctoral thesis, would occasionally add a word of advice. Whatever the truth, and Watson did at one stage admit to working hard, the base pairing was realised and the full structure immediately followed.

Crick has recently written that in their classic paper "the structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it." The nice thing about the programme was that it did show how, and in human, rather than scientific, terms. It was an excellent exercise in demonstrating the foibles and failings of scientists and acted as a splendid counterbalance to the reverent attitudes to which even Horizon is often prone. Nevertheless it was made quite clear that some three months before Mount Everest was first climbed, a scientific discovery of equal magnitude had been achieved.

The Valley of Paradise

by Allan Piper

LIKE Liverpool versus Leeds, and Royal Weddings, Vilcabamba—the "Valley of Paradise"—and its extraordinary inhabitants might have been made for television. In a beautiful, sun-filled bowl, 6,000 feet up in the Ecuadorian Andes, a community of gentle peasants farm the fertile land. Amazingly, in spite of a daily consumption of 40 cigarettes, four cups of locally distilled, 110° proof rum, and the occasional guinea pig, many of these strange people live to ages of more than 120 yr. And as if that were not enough to ensure a captive audience, there is a hint that it could be attributable to a healthy

sex life. But disappointingly, and uncharacteristically, Granada's "World in Action" team never quite made the best of what was bound to be a good documentary.

Arriving just ahead of the new road through the mountains, and the inevitable tourists, the camera crews have been beaten to Vilcabamba only by the scientists.

Many of their theories are predictable: "The air is so pure" and so on. Other theories are more unorthodox and intriguing. It has been suggested that centuries of close inbreeding have isolated a genetic abnormality, or more remarkable, that after a lifetime of climbing steep slopes and working the land, the calf muscles of these people have become so powerful that they operate as a secondary heart. But the inhabitants of Vilcabamba pay a price for their longevity: half of the children of the valley die before they are 10.

In spite of its unusual content, however, the documentary, (screened on July 8) lacked a sense of coherence. It was never cogent or compelling. Perhaps that was because "World in Action" tried to do too much in too little time. As a result, the programme was often vague and contradictory. We were told that, for example, all the peasants are vegetarian, and also that some eat donkey meat.

We were also presented with various nuggets of hollow information: There are two rivers flowing through Vilcabamba. One is warm, the other is cold. Ho hum. And we were treated on the basis of slender evidence to the thesis that an older woman might be so old because of her healthy attitude towards sex. "Did you have many lovers?" she was asked. Well, she was married once but her husband died a long time ago; just as well really, he used to be a devil—always beating her. Nearly killed her once. Eeee.

Miguel Carpio, 132 years old, tall and bronzed with long black hair and a snow white beard, retired from a life of farming at 125. Healthy and in command of all his mental faculties, he sits upright and impassive as the young intruders from the outside world try to come to terms with his only obvious handicap. "Do you", bawls the reporter, an impolite six inches from Miguel's left ear, "still like women?" Obviously, Miguel is going deaf. Probably, it doesn't bother him very much: at his age he's undoubtedly heard it all before.

Science broadcasting statistics

The proportion of BBC radio time spent on science has never been lower, in spite of the fact that the proportion of time devoted to talks and discussions has been increasing over the past ten years. Yet television has shown that there is a market for science, with ten million viewers watching *The Burke Special*.

James Friday, an archivist on the staff of the Royal Institution has been collecting statistics on the BBC's use of programme time. In 1923 Reith, with his brief to educate, inform and entertain devoted $\frac{1}{2}\%$ of radio time to

scientific programmes. Subsequently the emphasis shifted heavily to entertainment and science was relegated to schools programmes. In 1943 with science up to about 1%, the British Association, whose general secretary was then Richard Gregory, editor of *Nature*, sent a delegation to the BBC to suggest improvements in science broadcasting. The delegation recommended that the BBC should appoint a scientific advisory group and a scientific programme organiser. The BBC rejected both proposals. When similar proposals were made twenty years later by the Pilkington Commission they were accepted in part: a Science Consultative Group was appointed in 1964. But, its influence seems to have been negligible.

Television science has clearly not suffered from the BBC's dislike of having scientists in its midst. But radio producers seem to have become progressively more intimidated by the subject: science at present occupies $\frac{1}{4}\%$ of BBC radio time. With television science included, the proportion rises to slightly over 1%—little more than in 1943. Yet surely science impinges on most peoples' lives considerably more than it did in 1943? The BBC—and scientists—should consider, said Friday in a recent RI lecture, how they can best transmit science and how many hours a week they need to do it before they are overtaken by the report of the Annan Commission and the timidity of the programme producers.

Announcements

Appointments

Roy Gibson has been appointed Acting Director General of The Council of the European Space Research Organisation.

Erratum

In the article 'Nuclear fission as a general source of energy' by L. R. Shepherd (*Nature*, **249**, 717; 1974) the two figures were inadvertently printed above the wrong figure legends. The legends themselves are correct and in the right places.

International meetings

September 2–6, **International Symposium on Approaches to the Early Detection of Chemical Toxicity**, Guildford (Mrs J. King, Department of Biochemistry, University of Surrey, Guildford GU2 5XH).

October, **International Symposium on Andean and Antarctic Volcanology Problems**, Chile (Professor O. Gonzales-Ferran, Department of Geology, University of Chile, Casilla 13518, Correo 21, Santiago, Chile).

October 6–11 **Symposium of the International Society for Photogrammetry**, Banff, Canada (Dr L. Sayn-Wittgenstein, Canadian Forestry Service, Ottawa, Canada).

October 7–11, **First World Congress of Genetics Applied to Animal Production**, Madrid (Professor Dr Luis de Cuenca, Fac de Vet, University of Madrid, Ciudad University, Madrid, Spain).

October 10–12, **International Symposium on Thermogenesis and Depressed Metabolism**, Czechoslovakia (J. Meisner, MD, CSc, General Secretary, Faculty of Natural Sciences, Vinicna 7, 120 00 Praha 2, Czechoslovakia).

October 13–16, **Symposium on Temperature Regulation**, Israel (The Secretariat, POB 983, Jerusalem, Israel).

October 13–16, **Symposium on the Mechanisms of Synaptic Action**, Israel (The Secretariat, POB 983, Jerusalem, Israel).

October 13–17, **Symposium on Environmental Physiology** (The Secretariat, POB 983, Jerusalem, Israel).

October 13–19, **Fifth World Congress of Gastroenterology**, Mexico (V Congreso Mundial de Gastroenterología, Avenida Veracruz 93, Mexico 11 DF, Mexico).

October 14–18, **International Symposium on the Regulation of Growth and Differentiated Functions in Eukaryote Cells**, New Delhi (Professor G. P. Talwar, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, 11016, India).

October 20–22, **Sixth Meeting of the International Association for the Study of the Liver**, Acapulco (VI Reunion de la Asociacion Internacional para el Estudio del Higado, Avenida Veracruz 93, Mexico 11 DF, Mexico).

October 21–23, **3rd Conference on Application of Small Accelerators**, Texas (Dr Jerome L. Duggan, Department of Physics, N. Texas State University, Denton Texas 76203).

October 21–25, **Symposium of the International Atomic Energy Agency on the Thermodynamics of Nuclear Materials**, Vienna (International Atomic Energy Agency, PO Box 590, A-1011 Vienna, Austria).

October 21–25, **Second Conference of the Condensed Matter Division of the European Physical Society**, Budapest (EPS Condensed Matter Division, Conference Office, Research Institute for Technical Physics, 1325 Budapest, PO Box 76, Hungary).

Reports and Publications

Great Britain

Department of Trade Industry, Warren Spring Laboratory—Annual Review, 1972/1973. Pp. 32. (Stevenage, Herts: Warren Spring Laboratory, 1974.)

[116]
Bulletin of the British Museum (Natural History). Historical Series, Vol. 4, No. 5: Sir Joseph Banks and the Plant Collection from Kew Sent to the Empress Catherine II of Russia, 1795. By H. B. Carter. Pp. 281–385 + 4 plates. (London: British Museum (Natural History), 1974.) £5.45.

[126]
British Museum (Natural History). Entomology Leaflets. No. 1: Insects. Pp. 6. 5p. No. 2: Insects and Man. Pp. 12. 9p. No. 3: Aquatic Insects. Pp. 6. 5p. No. 4: Insect Flight. Pp. 6. 5p. No. 5: Butterflies and Moths. Pp. 6. 5p. Zoology Leaflet No. 8: The Domestic Dog. Pp. 6. 5p. (London: British Museum (Natural History), 1974.)

[126]
Microbiological Research Establishment—Abstracts of Work published in 1973. Pp. 19. (Porton Down, Salisbury: Microbiological Research Establishment, 1974.)

[126]
Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 267, No. 889: Late Quaternary History of Vegetation and Climate of the Rajasthan Desert, India. By G. Singer, R. D. Joshi, S. K. Chopra and A. B. Singh. Pp. 467–501. (London: The Royal Society, 1974.)

Other Countries

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 21: The Computation of Optimal Rates of Application of Fertilizers from Quadratic Response Functions. By J. D. Colwell. Pp. 15. (Melbourne: CSIRO, 1974.)

[106]
Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 224: Carboniferous and Permian Stratigraphy of Axel Island and Western Ellesmere Island, Canadian Arctic Archipelago. By R. Thorsteinsson. Pp. 115 (27 plates). \$6. Memoir 368: Surficial Geology of Avalon Peninsula, Newfoundland. By E. P. Henderson. Pp. 121. \$4. Paper 72–51: Surficial Geology of the Kananaskis Research Forest and Marmot Creek Vasin Region of Alberta. By A. MacS. Stakler. Pp. 25. \$2. (Ottawa: Information Canada, 1972, 1973 and 1974.)

[116]
World Health Organization. Technical Report Series. No. 541: Disposal of Community Wastewater—Report of a WHO Expert Committee. Pp. 72. Sw. fr. 6. No. 542: WHO Expert Committee on Filariasis—Third Report. Pp. 54. Sw. fr. 5. (Geneva: WHO; London: HMSO, 1974.)

[116]
CERN—European Organization for Nuclear Research. CERN 74–8: Topical Meeting on Intermediate Energy Physics. Lyceum Alpinum, Zuo, Engadin, Switzerland, 4–13 April 1973—Proceedings. Pp. ix + 220. (Geneva: CERN, 1974.)

[116]
Smithsonian Contributions to Zoology, No. 164: Synopsis of the Families and Genera of Crayfishes (Crustacea: Decapoda). By Horton H. Hobbs, Jr. Pp. iii + 32. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 80 cents.

[146]
Institut for Atomenergi, Kjeller. Kjeller Report 149: The Electrochemistry of Uniform Corrosion and Pitting of Aluminium. By K. Videm, Pp. 85. (Kjeller, Norway: Institut for Atomenergi, 1974.)

[176]
Bulletin of the Fisheries Research Board of Canada, No. 186: The Capelin (*Mallotus villosus*) Biology, Distribution, Exploitation, Utilisation, and Composition. By P. M. Jangaard. Pp. x + 70. (Ottawa: Information Canada, 1974.) \$3.60.

Classified Advertisements

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APPOINTMENTS VACANT
**UNIVERSITY OF ALBERTA
DEPARTMENT OF ENTOMOLOGY**

Applications are invited for the position of **ASSISTANT PROFESSOR**, effective April 1, 1975. Qualifications are Ph.D. with postdoctoral experience in insect physiology and interest in aspects of this field applicable to agricultural or forest entomology. Duties include teaching courses in general or applied entomology and insect physiology, the development and direction of a research program and supervision of graduate students in insect physiology and in application of some aspect of physiology to agricultural or forest entomology. Maximum starting salary \$14,043.

Please send full curriculum vitae and names of 3 referees by October 31, 1974 to: Dr George E. Ball, Chairman, Department of Entomology, 260 Agriculture Building, University of Alberta, Edmonton, Alberta T6G 2E3. (297)

UNIVERSITY COLLEGE GALWAY
**JUNIOR LECTURESHIP
IN ZOOLOGY**

Applications are invited for the above post. Salary scale £2,478 by 99 (10) to £3,468, plus Family Allowances. The closing date for receipt of applications is **August 8, 1974**. Prior to application, further information should be obtained from the Registrar of the College. (308)

**CHARING CROSS HOSPITAL
MEDICAL SCHOOL**

Applications are invited from persons with some experience of electron microscopy or graduates considering entering this field for a new appointment of technician in the Department of Histopathology of Charing Cross Hospital Medical School. Duties commencing September/October 1974 will include the maintenance and operation of transmission and scanning electron microscopes. Some initial training will be given which the successful applicant will be encouraged to supplement by external study. Whitley Council terms and conditions of service. Apply to Dr J. G. Jackson, Department of Histopathology, Charing Cross Hospital Medical School, Brandenburgh House, Fulham Palace Road, London W6 9HH. (352)

**THE UNIVERSITY OF SHEFFIELD
W.E.S. TURNER CHAIR OF
GLASS TECHNOLOGY**

Applications are invited for the **W.E.S. TURNER CHAIR OF GLASS TECHNOLOGY** which will become vacant in September 1975 on the retirement of Professor R. W. Douglas. Salary in the range approved for professorial appointments with superannuation provision. Further particulars may be obtained from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications (one copy only) should be sent by September 21, 1974. Please quote reference R116/G. (384)

**SOUTH AFRICAN SUGAR ASSOCIATION
EXPERIMENT STATION
MOUNT EDGECOMBE, NATAL
REPUBLIC OF SOUTH AFRICA**

CHIEF RESEARCH OFFICER

The Experiment Station of the South African Sugar Association at Mount Edgecombe, Natal, requires the services of a Chief Research Officer. The successful applicant will be responsible to the Director for the operations of the following departments: Agricultural Engineering; Agronomy; Biometry; Chemistry and Soils; Entomology and Nematology; and Land and Water Management. His duties will include the preparation and supervision of annual programmes of work, the administration of a specialist advisory service to all sugarcane growers and the motivation and co-ordination of special research projects. Applicants should preferably hold a Ph.D. or an equivalent degree in science or agriculture. Extensive experience in research will be an essential pre-requisite.

This appointment holds good prospects for an outstanding scientist with organizing ability. It will also include opportunities for overseas travel when visits to other institutes are warranted.

Salary will be commensurate with qualifications, experience and the senior grading of the appointment.

Application forms, a list of benefits (which include a 10% annual bonus) and conditions of service are obtainable from:

**South African Sugar Association,
Fountain House,
125-135 Fenchurch Street,
London,
England EC3M 5EH.**

Completed forms, together with curriculum vitae, should be air-mailed directly to:

**The Director,
S.A.S.A. Experiment Station,
P.O. Mount Edgecombe, 4300,
Natal,
Republic of South Africa.**

(332)

Pharmacologists

The Union International Company Ltd., invites applications for two new posts in the Department of Pharmacology at the St. Albans Research Centre:—

- (A) will be involved with work on mitotic inhibitors, malignancies and immuno-suppression.
- (B) will be involved mainly with various aspects of biological Quality Control work, though there will be opportunities in several of the Department's research projects.

Applications for both posts must have an Honours Degree in Pharmacology, Pharmacy or other appropriate discipline.

Please write, giving full personal, educational and career details to the Staff Manager, (AD 5835), Union International Company Ltd., 14 West Smithfield, London EC1A 9JN.

(428)

British Museum (Natural History)
Sub-department of Anthropology

SCIENTIST

to assist in experimental and bibliographic research in the fields of biometry, radiometry and serology. The work involves the anthropometric and anthroposcopic study of skeletal material in the Museum collection, and investigation of physical and genetic characters in contemporary populations. The successful candidate will also undertake some curatorial duties.

Candidates (aged under 27) must

have a degree, HNC or equivalent in an appropriate subject.

Appointment will be as Scientific Officer; salary in the range £1,900-£3,000.

Application forms (to be returned by 16 August 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service). Please quote SB/32/DK. (382)

COLAISTE NA hOLLSCOILE GAILLIMH (UNIVERSITY COLLEGE GALWAY, IRELAND)

Department of Physics

Applications are invited for a

POST-DOCTORAL FELLOWSHIP

to investigate the heterogeneous nucleation of the solid phase, and its application to:

- deliberate and inadvertent weather modification
- Measurement of the concentration of lead-bearing particles in the atmosphere.

The successful candidate will probably have a Ph.D. in Physics, Chemistry or Meteorology.

The Fellowship will be supported by the National Science Council of Ireland. The appointment will initially be for one year, from October 1st, 1974, with a possible renewal for one or two further years.

Salary will commence at £2,100 per annum.

Applications, accompanied by a curriculum vitae, and the names of two referees, should be sent to—

Dr. A. F. Roddy, Department of Physics, University College, Galway, Ireland, from who further details are available.

Closing date is August 6th, 1974.

(398)

CITY OF LONDON POLYTECHNIC

Applications are invited for a research assistantship (£1,544 to £1,654 per annum, plus payments under Threshold Agreement) to work on the biochemistry of parasitic castration in the roach by the tapeworm *Ligula intestinalis*. The post is tenable for three years and the successful candidate will be expected to register for a higher degree.

Application forms and further details can be obtained from Dr. G. L. Underwood, Department of Biological Sciences, City of London Polytechnic, 31 Jewry Street, London EC3N 2ET.

Closing date—September 16, 1974.

(434)

THE UNIVERSITY OF MANCHESTER Institute of Science and Technology DEPARTMENT OF CHEMISTRY

Experimental Officer (Ref: C/108/AI)

Applications are invited for the post of Experimental Officer in the Department of Chemistry to take responsibility for the running of an Electron Spin Resonance Spectrometer.

The instrument is to be used to provide an analytical service to research and teaching groups in the department and also to carry out development work in the use of E.S.R. and its application to departmental projects.

The post will be particularly attractive to candidates combining a chemical background with an interest and practical knowledge in electronics.

Salary within the scale: £1,758 to £3,114 with F.S.S.U.

Requests for application forms to the Registrar, U.M.I.S.T. Sackville Street, Manchester M60 1QD, to be returned not later than August 6, 1974.

(385)

UNIVERSITY OF EAST ANGLIA SENIOR DEMONSTRATORSHIP IN CHEMICAL PHYSICS

tenable for 2 years from October 1, 1974. Some preference will be given to candidates who have interests in surface chemistry. Salary within the range £2,118 to £2,931 p.a. depending on qualifications and experience, plus F.S.S.U. benefits. Applications should be submitted as soon as possible to Professor N. Sheppard, F.R.S., School of Chemical Sciences, University Plain, Norwich, NOR 88C.

(386)

JUNIOR TECHNICAL OFFICER

required at Institute of Cancer Research, Fulham Road, London SW3, to work in the Division of Molecular Biology. The work is concerned with the isolation and characterisation of proteins in relation to cancer. Candidates should have at least two 'A' levels passes in Chemistry, Physics, Mathematics or Biology. Salary in scale £1,173 to £2,073, plus London Allowance and threshold awards.

Apply in duplicate with the names of two referees, to the Secretary, 34 Sumner Place, London SW7 3NU, quoting ref. 301/B/527.

(387)

UNIVERSITY OF NEW SOUTH WALES PROFESSOR of PURE MATHEMATICS

Applications are invited for appointment to a chair of pure mathematics in the School of Mathematics; there are five chairs in the School, including another one in pure mathematics. Applicants should have substantial research interests in some branch of pure mathematics. The new professor will supervise and participate in teaching and examining undergraduate and postgraduate students in the School and engage in and promote research and advanced study in the field of his chair.

Salary, \$A19,614 per annum. Subject to the consent of the University Council, professors may undertake a limited amount of higher consultative work. The University reserves the right to fill any chair by invitation.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on September 30, 1974.

(392)

UNIVERSITY OF NEW ENGLAND ARMIDALE, NEW SOUTH WALES TEMPORARY LECTURESHIP — AGRICULTURAL BIOLOGY (12 Months)

Applications are invited from graduates in Agriculture, Biology, or Veterinary Science with a particular knowledge of host parasite relationships, epidemiology of livestock parasitic disease and integrated management control measures in agricultural enterprises. Ideally applicants will be familiar with and experienced in student directed learning, the group learning environment and enquiry projects. The appointee will be expected to work in co-operation with a senior lecturer in Pathology and Preventive Medicine to maintain a resourceful learning environment for third and fourth year students of the Faculty of Rural Science in a co-ordinated study of the expressions of disease by livestock through interaction with infective agents; the consequential effects upon productivity; and the means of controlling these with bio-economic constraints by integrated whole enterprise management.

Salary: \$A9,002 to \$A12,352.

Further information can be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on September 6, 1974.

(393)

**UNIVERSITY OF EAST ANGLIA
DEMONSTRATORSHIP
In INORGANIC and/or
PHYSICAL CHEMISTRY**

tenable for 1 year from October 1, 1974. Salary up to a maximum of £2,118 depending on qualifications and experience. Applications should be submitted as soon as possible to the Dean of the School of Chemical Sciences, Professor S.F.A. Kettle, University Plain, Norwich, NOR 88C. (388)

**MEDICAL LABORATORY
TECHNICIAN**

required for expanding diagnostic radioimmunoassay/saturation assay department. Experience in radioisotopic methods is desirable though not essential, but attention to detail and a high standard of orderliness in laboratory work are very important requirements. Successful candidates will be expected to display initiative and to contribute to the development of new techniques in this expanding and important field. Salary on Whitley Council scale according to age and experience (maximum £2,577 p.a.). Applications including a curriculum vitae and the names and addresses of two referees should be submitted to: Professor R. P. Ekins (N), Department of Nuclear Medicine, The Middlesex Hospital Medical School, London WIN 7RL by July 31, 1974. (396)

**THE POLYTECHNIC
OF NORTH LONDON
DEPARTMENT OF CHEMISTRY**

**SRC RESEARCH
TECHNICIAN**

Applications are invited for an S.R.C. research position in each of the following areas:

- Nmr investigations of organothallium compounds. Apply to Dr R. W. Matthews.
- The study of 'template synthesis' and catalysis by transition metal ions. Apply to Dr P. A. Tasker.

The appointments will be made for two years only with salary in the range £1,824 to £2,094. Applicants for either position should be chemistry graduates or have experience in synthetic chemistry and should write as soon as possible, giving details of qualifications and experience, with the names of two referees, to the above at the Department of Chemistry, The Polytechnic of North London, Halloway, London, N7 8DB. (383)

**GRIFFITH UNIVERSITY
Brisbane, Australia
SCHOOL OF SCIENCE
CHAIR IN BIOCHEMISTRY**

Griffith University will start undergraduate teaching in March 1975. The academic organisation is on a broad school of study basis and there is a commitment to interdisciplinary teaching.

Applications are now invited from men and women for the third chair in the School of Science, the existing one being in chemistry and in physics. Some preference will be given to a person with interests in the Biochemistry of metabolism or in physiological chemistry, but candidates with other interests will be considered. The appointment will be from January 1, 1976. In order that the appointee can participate in planning, selection will be completed by April 1975.

Salary will be \$A19,614 p.a. Rates of exchange as at June 1, 1974 are: \$A1=UK 62p, \$US 1.48.

Prospective applicants may care to note that the Chairman of the School of Science, Professor R. D. Guthrie, will be available for consultation in London at the offices of the Association of Commonwealth Universities from July 29 to August 2.

For further information and for details of the method application, please write to the Assistant Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

The closing date is September 21, 1974. (394)



**Fonction publique
Canada**

**Public Service
Canada**

**THIS COMPETITION IS OPEN TO BOTH MEN AND WOMEN
SYSTEMS RESEARCH OFFICERS**

Ecological Systems Research Division
Planning and Finance Branch
Environment Canada
Hull, Quebec
\$16,958 to \$22,880

DUTIES: To conduct research on a variety of subjects of national and international interest to develop environmental policies and programs. Current research programs include work on carrying capacity of Canada in terms of population, resources and environment, development of techniques to evaluate environmental intangibles, macro global modelling, the growth ethics and use of systems analysis to develop resources and environmental policies.

QUALIFICATIONS: At least a Master's degree in Economics or in a discipline related to the duties of the position, combined with adequate knowledge and experience in economic theory and analysis.

The knowledge of the English language is essential for these positions.

Please quote reference number: 74-430-20.

Forward resumé or "Application for Employment" (Form PSC 367-401) available at Post Offices, Canada Manpower Centres and offices of the Public Service Commission of Canada to:

E. S. GROUP
SOCIAL-ECONOMIC PROGRAM
PUBLIC SERVICE COMMISSION OF CANADA
TOWER "A" PLACE DE VILLE
OTTAWA, ONTARIO K1A 0M7.

Appointments as a result of this competition are subject to the provisions of the Public Service Employment Act. (389)

HAZLETON LABORATORIES EUROPE LIMITED

Director of Drug Safety Evaluation

H.L.E. has now set up its contract research organisation in the former Tobacco Research Council Laboratories at Harrogate. We are seeking to make key appointments in the Division of Drug Safety Evaluation, which comprises a Department of Toxicology and a Department of Pathology. In particular we are looking for a Director for the Division, whose responsibilities will include the development of the existing facilities for the full range of toxicological investigations required by current legislation.

Enquiries are invited from either Toxicologists or Pathologists who are experienced in the organisation, performance, evaluation and reporting of toxicology studies. Familiarity with the requirements of the C.S.M., F.D.A., etc. is essential and a knowledge of the contract research industry is desirable.

An excellent salary and the usual fringe benefits will be offered to the successful candidates.

Your application and Curriculum Vitae should be sent, in confidence, to the Personnel Officer, Hazleton Laboratories Europe Limited, Otley Road, Harrogate, HG3 1PY. (402)

AGRICULTURAL RESEARCH COUNCIL OF MALAWI

PATHOLOGIST

A vacancy exists in the Grain Legume Productivity Research Unit for a pathologist to develop improved methods of controlling groundnut diseases, especially of *Cercospora* leaf spot and fungi affecting kernel quality. The successful applicant will work in close association with the plant breeder and agronomist.

Applicants should possess a good degree in agriculture, agricultural botany or a related subject with specialist training in plant pathology. A practical knowledge of fungicide dusting and spraying techniques would be an advantage.

The appointment will be for a contract of three years at a salary and overseas allowance commensurate with qualifications and experience. A tax free gratuity of 25% is payable on completion of contract. Generous leave, free family passages, medical and educational allowances and free educational passages are provided together with a furnished house at low rental.

Applications, including curriculum vitae and names of two referees should be addressed to:

Administrative Officer, P/Bag 3, Thondwe, Malawi. (442)



Wellcome

Information Scientist

New Appointment

The Wellcome Foundation is an international research based company manufacturing and selling human and veterinary medicines, vaccines and drugs.

Through the continuous growth of our operations we need to increase the staff of our Clinical Information Department, by appointing an assistant to our Senior Information Scientist. We require a scientist with a M.Sc (Information Science) or postgraduate Diploma in Information Science, or an equivalent qualification. Some practical experience with computerised information retrieval systems would be an advantage. He will assist in the development and maintenance of computerised information storage and retrieval systems and the dissemination of information on a world wide basis. Initially the post will be based at our head office at Euston, London but the Department will move to Beckenham, Kent in three years time.

This is a good opportunity to join a major international company offering excellent terms and conditions of employment including assistance with relocation expenses where appropriate.

Please write quoting reference U/138 to:

Group Personnel,
The Wellcome Foundation Ltd,
183 Euston Road, London NW1 2BP.

(452)



SIMON FRASER UNIVERSITY British Columbia, Canada DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for the position of Assistant Professor.

Qualifications: Ph.D. with postdoctoral experience; a strong mathematical background and research interests in benthic marine communities.

Nature of duties: Teaching undergraduate courses in invertebrate biology and marine ecology; the development of an active research programme.

Salary: Negotiable.

Letters of application, including curriculum vitae and the names and addresses of three referees should be sent to Dr J. M. Webster, Chairman, Department of Biological Sciences, Simon Fraser University, Burnaby, B. C. V5A 1S6, Canada, before October 1, 1974. (403)

University of New South Wales WOLLONGONG UNIVERSITY

to become the

UNIVERSITY OF WOLLONGONG
1st January, 1975

LECTURER IN BIOLOGY (2 positions)

The appointees will be expected to help in the development of a course in biological energetics. One lecturer will be responsible for an instructional unit in biophysics with emphasis on cellular energy relations. The other lecturer will be responsible for an instructional unit in the physiology of multicellular organisms, again with emphasis on their energy relations.

Further information may be obtained from Dr. A. D. Brown, School of Microbiology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia.

LECTURERS (2 positions) DEPARTMENT OF ELECTRICAL ENGINEERING

Applicants should have high academic qualifications together with research or industrial experience in one or more of the following general areas: electronic circuits, devices and systems generally, logical design and digital techniques; computer engineering and applications; applications of modern control theory; materials science; communications.

Further information is available from Professor B. H. Smith, Department of Electrical Engineering, Wollongong University College, Box 1144, P.O., Wollongong, N.S.W. 2500, Australia.

Commencing salary for both posts according to qualifications and experience within the range \$A9,002 to \$A12,352 per annum.

Conditions of appointment and application forms available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close September 2, 1974. (395)

UNIVERSITY OF GLASGOW LECTURESHIPS IN BIOCHEMISTRY

Applications are invited for two Lectureships in the Department of Biochemistry. The salary will be within the range £2,118 to £3,285 per annum of the new Lecturers' scale of £2,118 to £4,896 per annum, which will be effective from October 1, 1974. Initial placement will be according to qualifications and experience. F.S.S.U.

Applications (eight copies) should be lodged not later than September 6, 1974, with the undersigned, from whom further particulars may be obtained. In reply please quote Ref. No. 3512M. Robert T. Hutcheson, Secretary of the University Court. (397)

Senior Biochemist

A leading British pharmaceutical Research Organisation has a vacancy for a SENIOR BIOCHEMIST to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines. Persons with appropriate experience are invited to apply in writing for an Application Form from The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB, marking the envelope CONFIDENTIAL. (405)

Pharmacologists

Bayer AG., one of the world's largest research orientated companies, wishes to recruit additional pharmacologists to work in Germany. Bayer's pharmaceutical research is carried out in a new £8 million research centre in Wuppertal near Düsseldorf. The successful applicants will either be involved in work on the pharmacology and biochemistry of the cardiovascular system or of blood clotting. Experience in these fields would be an advantage, but new graduates (M.D., Ph.D. or comparable degree required) are invited to apply. A knowledge of German is not essential.

The position will be permanent and offers an attractive and competitive salary. There is an annual bonus with yearly salary-related increments and an excellent group pension scheme. All relocation expenses will be paid.

Please apply in the first instance to:
Dr. H. B. Allen,
Medical Director,
Bayer U.K. Limited,
Pharmaceutical Division,
Haywards Heath, West Sussex.



Bayer

(420)

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY
AND CHEMISTRY**

Applications are invited from graduates, or others with suitable qualifications in biochemistry or related subjects, for the post of Research Technician to work on membrane biochemistry for 14 months from September 1, 1974 (S.R.C. Grant). Salary according to age and qualifications up to £1,833 p.a. plus £126 London Allowance and Threshold Payments. Applicants should send a curriculum vitae and the names of two referees, as soon as possible, to Professor J. A. Lucy, Royal Free Hospital School of Medicine, 8 Hunter Street, London, WC1N 1BP (Telephone 01-837 5385). (401)

**Pharmacologists/
Toxicologists**

Honours Graduates, some experience an advantage, required for pharmacological research and short and long term toxicological studies of compounds of potential therapeutic importance. Excellent opportunities for advancement in modern, well-equipped laboratory. Pension and Assurance Scheme. Application Forms from the Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (406)

**M.R.C. CLINICAL RESEARCH
CENTRE**

(Northwick Park Hospital), Watford Road,
Harrow, Middlesex, HA1 3UJ

SCIENTIST to work in CYTOGENETICS

with a team investigating factors in gametogenesis and early mammalian development that might lead to chromosomal abnormality. The successful applicant will work under the direction of Dr C. E. Ford of the Sir William Dunn School of Pathology, Oxford, with Dr R. D. Barnes in the Department of Infant Development at the Clinical Research Centre. Candidates should have experience in cytogenetics and preferably a Ph.D. in this subject.

Salary and conditions of appointment according to age and experience.

Application forms and further details may be obtained from Mrs J. Tucker-Bull.

Please quote ref. 119/1/EB6. (404)

**HISTOLOGY
TECHNICIAN**

required for Pharmacological and Toxicological Laboratory. Experience essential in the preparation and processing of animal tissues. Good working conditions. Pension and Assurance Scheme. Application forms from The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB. (407)

**NATIONAL VEGETABLE RESEARCH
STATION**

PLANT PATHOLOGIST

There is a vacancy for an officer to assist with research on vegetable virus diseases. Appointment will be as a Scientific Officer (£1,702-£2,675) or Higher Scientific Officer (£2,461 to £3,371), grade and starting salary being determined according to qualifications and experience. Superannuation scheme with allowance to offset personal contributions. The minimum qualification is a degree (or equivalent) in Botany, or horticultural or agricultural science. Candidates for appointment as H.S.O. will normally be expected to have at least five years' postgraduate experience, including experience in plant virus techniques; training in such work will however be given to a less-experienced appointee. Full particulars and application form (to be returned by August 22, 1974) from the Secretary, N.V.R.S., Wellesbourne, Warwick CV35 9EF. (417)



Wellcome

**Senior Biochemist
Enzymology**

The Department of Biophysics and Biochemistry at The Wellcome Research Laboratories, part of the multi-million international Wellcome Organisation, requires a Senior Biochemist to collaborate with a small inter-disciplinary group, who are studying the ways in which drugs react with enzymes.

Candidates should have had several years practical post-doctoral experience in enzymology, and will be able to interpret their results in line with modern biochemical concepts.

The Laboratories are located in beautiful parkland surroundings at Beckenham in Kent, near attractive residential areas and yet within easy reach of London.

Salary and conditions of employment are attractive, including generous assistance with relocation expenses, where appropriate.

Please write, with brief career details, to the
**Personnel Manager,
THE WELLCOME RESEARCH LABORATORIES,
Langley Court,
Beckenham, Kent, BR3 3BS.**



(464)

GREATER GLASGOW HEALTH BOARD—WESTERN DISTRICT

Research Assistant in Renal Transplantation in the Western Infirmary

Salary Scale £1,497-£2,259 (Honours Graduates commence at £1,797).

Applications are invited from Science Graduates for the above post. The work will involve the setting up and supervision of immunological tests used in patients who have undergone kidney transplants. No special experience in this field is required but some training in immunology would be an advantage.

Further details can be obtained from Dr. J. D. Briggs, Renal Unit, Western Infirmary, Glasgow, G11 6NT.

Applications should be addressed to Dr. I. Macleod, District Medical Officer, Greater Glasgow Health Board—Western District, Western Infirmary, Glasgow, G11 6NT, not later than 15th September, 1974. (424)

**UNIVERSITY OF HOHENHEIM (LH)
GERMANY**

Institute of Plant Pathology

The post of a Wissenschaftlicher Rat and Professor (AH 2/3) for "Plant Virology and Bacteriology" is to be filled. The main field shall be virology; the problems of practical plant protection in this field are also to be considered. Experiences in the Tropics and Subtropics are desired.

Applications including a list of publications and curriculum vitae should be sent by September 30, 1974, or as soon as possible thereafter, to:

Dekan
des Fachbereichs Agrarbiologie
der Universität Hohenheim
D-7000 Stuttgart 70
Postfach 106
W. Germany

(433)

HILL FARMING RESEARCH ORGANISATION

PLANTS AND SOILS DEPARTMENT

Applications are invited for a SCIENTIFIC OFFICER to join a team studying the nutritional problems of grasses and white clover growing in hill soils with the objective of improving pasture production.

Minimum qualifications: A pass Degree or Higher National Certificate in Botany or an Agricultural Science subject.

Salary depending on qualifications and experience in scale £1,592 to £2,675 with a superannuation scheme which attracts a 5½% non-pensionable allowance to offset personal contribution.

Further particulars and application form may be obtained from the Secretary, H.F.R.O., Bush Estate, Penicuik, Midlothian. Closing date August 21, 1974. Please quote ref: A/6/192. (480)


Nicholas

Pharmaceuticals, Toiletries
Hospital Supplies

Section Head anti-inflammatory research

The pharmacology department of the research division is expanding and an experienced scientist is required to take charge of the existing anti-inflammatory section. The work entails the direction and conduct of the current biological research programme, research into fundamental aspects of inflammation and the development of new testing procedures.

Applicants should be graduates in pharmacology or a closely related discipline with a Ph.D. and several years post-graduate experience in the inflammatory field.

A good salary is offered and will depend on qualifications and experience.

Publications and attendance at scientific meetings are encouraged.

Benefits include a contributory pension scheme with free life insurance, four weeks holiday and assistance with re-location expenses.

Applications in writing to:
A. J. Strathdee,
Personnel Operations Manager,
Nicholas Laboratories Ltd.,
225 Bath Road, Slough. Berks. SL1 4AU.

(471)

AGRICULTURAL RESEARCH COUNCIL OF MALAWI AGRONOMIST

A vacancy exists in the Grain Legume Productivity Research Unit for an energetic and practical agronomist to conduct a research programme on groundnuts. The successful applicant will work in close association with the plant breeder and pathologist and undertake a study of the fertilizer requirements of groundnuts and the influence of improved varieties and disease control methods on cultural practices. He will also be required to assist with the development and evaluation of harvesting methods.

Applicants should possess a good degree in agriculture, agricultural botany or a related subject. Experience of tropical agriculture would be an advantage.

The appointment will be for a contract of three years at a salary and overseas allowance commensurate with qualifications and experience. A tax free gratuity of 25% is payable on completion of contract. Generous leave, free family passages, medical and educational allowances and free educational passages are provided together with a furnished house at low rental.

Applications including curriculum vitae and names of two referees should be addressed to:

Administrative Officer, P/Bag 3, Thondwe, Malawi.

(443)

UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS

Applications are invited for the post of Post Doctoral Senior Research Assistant from October 1, 1974. The successful candidate will be expected to pursue research in Cosmic Ray Physics involving calculations of the night sky Cerenkov radiation and measurements at the Mount Hopkins Observatory of the Smithsonian Institution, Tucson, New Mexico. The periods of work in the United States will be for up to 12 months.

The appointment, which is funded by the Science Research Council, will be for a period of two years.

The salary will be on the scale £2,055 to £2,793 with F.S.S.U. benefits.

Applications (3 copies) including the names and addresses of three referees should be sent to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained.

Closing date August 15, 1974.

(411)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF OPERATIVE DENTAL SURGERY

SENIOR RESEARCH ASSOCIATE

Applications are invited for a postdoctoral post as Senior Research Associate to work on research into the chemical characterisation of bioadhesive secretions in the common sea mussel in collaboration with Mr. M. Cook and Dr. P. W. Kent in the Universities of Newcastle upon Tyne and Durham. The research which is supported by an M.R.C. grant will be carried out at the Dental School, Newcastle upon Tyne, and the appointment will be for a period of up to three years from October 1, 1974. Commencing salary up to £3,108 per annum with F.S.S.U. Applicants should have had experience in biochemistry or relevant chemistry of natural products.

Applications giving the names of two referees should be sent to Mr. M. Cook, the Dental School, Northumberland Road, Newcastle upon Tyne, NE1 8TA within three weeks of this advertisement.

(415)

UNIVERSITY OF STERLING DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH FELLOWSHIPS

Applications are invited for Postdoctoral Research Fellowships for work on (a) The Biosynthesis of a fungal antibiotics and (b) In Vitro Analogies for the Biosynthesis of certain Terpenes.

The appointments would be for one year in the first instance, commencing October 1, 1974. Salary will be on the scale £2,118 to £2,412 with placement according to age, and is superannuable (F.S.S.U.).

Applications together with the names of two referees should be sent to The Secretary () Department of Chemistry, University of Stirling, Stirling by September 30, 1974.

(410)

LEEDS AREA HEALTH AUTHORITY (TEACHING)

Applications are invited from graduates in one of the Biological Sciences for the post of SCIENTIFIC OFFICER in the Regional Cytogenetic Unit established in the Pathology Department of this hospital. The Centre undertakes the cytogenetic investigations required for patients living within the area of the Yorkshire Regional Health Authority. Previous experience in cytogenetics is desirable but not essential.

Application forms obtainable by ringing Leeds 33144, extension 203 or 293 and should be returned to the Personnel Manager, St James's Hospital, Leeds LS9 7TF not later than 10 days after the appearance of this advertisement.

(409)

UNIVERSITY OF SOUTHAMPTON MEDICAL LABORATORY TECHNICIAN

to work for Department of Child Health in the University Medical Sciences Building. Duties involve operation and maintenance of cell culture apparatus and enzyme analysis and include development work and assistance with student research.

Qualification H.N.C. with related experience. Salary scale £1,848 to £2,163 or £2,007 to £2,382 according to qualifications and experience.

Applications in writing, giving details of age, qualifications and experience and the names and addresses of two referees, should be sent as soon as possible to the Deputy Secretary's Section, The University, Southampton SO9 5NH, quoting reference 262/T.

(430)

THE ROYAL DENTAL HOSPITAL
OF LONDON
SCHOOL OF DENTAL SURGERY
(University of London)
RESEARCH ASSISTANTS

Department of Physiology

Applications are invited for the appointment of Research Assistant in Physiology in relation to Dentistry. Applicants must be graduates in biological science preferably physiology and previous research experience is not essential. The post will be tenable for three years in the first instance and would permit registration for a higher degree as an internal student of the University of London. Salary range according to age and experience £1,400 to £1,713 (under review) plus L.A. and F.S.S.U. benefits. The successful applicant will be required to assist in work on the neurophysiological control of jaw function which involves laboratory experiments and the eventual application of data to clinical diagnostic procedures. As the School is in association with St. George's Hospital Medical School and Chelsea College opportunities for collaborative work exist with these institutions.

Department of Physical Sciences

A graduate Research Assistant is required by the Department of Physical Sciences to study ion-exchange and adsorption phenomena at the surface of hydroxyapatite, the mineral constituent of bones and teeth. Applicants are expected to have or to obtain a good degree in Physics or Chemistry. The appointment, which is for three years in the first instance, will be on a salary scale from £1,400 to £1,713 (under review) plus a London Allowance and F.S.S.U. benefits. Registration for a higher degree will be expected. Alternatively a part-time appointment would be considered.

Applications with curriculum vitae and the names of two referees should be submitted to the School Secretary, Royal Dental Hospital of London (School of Dental Surgery) 32, Leicester Square, London, W.C.2. and received not later than September 6, 1974. (418)

UNIVERSITY OF LEEDS
DEPARTMENT OF GENETICS

Applications are invited for a post-doctoral fellowship to work on amino acid replacements in mutant forms of *Neurospora* glutamate dehydrogenase. The post is available for one year (possibly renewable) starting as soon as possible, but no later than October 1, 1974; salary on the scale £1,929 to £2,388 (to be reviewed October 1, 1974).

Applications, together with the names of two referees, should be sent to Professor J. R. S. Fincham, Department of Genetics, University of Leeds, Leeds LS2 9JT, from whom particulars may be obtained. (426)

SIR GEORGE WILLIAMS
UNIVERSITY

Montreal, Quebec, Canada

DEPT. OF BIOLOGICAL SCIENCES
THREE FACULTY POSITIONS

Three faculty positions available, July 1, 1974: 1 Associate Professor (\$15,400 to \$17,000), 2 Assistant Professors (\$12,000 to \$15,000) depending upon experience and qualifications. Research orientated persons qualified to teach in one or more of the following areas will be considered: Embryology, Plant Ecology, Biostatistics, Introductory Biology. Ph.D. required. Send resume and names of 3 references to: Dr. H. Enesco, Chairman, Dept. of Biological Sciences, Sir George Williams University, Montreal, Que. H3G 1M8. (429)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF SURGERY
MEDICAL RESEARCH TECHNICIAN

required to assist with research into the biochemical aspects of liver disease. Experience with radio active isotopes an advantage but not essential. The laboratories are well equipped and will move into the new teaching hospital in the near future.

Candidates should be State Registered and salary will be on the Whitley Council Scale £1,557 to £2,451 per annum (plus threshold payments) according to qualifications and experience. Application forms may be obtained from the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref RV/N/276145. (425)

Opportunities Overseas

Afghanistan

Cotton Adviser (Production and Extension)

To increase the production of cotton in the Helmand Valley by conducting extension work among farmers, advising on cotton production, controlling demonstration plots and helping to train local personnel. Involves liaison with extension staffs of the Cotton and Vegetable Oil Corporation and the Arghandab Valley Authority.

Appointment 2 years. Candidates should possess a degree or diploma in agriculture with extensive experience of the prescribed duties of the post.

Salary in scale £3,000 to £5,000 p.a. plus a variable tax-free overseas allowance in scale £365 to £1,295 p.a.

For full details together with an application form and booklet on Afghanistan, please apply giving age and brief details of qualifications and experience to:—

Appointments Officer

Ministry of Overseas Development

Room E 301, Eland House
Stag Place, LONDON SW1E 5DH



(478)

Tropical Products Institute
London

Microbiologist

■ Direct routine microbiological studies on spoilage of fresh and processed food of tropical origin ■ Investigate new microbiological testing methods ■ Train staff, and students from overseas ■ Possibility of work overseas for short periods.

☐ Degree, HND, HNC or equivalent in appropriate subject ☐ Skill in practical techniques of bacteriology and, preferably, mycology ☐ Age under 30 ☐ Appointment as Higher Scientific Officer (around £2800-£3700) or Scientific Officer (over £1900-£3000) ☐ Ref: SA/28/JD. ☐ Application forms (for return by 16 August 1974) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

**Science
group**
CIVIL SERVICE

(474)

Medical Laboratory Technologists

3 years here could add a new dimension to your kind of work

As agents for Nchanga Consolidated Copper Mines, Zambia's largest mining group, we are looking for Medical Laboratory Technologists to work – on a 3 year renewable contract basis – at the Company's hospitals in Chingola and Kitwe, two important towns on the Copperbelt.

These hospitals serve not only the local and expatriate mining staff and their families, but also everyone for miles around. As a consequence, preventive medicine and also the public health and hygiene of whole communities have become major responsibilities. You will, in the course of your work, find yourself involved with the whole gamut of conditions and diseases peculiar to the Tropics – the kind of experience you might find hard to acquire elsewhere in one locality.

Take into account also the attractive life in a Copperbelt town, and the marvellously sunny Zambian weather and you can see how we can promise you an enjoyable time as well as valuable professional experience.

Qualifications: Applicants must possess at least FIMLT, offer practical experience in hospital laboratory routines, and be knowledgeable on modern bacteriological, bio-chemical and haematological techniques.

SALARY K 7,069 p.a. (£4,594 based on an exchange rate of K 1. = £0.65). Benefits include currency regulations advantages – up to 33½% of gross income can be sent home each month, with an additional end-of-contract allowance. Free passages to and from Zambia. A tax-free settling-in allowance of £390 if married and £195 if single. Free Life Assurance. A house or flat at a very low rent, and domestic help easily available. Generous paid annual leave. Company private primary schools available. Tax-free allowances for children's education and travel. Loan facilities to assist in car purchase. Medical aid scheme.

Write (enclosing details of professional qualifications and experience) for an application form and booklet to:

Anglo Charter International Services Limited
(Appointments Division), Department
7 Rolls Buildings, London EC4A 1HX.



Zambia's Copperbelt

an experience that counts for a lifetime

(488)

UNIVERSITY OF OTAGO

Dunedin, New Zealand

LECTURESHIPS IN ANATOMY

(Visiting or Established Posts)

Applications are invited from suitably qualified persons who can carry out research and teaching in neuroanatomy, or in embryology or in histology including cytology and who, in addition, either already have or are prepared to acquire experience in teaching gross anatomy. Demonstrated research capacity in some aspect of embryology or developmental biology would be an advantage.

While the vacancies are for established posts normally leading to tenure, the University is prepared to enter into discussions for short-term appointments for two years (although one year would be considered) with return air fares provided. The latter might interest recently retired anatomists.

Salary according to qualifications and experience within the relevant scale as under:

Science Graduate

Lecturer: NZ\$7,361 to \$9,339 per annum
Senior Lecturer: NZ\$9,503 to \$12,142 per annum with a bar at \$11,153 per annum.

Dental Graduate

Lecturer NZ\$8,303 to \$11,517 per annum
Senior Lecturer: NZ\$11,918 to \$13,121 per annum

Medical Graduate

Lecturer: NZ\$9,997 to \$14,287 per annum with a bar at \$11,811 per annum
Senior Lecturer: NZ\$14,699 to \$16,926 per annum

Further particulars should be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications, quoting ref. no. A.74/24, close in New Zealand and London on September 30, 1974. (431)

UNIVERSITY COLLEGE DUBLIN

Research Assistantship (full time)

or

Research Demonstratorship (part time)

Applications are invited for the above—which operate under a National Science Council Grant—in the Department of Geology. The appointments are for one year but may be renewed subsequently. A requirement is a good Honours Degree in Geology and preference will be given to candidates with practical experience in field or laboratory.

Salary:—

Research Assistant = £1,782 p.a.
Research Demonstrators = £891 p.a.

The latter post will permit demonstrating for which extra remuneration is made. Closing date for applications August 26, 1974.

Applications to

The Secretary,
Department of Geology,
University College,
Belfield,
Stillorgan Road,
Dublin, 4.

(423)

UNIVERSITY COLLEGE DUBLIN

RESEARCH POSTS IN INVERTEBRATE ECOLOGY

A post doctoral fellow or research assistant or postgraduate student is required for a 3-year project sponsored by the National Science Council on the organisation and dynamics of grassland invertebrate communities. Applicants should hold an initial honours degree in Agriculture or Zoology and preferably have research experience in quantitative ecology. Applicants graduating this Autumn will be considered.

Full details from:—

Dr. J. P. Curry,
Department of Agricultural Biology,
Faculty of Agriculture,
University College,
Glasnevin,
Dublin, 9.

(422)

QUEEN ELIZABETH HOSPITAL FOR CHILDREN

HACKNEY ROAD, LONDON E2 8PS

Applications are invited for the post of RESEARCH ASSISTANT in the Academic Department of Child Health at this hospital. The post is grant-supported and the successful applicant, who should have a good honours degree in biological sciences, will be expected to undertake a study of reaginic antibody in childhood asthma. Salary will be on the scale of £1,440 to £1,883. Applications with the names of two referees should be addressed to Pro. C. B. S. Wood, Academic Department of Child Health. (421)

AGRICULTURAL RESEARCH COUNCIL

UNIT OF DEVELOPMENTAL BOTANY

Application are invited from PLANT PHYSIOLOGISTS and BIOCHEMISTS for a two year temporary appointment as a SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER at the Agricultural Research Council's Unit of Developmental Botany to investigate an aspect of the hormonal control of plant growth development. Candidates should hold post graduate qualifications and have some further experience in original research.

Appointment in grade of Scientific Officer £1,707 to £2,329 p.a. or Higher Scientific Officer £2,221 to £2,854 p.a. Starting salary in accordance with qualifications and experience. At least two years post graduate experience is required for appointment to H.S.O. Superannuation under F.S.S.U. with an allowance of 4½% of basic salary to off-set contributions.

The unit is attached to the Botany School of Cambridge University and is under the Direction of Professor P. W. Brian. Applications should be addressed to the Deputy Director, Dr. D. J. Osborne, Unit of Developmental Botany, 181A Huntingdon Road, Cambridge CB3 0DY. (435)

CHELSEA COLLEGE
University of London

TECHNICIAN GRADE 4

A TECHNICIAN (GRADE 4) is required for the Microbiology Section of the Department of Pharmacy, to undertake work involving the maintenance of the culture collection, preparation of cultures for undergraduate and post-graduate students and some field work for new experiments. Relevant experience and a List B qualification are essential. Salary in the range £2,023 to £2,338 per annum (including £228 London Allowance).

Application forms from the Manager of Technical Services, Department of Pharmacy, Chelsea College, Manresa Road, London SW3 6LX. (419)

UNIVERSITY COLLEGE, CARDIFF
DEPARTMENT OF BOTANY
RESEARCH ASSISTANT IN
PLANT BIOCHEMISTRY

Applications are invited from graduates in Biology, Biochemistry or Botany for the post of Research Assistant, to work on D.N.A. polymerase in higher plants, under the supervision of Dr. J. A. Bryant. The post is for a period of three years from October 1, 1974, with a starting salary of £1,422 (except in the case of an applicant who is allowed to register for a higher degree, for whom the salary will be adjusted to be equivalent to a post-graduate studentship). Further details are available from: The Registrar, University College P.O. Box 78, Cardiff CF1 1XL, to whom applications (including the names of two referees) should be sent by August 19, 1974. Please quote reference 0615. (436)

UNIVERSITY COLLEGE CARDIFF
Applications are invited for the following vacancy:
TECHNICIAN GRADE 3

in the Department of Physiology. Salary Range: £1,650 to £1,920 per annum. Duties to commence September 2, 1974. The post is for 15 months.

Applications, together with the names and addresses of two referees, should be forwarded to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing date August 9, 1974. Please quote reference 0616. (438)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PHYSICS

The Physics and Chemistry of Solids Research Group (Professor D. Tabor) of the Cavendish Laboratory hopes shortly to make appointments against posts concerned with the following projects starting in October:—

- The friction and wear properties of polymers and the influence of specific additives and fillers.
- Micromechanical properties in ultra-high vacuum of well characterised surfaces.
- The effect of minor constituents on the surface properties of solids.

Applications are invited from graduate and post-doctoral scientists with appropriate experience. Salaries on a scale between £2,118 and £2,931 p.a. depending upon age and experience.

Applications, incorporating a curriculum vitae and the names of two referees and noting the project(s) of interest, should be sent to The Secretary, Cavendish Laboratory, Madingley Road, Cambridge. CB3 0HE. (439)

UNIVERSITY OF ST ANDREWS
RESEARCH ASSISTANT IN
LASER PHYSICS

Immediate vacancy for a Research Assistant to work in the laser group on a CO₂ TEA laser contract. Previous experience in laser physics and high vacuum technology an advantage. Salary in the range £1,600 to £1,800 p.a., tenable for one year, possibly renewable.

Applications including a curriculum vitae and the names of two referees should be sent as soon as possible to Dr. A. L. S. Smith, Physics Department, University of St. Andrews, Fife from whom further details can be obtained. (441)

UNIVERSITY OF LONDON
CHAIR OF STRUCTURAL BIOLOGY AT ST.
GEORGE'S HOSPITAL MEDICAL SCHOOL

The Senate invite applications for this newly established Chair. Initial salary to be agreed but not less than £5,973 a year, plus £162 London Allowance. Applications (11 copies) should be received not later than September 10, 1974 by the Academic Registrar, (N) University of London, Senate House, WC1E 7HU, from whom further particulars may be obtained. (444)

LABORATORY ASSISTANTS OR TECHNICIANS
(CHEMISTS)

Two vacancies exist for young people in our Special Polymers laboratory and Sterile Solutions division to assist in production, research and development, covering a wide range of activities.

Candidates should ideally be qualified to HNC or equivalent in Chemistry or related scientific subject or alternatively be qualified by experience.

A realistic and progressive salary will be paid commensurate with age and qualifications. The usual Company fringe benefits apply.

Write in the first instance to:

Mr K. Gent,
Personnel Manager,
Contact Lenses (Mfg) Limited,
14/16 Child's Place,
Earls Court, London SW5 9RX
Tel: 01-370 4455.

(479)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF MATHEMATICS

RESEARCH ASSISTANTSHIP

Applications are invited for a Research Assistantship in the Department of Mathematics to take part in a theoretical investigation of rheological properties of pigment suspensions. Applicants should be well qualified in applied mathematics, or a related discipline, and have a good knowledge of contemporary continuum mechanics.

The post is for one year with salary up to £2,757 per annum, placing depending on age, qualifications and experience.

Further information (quoting R27/74) may be obtained from Dr F. M. Leslie, Department of Mathematics, University of Strathclyde, Livingstone Tower, 26 Richmond Street, Glasgow G1 1HX, with whom applications should be lodged by August 14, 1974.

(487)

UNIVERSITY OF STRATHCLYDE
DEPARTMENT OF APPLIED MICROBIOLOGY

Research Assistant

Applicants are invited for Research Assistantship to carry out an investigation on RNA dependent RNA polymerases in different strains of yeast. The work will involve the use of techniques in cell biology, enzymology and virology. Applicants should have a good Honours degree in Biochemistry or a related subject.

The appointment, starting on October 1, 1974, is supported by the Science Research Council until September 30, 1977, and the salary is £1,500 by £100 (2) to £1,700 per annum.

Applications (quoting R26/74) giving details of qualifications and experience, together with the names of two referees should be sent to Dr E. A. Berry, Department of Applied Microbiology, University of Strathclyde, Royal College Building, 204 George Street, Glasgow, G1 1XW.

(472)

TAX-FREE HOSPITAL OPPORTUNITY in SAUDI ARABIA

with **Whittaker**

The Whittaker Corp. represents one of the largest companies in the USA, which has diversified internationally into health care and environmental projects. We have been awarded a \$100 million contract to staff and manage three newly-built hospitals in Jiddah (60 beds), Tabuk (135 beds) and Khamis Mushayt (135 beds). The hospitals have been built to top US standards. Equipment is the latest and best from the USA and Europe, covering the full range of medical and surgical treatment.

There is an immediate opportunity on a two-year contract basis for:

Epidemiologist \$18,000

to study the incidence of communicable and chronic diseases and develop programmes and recommend courses of action for their control.

Candidates should be qualified in Public Health or Epidemiology with at least 2 years' experience in this field.

In addition to a tax-free salary, negotiable up to the maximum indicated, benefits include: free housing and messing; return passages for employees and families; generous baggage allowance and household storage provision; and 30 days' annual holiday.

ACTION—To meet our initial staffing requirements, this post must be filled quickly. Please URGENT write immediately with full biographical details, quoting reference U.123, to F. Sicher, Whittaker Corp., c/o MSL ADVERTISING SERVICES LTD. 17 Stratton Street, London W1X 6DB.

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL DEPARTMENT OF BIOCHEMISTRY M.R.C. Research Studentship

Applications are invited for an M.R.C. studentship which is available from October, 1974. Candidates should hold a good Honours degree in chemistry or a biological science. The successful candidate will pursue a course of research, leading to a Ph.D. degree, involving a study of changes which occur in the protoplast membrane of *Bacillus amyloliquefaciens* during the secretion of extracellular enzymes with particular regard to the metabolism of membrane phospholipids.

Applications and enquiries should be made to Dr. G. Coleman or Dr. D. White, Department of Biochemistry, University of Nottingham Medical School, University Walk, Nottingham NG7 2RD. Telephone 0602-56101. (446)

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH BIOCHEMIST/MICROBIOLOGIST

A postdoctoral biochemist or microbiologist is required by the LABORATORY FOR LEPROSY AND MYCOBACTERIAL RESEARCH to investigate killing and degradation of mycobacteria by phagocytic cells. The work of the Laboratory includes several aspects of the relationship between pathogenic mycobacteria and their host cells. The appointment, which is for up to three years, is in the salary range £2,223 to £3,378 p.a. plus £162 p.a. London Allowance; superannuation provision under F.S.S.U.

Applications giving detail of qualifications, research experience and the names of two professional referees should be sent to The Director, National Institute for Medical Research, Mill Hill, London, NW7 1AA. (456)

UNIVERSITY OF SASKATCHEWAN

SASKATOON, SASKATCHEWAN,
Canada

Applications are invited for the following positions in the Department of Crop Science
PROFESSIONAL RESEARCH ASSOCIATE

A plant physiologist with experience in the field of identification and physiology of naturally-occurring plant growth regulators.
PROFESSIONAL RESEARCH ASSOCIATE

A plant physiologist with training and experience in the area of water relations and/or stress physiology.

The successful applicants will be part of a team of three scientists and three technicians who will undertake a comprehensive analysis of the role of plant hormones in drought stress in sorghum. The study, supported by the International Development Research Centre, Ottawa is designed to provide cereal scientists working in the semi-arid tropics with a more complete knowledge of the manner in which plant growth and productivity are influenced by drought stress. The project will take approximately five years.

The commencing salary for each position is \$12,924 plus a pension and annual increments currently equivalent to 5%. Minimum qualification is a Ph.D.

Further enquiries and applications, which close August 31, 1974, and letters from three referees should be sent to:
Prof. G. M. Simpson,
Crop Science Department,
University of Saskatchewan,
Saskatoon, Saskatchewan, Canada S7N 0W0
(440)

UNIVERSITY OF SOUTHAMPTON Senior Programmer

Applications are invited from graduates in Mathematics, or with a strong mathematical background, for the post of Senior Programmer in the Biology Department. The successful applicant will be expected to act as Computer Liaison Officer for the Department as well as developing and servicing computer programs. An interest in the application of computer-based techniques to biological problems is essential, and some knowledge of multivariate statistical methods would be an advantage.

Salary on scale: £2,580 to £3,636 per annum. Further particulars may be obtained from the Deputy Secretary's Section (Ext. 731), The University, Southampton SO9 5NH, to whom applications, with the names of three referees, should be sent by August 12, 1974. Please quote reference Na 902/0. (445)

THE QUEEN'S UNIVERSITY OF BELFAST

Research Officer/ Senior Research Officer

DEPARTMENT OF MICROBIOLOGY

Applications are invited for the post of Research Officer/Senior Research Officer in the Department of Microbiology. The duties of the post include assistance with investigations into persistent infection and with the preparation of teaching materials for classes in microbiology. Candidates for the senior post should have an ordinary science degree or equivalent, and the particular requirements for both grades of appointment are a knowledge of virology, tissue culture and experience with immunofluorescent and/or radio-active tracing of biological materials. In addition, candidates for the senior post should have experience in cellular immunology and the culture of cells from the immune system of animals and man.

The grade of appointment will depend on age and qualifications. The salary range for the Research Officer grade is £1,932 to £2,910 for the Senior Research Officer grade £2,952 to £3,990 both posts carry superannuation within the F.S.S.U.

Letters of application, giving the names of two referees, should reach the Personnel Officer, The Queen's University, Belfast, BT7 1NN, Northern Ireland by August 16, 1974. (437)

(489)

Royal Postgraduate Medical School TECHNICIAN, (Organic Chemist)

required in the peptide chemistry section of the Endocrine Unit, to assist with the synthesis of biologically active peptides. Qualifications: relevant 'A' levels, O.N.C. or H.N.C. in chemistry. Starting salary and grade according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammer-smith Hospital, Du Cane Road, London W12 0HS, quoting ref. no. 2/322.N. (449)

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG, SOUTH AFRICA DEPARTMENT OF PHYSICS

Applications are invited for the following posts:

- (A) Senior Lecturer
- (B) Lecturer
- (C) Junior Lecturer

- (D) Senior Lecturer with special responsibilities in the Faculty of Education

The persons appointed to posts (A), (B) and (C) above will take part in the general teaching and research work of the Department of Physics. Solid State Physics and Nuclear Physics are the two main research interests.

The principal duty attached to post (D) will be to organise in-service training for teachers of physical science in high schools.

Salary will be determined according to qualifications and experience within the following ranges:

Senior Lecturer: R7,245 to R9,315

Lecturer: R5,520 to R7,935

Junior Lecturer: R4,140 to R5,175

Improved salary scales are to be introduced shortly.

Benefits include an annual bonus, pension and medical aid, and a housing subsidy (if eligible).

Intending applicants should obtain the information sheet relating to these posts from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than September 20, 1974. (Please quote the post for which you are applying). U.K. applicants should obtain the information sheet from the London Representative, University of the Witwatersrand, Chichester House, 278 High Holborn, London WC1, to whom a copy of the application should be sent. (450)

EAST MALLING RESEARCH STATION

Head of Plant Protective Chemistry Section

A chemist with experience in pesticides required to lead the section concerned with pesticide usage on fruit and hops.

Appointment, according to age and experience, in the Principal Scientific Officer scale £4,227 by 7 increments to £5,550 together with the cost of living allowance and 4½% of gross salary to offset superannuation contributions. Superannuation under F.S.S.U.

Application form and further details from Assistant to the Secretary, East Malling Research Station, East Malling, Maidstone, Kent, ME19 6BJ. (451)

THE EDINBURGH SCHOOL OF AGRICULTURE AGRICULTURAL ENTOMOLOGIST

Applications are invited for a post in the Crop Health Department from candidates with post-graduate experience in Entomology/Nematology.

Salary scale (under review) Grade III £2,233 to £3,895 plus a cost-of-living safeguard and a non-pensionable supplement of 4½% to offset superannuation contributions.

Further particulars and application form from The Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (468)

NRDC

National Research Development Corporation Fellowship

Chemist/Biochemist

Applications are invited for a two-year NRDC Fellowship to work at the Microbiological Research Establishment, Porton Down (under the direction of Dr K. D. Macdonald), on the isolation, purification and characterisation of microbial metabolites with antibiotic activity.

Applicants should have two to four years' post-graduate experience, preferably with a knowledge of the chemistry of antibiotics both at a comparative and analytical level. Salary starting at £2,568 per annum rising to £2,675.

The appointment of a recent graduate with an interest in this field is not excluded and the salary would be in the range of £1,592 to £2,038.

'Threshold' payments will be made and annual leave will be 22 days.

Applications, with details of qualifications and naming two referees, to: The Assistant Director, Microbiological Research Establishment, Porton Down, Salisbury, Wiltshire SP4 0JG. (490)

NEW ZEALAND UNIVERSITY OF CANTERBURY CHRISTCHURCH

SENIOR LECTURERS or LECTURERS IN CIVIL ENGINEERING

Applications are invited for vacancies in the following fields: Highway Engineering; Public Health Engineering; Surveying and Town Planning.

The salary for Lecturers is on a scale from NZ\$7,361 to \$9,339 per annum and for Senior Lecturers NZ\$9,503 to \$11,153 (bar) \$11,484 to \$12,142 per annum.

Particulars, including information on travel and removal allowances, study leave, housing and superannuation may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **October 31, 1974.** (461)

West Berkshire Health District MEDICAL PHYSICS TECHNICIAN

required for modern Isotope Laboratory at the Royal Berkshire Hospital, Reading. The post will be on Grade IV (£1,590 to £1,953) or Grade V (£1,308 to £1,677), and involves interesting work on the chemistry and physics of medical isotope techniques.

Candidates should normally have appropriate O.N.C. or 'A' levels. Day release for further training may be available.

Written applications to:

The Hospital Secretary,
Royal Berkshire Hospital,
London Road,
Reading,
Berks.

(455)

FRAME

which is concerned with disseminating information through its twice yearly Journal ATLA ABSTRACTS (Alternatives To Laboratory Animals) seeks a part-time scientific literature searcher and abstractor in the London area. For further details write to: The Secretary, FRAME (Fund for the Replacement of Animals in Medical Experiments) 312a Worple Road, London SW20 8QU. (459)

UNIVERSITY OF NEWCASTLE UPON TYNE

RESEARCH ASSOCIATE (BIOCHEMIST)

A Post-Doctoral Biochemist is required to participate in a three year project investigating the metabolism of foreign compounds by human skin, commencing in Autumn 1974 and being conducted jointly in the Departments of Dermatology and Pharmacology. Commencing salary up to £2,247 per annum with F.S.S.U.

Applications giving the names of two referees should be sent to Professor M. D. Rawlins, Department of Pharmacology, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, from whom further information may be obtained. (447)

University of Edinburgh RESEARCH ASSISTANTS

Required by the DEPARTMENT OF MOLECULAR BIOLOGY to work on genetic and biochemical aspects of prokaryotic molecular biology. The posts are grant supported for limited terms varying from two to seven years, and should appeal to holders of H.N.C., B.Sc., (Hons.) or a Ph.D. Salaries will be on scale £1,848 to £2,163 per annum. Threshold payment. Applications with names of two referees should be sent to THE PERSONNEL OFFICER, UNIVERSITY OF EDINBURGH, 63 SOUTH BRIDGE, EDINBURGH, EH1 1LS. TELEPHONE NUMBER 031 667 1011 Ext. 4446. Please quote Reference number A038. (454)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF PHYSICS RESEARCH ASSOCIATE

Applications are invited for a Research Associateship for up to three years under the terms of an S.R.C. grant in the Low Temperatures Group for the experimental study of electronic properties of quasi two-dimensional metallic compounds. Candidates should have a degree in physics and good experimental ability.

Salary:— £1,758 to £1,974 + F.S.S.U.

Applications (3 copies) naming 3 referees should be sent to the Assistant Registrar (S), P.O. Box 363, Birmingham B15 2TT, by August 26 1974. Please quote ref:— NP3. (476)

SURREY area health authority

West Surrey/North East Hampshire
Health District

DISTRICT PHARMACEUTICAL OFFICER

(PRINCIPAL PHARMACIST)

£3,522 to £4,185

To be responsible to the Area Pharmacist for the Hospital Pharmacy Service in the West Surrey/North East Hampshire Health District. The District Pharmaceutical Officer will be based at Frimley Park District General Hospital. The new department of health "best buy" hospital of 583 beds which is opening to out-patients on August 1, 1974.

Frimley Park Hospital is within short distance of London by train or road. Handy for reaching the South Coast Resorts and near the first class shopping facilities of Camberley.

Candidates with previous hospital experience who may now be working in other fields will be considered.

Application forms and job description from the District Administrator, West Surrey/North East Hampshire Health District, 3rd Floor, Abbey House, 282-292 Farnborough Road, Farnborough, Hants GU14 7NE.

Closing date August 9, 1974.

(492)

UNIVERSITY OF BRISTOL GRADUATE RESEARCH ASSISTANT in the DEPARTMENT OF BACTERIOLOGY

To start work in October on the role played by microorganisms in the production of pheromones. This three year investigation is part of a multidisciplinary environmental study of the Red Fox.

Initial salary up to £1,686 per annum.

Applications, preferably from microbiologists having some knowledge of Organic Chemistry (glc) should be sent to Dr G. C. Ware, Department of Bacteriology, Medical School, University Walk, Bristol BS8 1TD. (453)

MASSEY UNIVERSITY Palmerston North, New Zealand DEPARTMENT OF SOIL SCIENCE POST GRADUATE RESEARCH ASSISTANTSHIPS

Applications are invited from suitably qualified students for Post-graduate Research Assistantships in the Department of Soil Science. Applicants should have completed an Honours, or Master's Degree in Soil Science, Chemistry, or Microbiology and be interested in enrolling for a Ph.D. degree in Soil Science.

The areas of research relate to the transformation of nitrogen in soils, movement of nitrogen and phosphorus in soils as influenced by drainage and irrigation, and the biological availability of phosphorus in streams.

The appointments are for two years initially, commencing October 1, 1974 but extension to a third year is possible. No teaching duties are required.

Award: NZ\$2,400 per annum. (NZ\$1=\$A1=62p).

Further details of the positions and of the University may be obtained from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on **August 23, 1974.** (463)

UNIVERSITY OF LEUVEN PHYSIOLOGICAL LABORATORY RESEARCH ASSISTANT

Applications are invited for three pre- or postdoctoral research assistantships. The persons appointed will be required to do research in electrophysiology and excitation-contraction-coupling of the heart (Prof. E. Carmeliet) or in renal and membrane physiology (Prof. R. Borghgraef) and to do some teaching to medical students. The appointments will be for two years. Salary between BF.390.000 and 460.000 per annum.

Further information may be obtained from Laboratorium voor Fysiologie, Dekenstraat, 6, B-3000 Leuven, Belgium, to which applications should be sent together with a curriculum vitae and the names of two referees. (482)

UNIVERSITY OF EDINBURGH DEPARTMENT OF CLINICAL SURGERY RESEARCH ASSOCIATE

Applications are invited for a post of Research Associate in the above department to take part in a project investigating the relationship between biochemical parameters and mammary cancer.

Applicants should have a degree in either biochemistry or biology. Experience in endocrine or biochemical techniques and work with experimental animals would be advantageous, but not essential.

Salary on scale rising to £2,757 with placement according to qualifications and experience.

Applications in writing, giving full details of qualifications and experience, and giving the names and addresses of two referees, should be sent to Professor A. P. M. Forrest, Department of Clinical Surgery, The Royal Infirmary, Edinburgh, 3. Please quote reference number 5038. (469)

THE INSTITUTE OF DEVELOPMENT STUDIES

at
University of Sussex

BRIGHTON BN1 9RE

Two Research Officers

Needed immediately to work with Michael Lipton on three year grain storage project in Rual India (Andra Pradesh) pilot project already completed.

(A) Crop Storage Specialist

(B) Agricultural Economist

Salary in range £1,761 to £3,378 (under review) with threshold agreements and overseas allowances.

Applications with C.V. and addresses of referees to Administrator Research within one week of publication date. (475)

UNIVERSITY OF OXFORD

DEPARTMENT OF ZOOLOGY

There is a vacancy for a research assistant to work on some aspects of insect neurophysiology with Dr. P. L. Miller for one year from October 1974. Applicants should have a good Honours Degree in Zoology. Salary in accordance with age and experience. Applications, with names of two referees, to Dr. P. L. Miller, Dept of Zoology, South Parks Road, Oxford. (481)

UNIVERSITY OF EDINBURGH DEPARTMENT OF SURGERY RESEARCH ASSISTANT

Applications are invited from biological science graduates for the above post to work on the association of alpha₂-macroglobulin with lymphocytes and its possible immunological significance.

The appointment is for three years and the commencing salary is up to £1,659 per annum, according to qualifications and experience.

Applications, together with the names of two referees should be submitted to Dr Keith James, Department of Surgery, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG. Please quote reference number 5036. (470)

GEOPHYSICIST

The Lamont-Doherty Geological Observatory of Columbia University announces a position with the seismology group as a Research Associate or Senior Research Associate. Candidates must have a strong background in seismic instrumentation and data analysis and will be expected to lead existing and initiate new programs of research involving observations of earth strain, tilt, and long-period seismic waves. Qualified candidates are invited to submit a resume of their qualifications, publications, names and addresses of three references by August 15, 1974. (460)

UNIVERSITY OF DUNDEE
DEPARTMENT OF PHARMACOLOGY
AND THERAPEUTICS
RESEARCH ASSISTANTSHIP

Applications are invited from University graduates or holders of equivalent qualifications for a Research Assistantship in the Department of Pharmacology and Therapeutics, located in the new Ninewells Hospital and Medical School complex, Dundee. The holder of this post, which is available immediately, will be required to provide a Gas-Liquid Chromatographic Service within the Department and to collaborate as required with other staff in research and teaching and will be afforded facilities to carry out his own research work on a part-time basis for a higher degree.

Applicants should ideally have had experience of gas-liquid chromatography but training in this will be available.

The salary attached to the post will be within the range £1,311 to £2,052 depending on qualifications and experience.

Applicants should apply, giving their qualifications, the names of two referees and quoting reference Est/39/74 J by August 9, 1974 to The Secretary, The University, Dundee DD1 4HN, from whom further particulars may be obtained. (477)

NATIONAL HEART HOSPITAL
WESTMORELAND ST, LONDON W1

**QUALIFIED TECHNICIAN
OR TRAINEE**

required to train in operation of Heart/lung machine and monitoring equipment in operating theatre and intensive care unit. Preference given to graduates studying for higher degrees or students attending evening classes. Day release facilities, however, may be given. Enquiries and applications to Assistant House Governor, telephone 01-486 0824 Ext 123. (491)

UNIVERSITY OF LONDON
KING'S COLLEGE

DEPARTMENT OF BIOPHYSICS

Applications are invited for the post of

RESEARCH ASSISTANT

to join a group working on the fractionation and crystallisation of transfer-RNA. The post is limited to two years, and would suit a recent graduate. Depending on qualifications and experience, the starting salary (including London Allowance) would be £1,746 or £1,920 till October 1, 1974, when new national scales will be effective.

Applicants should write (giving the names of two referees), to Dr N. Spencer, Department of Biophysics, (N) King's College, 26-29 Drury Lane, London WC2B 5RL. (483)

TISSUE CULTURE
Scientific Officer

required for work in Edinburgh on Animal Diseases. The appointee will be responsible for a wide range of experiments mainly involving nervous and lymphoid tissues as primary cultures and existing cell lines. Tissue culture experience is essential. Qualifications: a degree, H.N.C. or equivalent. Salary: £1,592 to £2,675, plus 5½% Superannuation allowance, according to qualifications and experience. Superannuation. Apply with names of two referees to: The Secretary, ARC Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ. (486)

**FELLOWSHIPS AND
STUDENTSHIPS**

INSTITUTE OF UROLOGY
(UNIVERSITY OF LONDON)

in association with St. Peter's Hospitals

Applications are invited for a Research Fellow (with medical and/or scientific qualifications), in the grade of Lecturer (Senior Registrar status), for an investigation into the ultrastructure of the kidney and urinary tract epithelium. The successful applicant will be attached to the Electronmicroscopy Unit in the Department of Pathology, St. Paul's Hospital. Appointment for one year in the first instance, subject to renewal for a further period of two years. Applications to the Pathologist (L), St. Paul's Hospital, Endell Street, London, W.C.2. (391)

COLAISTE NA hOLLSCOILE GAILLIMH

(University College Galway, Ireland)

Department of Physics

RESEARCH FELLOWSHIP—A graduate in Science or Engineering is invited to join a project on the utilisation of environmental energy especially wind energy.

The salary is £2,100 per annum and the post is tenable for at least two years.

Written applications with curriculum vitae and the names of two referees should be sent to—Dr T. C. O'Connor, Department of Physics, University College, Galway, from whom further particulars may be had. (399)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF BIOLOGY

Postgraduate Studentship

Applications are invited for a three year research studentship sponsored by May and Baker Limited to study aspects of the mode of action of foliage-applied herbicides, using radio tracer, chromatography and other techniques. The value of the award will be in line with normal postgraduate rates.

The successful candidate should possess a 1st or upper 2nd class honours degree in Biology, Biochemistry or Botany, and will be required to register for a higher degree of the University.

Applications, including a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Professor W. W. Fletcher or Dr R. C. Kirkwood, Department of Biology, University of Strathclyde, Royal College, 204 George Street, Glasgow, G1 1XL. (473)

INSTITUTE OF UROLOGY
(UNIVERSITY OF LONDON)

in association with St. Peter's Hospitals

Applications are invited for a Research Fellow (with medical and/or scientific qualifications), in the grade of Lecturer (Senior Registrar status), for an investigation into the ultrastructure of the kidney and urinary tract epithelium. The successful applicant will be attached to the Electronmicroscopy Unit in the Department of Pathology, St. Paul's Hospital. Appointment for one year in the first instance, subject to renewal for a further period of two years. Applications to the Pathologist (N), St. Paul's Hospital, Endell Street, London, W.C.2. (390)

UNIVERSITY OF NOTTINGHAM
ALLIED BREWERIES RESEARCH
GRANT STUDENTSHIP 1974

Applications are invited for this studentship, tenable from September 1, to work under the supervision of Dr L. G. Briarty and Dr M. C. Pearson in the Department of Botany, School of Biological Sciences. The research project is concerned with the effects of atmospheric pollution on selected tree species in Sherwood Forest. The successful candidate will be expected to register for a higher degree. The value of the studentship is equivalent to the normal Research Council Studentships.

Applications and enquiries for further details should be addressed to the Department of Botany, University of Nottingham, University Park, Nottingham, NG7 2RD. Closing date for applications Wednesday, August 7, 1974. (400)

Department of Zoology,

UNIVERSITY OF READING

ICI Plant Protection Research Station
Jealotts Hill

Applications are invited from graduate chemists/biochemists for a Science Research Council C.A.S.E. studentship to investigate the mode of action of chemicals which interfere with chitin synthesis in insects. Candidates should write, giving the names of two referees, to Professor G. Williams, Department of Zoology, University of Reading, Whiteknights, Reading. (408)

UNIVERSITY OF SOUTHAMPTON
RESEARCH FELLOW
IN GAMMA RAY ASTRONOMY

Application is invited for the post of Research Fellow to work on the development of a balloon borne instrument to study direction of arrival and the temporal spectral characteristics of gamma ray bursts. Applicants should be familiar with particle counting techniques and associated electronic circuitry.

The post is initially for two years. Candidates should possess a Ph.D degree or equivalent qualification. Salary in range £1,929 to £2,388 (under review) plus F.S.S.U.

Applications including curriculum vitae and the names and addresses of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH, quoting reference number: Na261/R. (412)

UNIVERSITY OF KENT AT CANTERBURY

Applications are invited for TWO POST-DOCTORAL RESEARCH FELLOWSHIPS in S.R.C. sponsored work on transport processes and point defect structure of fluorite lattices in collaboration with Dr. A. V. Chadwick (Chemical Laboratory). One appointment will be mainly involved with ionic conductivity measurements and the other with pulsed n.m.r. techniques. Previous experience in one of these fields, though not essential is desirable. Starting salary will be £2,118 p.a. Applications with curriculum vitae and the names of two referees should be sent to the Assistant Registrar, Chemical Laboratory The University, Canterbury, Kent CT2 7NH quoting ref. A64/74. Closing date August 23, 1974. (416)

MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH FELLOW

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Applications should be submitted in duplicate to the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia, and should give age, qualifications and experience, and the names and addresses of two referees. Applicants should quote reference No. 41063.

Closing Date August 13, 1974.

The University reserves the right to make no appointment or to appoint by invitation. (432)

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Applications, with curriculum vitae and two referees, should be sent to Dr M. G. Rumsby, Department of Biology, University of York, Heslington, York, YO1 5DD, from whom further information is available. Please quote reference number 11/6025. (413)

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Qualifications in Chemistry, Physics or an Engineering subject would be appropriate. Further details are available from Professor J. N. Bradley, Department of Chemistry, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ. Tel: 0261 44144 Ext. 2030. (457)

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Enquiries and applications to Professor J. B. Lloyd, Biochemistry Research Unit, Keele University, Staffordshire, ST5 5BG, to whom applications should be sent by August 31, 1974. (465)

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about a recent eruption of Mount
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100 years ago

THE additions to the Zoological Society's Gardens during the past week include a Laughing Kingfisher (*Dacelo gigantea*) from Australia, presented by Mr. J. S. White; two Black-handed Spider Monkeys (*Ateles melanochir*) from Central America, presented by Mr. S. W. Rix; a Greater Sulphur-crested Cockatoo (*Cacatua galerita*) from Australia, presented by Miss S. Hooper; a Tamandua Ant-eater (*Tamandua tetradactyla*) from South America, deposited; and three Blotched Genets (*Genetta tigrina*), born in the Gardens.



Volume 250

August 2, 1974

Medical policymaking in Britain

If there is any subject that is guaranteed to generate boredom amongst scientists it is science policy. The average practitioner believes, with some justification, that whereas it would be most undesirable for a country to eschew a defence policy and leave all decisions, from procurement to strategy, to the soldier in the field, the scientist should be immune from policy constraints.

This system, in which the majority of scientists have received the majority of what they asked for and have in general been answerable only to their own consciences and to a relatively genial sponsor, is beginning to crumble as the growth rates for expenditure on research drop dramatically and as the demand for accountability rises sharply. Within the next year or two many scientists will indeed discover that there is such a subject as science policy, and arid and irrelevant as it may have seemed in the past, it is science policy that will decide whether their project will continue to be funded. The recent publication of the Medical Research Council's Annual Report (HMSO, 97p) is an appropriate time to look at the policymaking machinery in the biomedical field, and the report is generous in its attention to policy. The council is still able to say that "the ideas and interests of the individual scientists at the bench are the driving force behind science", although it adds in the next sentence "there may also need to be active discouragement of over-populated but low priority areas"—a fairly even-handed way of mixing good and bad news.

And yet there is something strangely and almost intangibly missing from the report. It is most easily seen in the section describing progress in research on selected topics. One gets the unmistakable impression that the Medical Research Council is the last bastion the world has against disease and death. The report is of course addressed primarily to the Secretary of State for Education and Science (who is told in the second paragraph of the transmittal that for £1.00 he can buy the council's Handbook from headquarters), and as such necessarily must remark on the successes that government money has bought. Nevertheless there remains just the vaguest feeling throughout the policy section that this is policy for medical research determined by the Medical Research Council without mention of the fact that a quite substantial amount of research is done by other research councils, by charities and trust funds, and by the pharmaceutical industry. Of course, excellent liaison may exist, although it doesn't shine through. And it is obviously desirable to allow a multiplicity of funding sources to exist so that power does not reside solely in one council, however appointed. But if it is indeed the function of the MRC to promote the balanced development of medical and related biological research and if all research programmes are going to fall on harder times in the next year or two it would be good to know that policymaking reflects the 'mixed economy' nature of Britain's support of medical science.

Dear Mr Turner . . .

By August 1 you had hoped to have appointed several research fellows to pursue fundamental biological research. Instead, as everyone now knows, you came into conflict during the selection procedure with potential recipients of the awards who objected to your refusal to consider those who had religious beliefs of one kind or another. No doubt you feel sore at all this and believe that there are fundamental inconsistencies between religion and science which cannot be glossed over by research scientists.

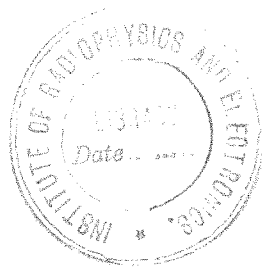
You are by no means alone in your doubts that the pursuits of science and religion are incompatible occupations; indeed I suspect that amongst businessmen it may be a majority view. Of course, there are many scientists whom you would probably happily exclude from your strictures—those working in agriculture, medicine, materials and so on are presumably exempt, but you would probably wish to retain those subjects which move close to the problems of man and the universe, such as astronomy, geology and those branches of biology that are concerned with the nature of life. How, you might say, could someone dare to contemplate an idea in these fields which struck at his own personal convictions.

As you know, there have been scientists of great

distinction who have held very strongly to religion and, one suspects, many more who have believed in some underlying purpose to the universe without feeling that they had to devote an hour to it every Sunday. Perhaps some were inhibited from peeping too far behind the scenes, although I know of none, but many more were positively driven on by their beliefs to understand more about a universe at which they marvelled.

Furthermore successful scientists learn flexibility early in their training—they cannot afford to become too committed to conventional wisdom because they are there to discover, not to reinforce. Thus they are unlikely to hold fast to any religious beliefs with which they know their research might bring them into conflict. They are also pragmatic and like fame as much as anyone else, so they are not going to stand by for the sake of religious beliefs whilst someone else finds a cure for cancer or a recipe for longevity.

Most scientists would have found it extraordinarily difficult from their personal experiences in the fields I have mentioned to categorise scientists as religious or otherwise from their mode of working and their approach to fundamental questions. You would probably have found the same.



Call for biohazard legislation

In this article Brian J. Ford argues that the law does not concern itself sufficiently with the various hazards that can arise when microorganisms are mishandled and suggests areas in which legislation should be introduced.

LEGAL restraints and regulations are the bane of research workers in many spheres. All of us must have felt some kind of annoyance at the filling in of forms or the signing of registers that have the aura of state bureaucracy, but the justification lies in the resulting safety of research. If that is the case, it is odd that legislation covering the handling and use of microorganisms and viruses that are pathogenic to mankind is virtually nonexistent.

At the moment moves are afoot in Britain to make hepatitis a recognised industrial disease amongst hospital and laboratory staff, and there is in the United States a call for a moratorium on microbial genetic manipulation. Such piecemeal short term measures could be superseded by the greater controls of a generally accepted legal framework of safety.

In other fields of scientific endeavour there are laws that regularise the use of potentially dangerous materials. Strict controls govern the use and distribution of radioactive substances, even those of the lowest potency used in student demonstrations. Toxic chemicals are subject to a range of long standing controls including the pharmacy and poisons legislation, the poisons rules and the legal restraints on dangerous drugs. In the First Schedule of poisons appear compounds great and small, from TEPP to 2-methoxycarbonyl-1-methylvinyl-diethyl phosphate; yet there is no reference to the bacterial toxins which are considerably more dangerous and also, in many respects, easier to produce.

In the regulations governing the security of the experimental animal appear rats, mice, rabbits; even dogs and cats. There is no mention, however, of pathogens. In the world of agriculture there are restrictions on the handling of some plant pathogens of economic significance and on the culture of viruses such as foot-and-mouth, though limitations on human pathogens are absent.

In philosophical terms the greatest hazard posed by the lack of controls

over pathogens is their infectivity: poisons do not replicate. A technician who takes home an isotope may damage himself as a result, whereas a mis-handled culture of virulent microorganisms could expose entire communities to widespread hazard. Yet isotopes are kept under lock and key. It is a culture of pathogenic microorganisms that needs no such safeguard in the eyes of the law.

Since I first published an outline of proposals for biohazard legislation (*New Law Journal*, 121 (5511), 823-824; 1971) there have been several examples of the misuse or abuse of cultures pathogenic to mankind. That paper referred to the virus of smallpox (variola) and the occasional accidents that occur when unsupervised or inexperienced staff come into contact with pathogens. Last year's outbreak in the London area is a tragic illustration of the hazard.

Children have recently been found playing with discarded culture vessels in the Midlands; and others were found to have obtained contaminated syringes casually discarded by a pharmaceutical concern in Kent. Occasionally cultures are wilfully misused. In the United States it has been reported that an underground group planned to immunise themselves against the effects of bacteria that were then to be introduced into the public water supply, and in Australia a culture of a meningitis virus was stolen (in its incubator) from a research laboratory.

Examples are known where disease has resulted from the use of unplugged mouth pipettes. A recent example in Britain involved a supposedly non-pathogenic bacterium ingested when

the tip of a pipette under suction was momentarily withdrawn from a thin agar culture. Elsewhere an assistant handling positive sera from syphilitics made a similar error, but did not develop the disease. I have seen a technician attempt to open a phial of polyvalent poliovirus, believing the glass to be scored. It was not, and the neck fractured and lacerated his finger.

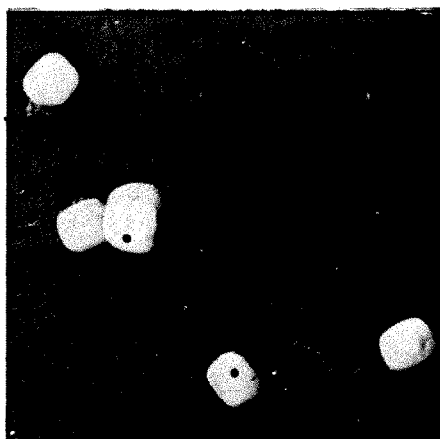
It had not been ascertained whether he was immune to polio before commencing his work with the viruses. A similar case concerned a girl technician who saw her doctor with a sore throat, contracted shortly after she began to work with diphtheria. No check had been made on her immunological status and following representations made by the doctor to the college department the entire research team was immunised as a precaution. At a university zoology department, to cite another example, staff set up a tank for the rearing of water snails infected with bilharzia. A small child related to one of the staff dabbled its hand in the water on the morning before the parasites were introduced.

In each of these episodes some safety measures were subsequently introduced to prevent a recurrence. But retrospective, custom-built regulations are no way to prevent accidents entirely. We now have a situation where safety codes in a laboratory tend to be based on the past history of accidents in the establishment. Biohazard law would enable general safety measures to be introduced and enforced for the protection of all.

The literature of the past five years has included several examples where widespread hazards might have been involved. A form of infectious choriomeningitis reportedly caused five cases with three fatalities during experiments in the United States, and an unidentified virus from vervet monkeys has been stated to have caused over 30 cases with seven deaths despite the most rigorous attempts to contain the virus. Venezuelan equine encephalitis is now said to be epizootic in Utah, having originated in biological warfare experiments.

Many hazards arise from the failure to maintain adequate supervision in laboratories. Junior or untrained staff may be given access to culture materials they do not understand. In some cases infected materials are disposed of by being poured down the same sink as

Smallpox virus particles



*The revealing lens, 19th century version.
A William Heath etching of the 1820's
depicts the contemporary view of
microbiological hazards*

that used for the preparation of tea and coffee.

There are interesting precedents in British law for the kind of proposals I envisage. The Factories Act 1961, Section 64, provides for the minister to extend the provisions banning eating in places where fumes or dangerous industrial dusts are produced; there is no mention of the far greater hazards that have biological origins (and, as the important case of *Weston v LCC* (1941) showed, a technical institute is not a factory).

The conduct of workers in laboratories has an interesting precedent in the Agriculture (Poisonous Substances) Act 1952. Section 1 Subsection (c) calls for the "abstention from eating, drinking and smoking in circumstances involving the risk of poisoning" and Section 2 Subsection 1 (b) makes it a culpable offence to do anything to "wilfully . . . cause risk of poisoning". Similar precautions with respect to microorganisms and viruses would not be hard to define.

The Radioactive Substances Acts, 1948 and 1960, define in detail the conditions under which radioactive substances should be handled, distributed, stored and controlled. The clear restriction of the availability of radioactive materials to "duly qualified medical practitioners or registered dental practitioners" is similar to what might be proposed for virulent pathogens. The Factories Act 1961 Section 18 Subsection (2) refers to the avoidance of dangers from "scalding, corrosive or poisonous" materials, to which list the infection risk could be appended. Section 82 of the same Act contains an anomalous, isolated reference to bacterial hazards when it refers to illnesses caused by lead, phosphorous, arsenic, mercury "and anthrax" as notifiable conditions, without reference to other infections of an occupational origin. The widespread use of microorganisms in industry, which an extension of biological engineering will inevitably entail, makes safeguards timely.

Conditions of storage have been covered in respect of phials containing vaccines, antibiotics and related biological products under Part III Section 5 (i) of the Therapeutic Substances Act 1956, which states that such containers must be sealed to "preclude the access of microorganisms" and later specifies the labelling of the outsides of the



containers. A universal biohazard mark for glassware and other apparatus used to contain pathogens should be found. Perhaps a yellow spot or disk could be fused into glassware during manufacture as a permanent warning against the casual use of beakers or flasks for the preparation of refreshments. The recently introduced biohazard symbol displayed in some laboratories, or something like it modelled on the radiation sign, should be obligatory in all establishments handling pathogens.

Certain diseases are already notifiable, of course; though it is interesting that an organism in one's gall bladder may lead to restrictions by the health authorities which they would be powerless to impose if the organisms were carried, in a bottle, in one's trouser pocket instead. The only substantive reference to microorganisms occurs in the Therapeutic Substances Act Part II Section 4 (b) of 1956. It requires that separate laboratories be provided by licensees producing medical products if they "engage in the culture or manipulation of pathogenic spore-bearing microorganisms". It is already recognised by this Act that pathogenic contaminants must be kept out of containers of vaccines and the like, though the converse possibility—that organisms allowed to escape from within containers may pose even greater problems—is not referred to.

By far the greatest indictment of the current situation is the framework of controls within the Public Health Act 1936 and 1965; and the Health Services and Public Health Act 1968 (as amended by Schedule 14 and para 4 of Schedule 29 to the Local Government Act 1972).

These regulations contain a host of strictures on those unfortunate enough to contract smallpox, with limitations

on the movement of people concerned and the use of library books and dustbins during an outbreak. The possibility that restrictions should be placed on the *in vitro* virus remains ignored.

Legislation should cover several specific areas:

- It should define groups of organisms, such as those of high pathogenicity (variola, tubercle, typhoid) which are not normally present in the environment but which pose problems to communities. These I have grouped as 'Schedule A' pathogens. The remainder, including streptococci and staphylococci, are widespread and should be subject to more basic safety regulations in the laboratory: these would constitute 'Schedule B' and would alone be available for school and teaching use.

- The status of licensed holders of Schedule A organisms and viruses would be defined in terms of academic training responsibility and seniority. Less severe restrictions would be placed on laboratories classified for the culture of Schedule B pathogens.

- Standards of safety in laboratories would be laid down and codes of conduct made mandatory. The registration of holders of specified pathogens would doubtless aid the coordination of the research effort, and clear sets of agreed criteria would apply to genetic manipulation of bacteria and viruses, and to the disposal of potentially hazardous materials.

It is indeed unfortunate that bacteria and viruses are still so widely seen as harmful. To many of them we owe our very existence. Yet they confer on the research worker considerable responsibility when he comes to work with pathogens *en masse*. A legal code is now overdue for academic, practical and humanitarian reasons. □

international news

Two groups which have long been advocates of arms control and of detente between the United States and the Soviet Union last week condemned as "a counterproductive sham" the so-called test ban agreement which the besieged Mr Nixon brought back from Moscow. Suggesting that the agreement will do more harm than good in preventing the spread of nuclear weapons, the two groups—the Federation of American Scientists (FAS) and the Arms Control Association—called on Nixon to renegotiate a more meaningful arms control measure instead of sending the Moscow test ban treaty to the Senate for ratification.

A so-called "threshold" ban, the agreement would prohibit the United States and the Soviet Union from testing nuclear weapons with a yield greater than 150 kton. It is not due to be brought into effect until March 31, 1976. But it does not cover so-called "peaceful" nuclear explosions and it is simply a bilateral agreement between the Soviet Union and the United States, with no provision for other countries to become parties to it.

The FAS and the Arms Control Association believe that the treaty will do nothing to limit the number of tests conducted by both countries and that it will be seen as "a complete and cynical fraud" by countries which do not now possess nuclear weapons and which are looking to the nuclear powers to show some restraint in weapons development. In short, they argue that the Moscow agreement is worse than nothing.

They base their condemnation of the measure on four chief grounds.

- The 150 kton threshold—which is ten times greater than the bomb which devastated Hiroshima—is so ludicrously high that it will allow the United States and the Soviet Union to continue testing virtually unchecked. Furthermore, since it will not be introduced for nearly two years, both countries will probably carry out what the Arms Control Association calls an "orgy of intensive nuclear testing" in order to get some large tests in before the deadline.

- There are at least two, and possibly three, weapons systems which will have to be tested before the limitation comes into effect. First, a new warhead which is being developed for the Minuteman III, with a yield of about 400 kton, will have to be extensively

Test ban treaty condemned

by Colin Norman, Washington

tested. Second, a new bomb or perhaps an air-launched missile will have to be developed for the B-1 bomber. And finally it has been suggested that the missile for the new Trident submarine may have a yield of 200 kton; that too will have to be developed before the treaty takes effect.

- As for the Soviet Union, the chief programme which will be affected will be the development of MIRVs for the big SS-18 missile. Although according to some accounts testing for that device may already be completed.

- Aside from these weapons, there is little need for either country to conduct large tests—indeed, since the partial test ban was signed in 1963 the vast majority of tests have been below 150 kton—and so the FAS believes that the treaty will have little effect on weapons development. Thus seen from the viewpoint of non-nuclear countries, the agreement is a sham which does nothing to fulfil the commitment enshrined in both the partial test ban treaty and in the nuclear nonproliferation treaty to bring about a complete halt to weapons testing.

Equally counterproductive as far as stopping the spread of nuclear weapons is concerned is the fact that the agreement does not cover explosions for peaceful purposes. Since India justified its recent nuclear test as a perfectly peaceful enterprise—an interpretation which has been greeted with great scepticism outside India—FAS and the Arms Control Association believe that the Moscow agreement will increase the chances of other countries following India's example. As Adrian Fisher, former deputy director of the Arms Control and Disarmament Agency, put it last week, the agreement "legitimises the Indian position" at the time when everybody is attacking it.

The FAS also points out that the test agreement may pose severe political problems because of difficulties in ensuring that it is not violated. For one thing the treaty provides for calibration shots to be carried out at each country's test site, but FAS notes that "we cannot know the yield of the Russian weapon from examination of seismological data

for reconnaissance. It could be 300 kton instead of the stated 150 kton". And for another, the same explosive force can give different seismological signals of differing strength, depending on where and how it is fired.

Finally, and perhaps most important, there is considerable concern that if this essentially meaningless agreement is ratified it will, in the words of the FAS, "sell out the efforts to reach a comprehensive ban" on nuclear testing by "retreating on policy grounds and taking the matter off the national agenda".

Thus FAS and the Arms Control Association are suggesting that the treaty should be renegotiated, with the objective of securing a complete embargo on nuclear tests. In any case, it is worth pointing out that some 37 senators, led by Edward Kennedy, sent a letter to Secretary of State Henry Kissinger during negotiations on the Moscow agreement asking that a comprehensive rather than a threshold agreement be negotiated. If they all stand firm in their belief that the Moscow agreement is meaningless in terms of control, they have enough votes to block its ratification if it is referred to the Senate.

If the matter is renegotiated, what grounds are there for believing that the Soviet Union would be prepared to negotiate a complete test ban? Two statements of Chairman Brezhnev are being widely quoted as giving a hopeful indication. First, on June 14 he said that the Soviet Union "was prepared to reach agreement now with the United States on the limitation of underground nuclear tests, proceeding to their full termination according to a coordinated timetable". And second he said in a speech on July 21 that "we would like to achieve something more and are prepared to go further; the Soviet Union is ready in particular to conclude an agreement on complete cessation of all underground tests of nuclear weapons".

It is widely assumed, however, that it was the Soviet negotiators who were most insistent in exempting peaceful nuclear explosions from the Moscow agreement, and that it could present a huge problem in any future negotiations. As the FAS succinctly put it, "the question arises of either talking the Soviet government out of its interest in peaceful uses or deriving an acceptable method of verification".

FOLLOWING hard on the heels of long awaited decisions about British nuclear power and oil comes the not unexpected news that the Maplin project for a 'third' London airport is to be abandoned. The decision has been made in the light of a reappraisal of the project made since the British election earlier this year, and comes just after the publication of the British Airports Authority (BAA) *Annual Report 1973/74* (HMSO, £2).

In that report, the Chairman of the BAA, Nigel Foulkes, commented: "The financial year brought a series of events which reduced the BAA's profits, slowed down the growth rate of the air transport industry sharply, and put all long-term plans for the future development of our airports back into the melting pot". What has now emerged from that melting pot is evidence that no further main runways will be required to handle expected traffic at the four airports in the London area

Maplin project abandoned

(Heathrow, Gatwick, Stansted and Luton) before 1990. With the noise nuisance now likely to be lower than forecast by the Roskill Commission (thanks to the advent of quiet wide-bodied jets) there is no need for a new airport at least until that time.

Capacity at both the principal London airports would have to be increased whether or not the Maplin project went ahead, the Secretary of State for Trade, Mr Peter Shore, told the House of Commons on July 17. The growth envisaged is from a capacity to handle 20 million passengers a year to 38 million in the case of Heathrow, and from the present 6 million passengers a year to 16 million for Gatwick.

If air traffic expands so that even this capacity is insufficient to meet the demand, various possibilities will be

open to the planners of the mid-1980s. Stansted, which has been referred to as the "expansion chamber" of the existing British airport system, must once again seem high on the list for future development, and although Mr Shore commented in the House that major diversion of traffic from the south-east to airports beyond Bournemouth, Birmingham and the East Midlands does not seem attractive at present, in the long term there is still a possibility of developing airports as far away as Prestwick and Edinburgh. With the higher speed rail links which would be possible by the 1990s, such a diversion might then have more appeal.

Whatever happens in the long term, in the present atmosphere of uncertainty about air traffic and with general economic stringency, many will, no doubt, echo the words of Mr Eric Moonman, Member of Parliament for Basildon: "To save £650 million is a good afternoon's work".

LORD ROTHSCHILD said in 1971, a year after the appointment of a Central Policy Review Staff (CPRS) or 'think tank', that its and his success could be measured in terms of its survival. It had then survived somewhat longer than the equally novel Department of Economic Affairs—a parallel he drew—and this was despite the unnecessarily disruptive rumpus caused by the blunt instrument of the White Paper on the reorganisation of government research funding. The think-tank's continuation has now been officially endorsed by the present Labour administration and so tacitly subsumed as a permanent piece of government machinery, but Lord Rothschild is opting out. His successor as head of the CPRS is Sir Kenneth Berrill, a Treasury economist and former Chairman of the University Grants Committee.

The virtue of the original think-tank (whatever its shortcomings) was that it was Lord Rothschild 'writ large'. The fifteen or so bright young men that formed its analytical staff were all of his personal choosing. He was not, nor ever could be, a conditioned civil servant. His independent means removed him from the servitude of security in presenting his opinions—though he has questioned the relevance of this. His background lay in demanding fields of observational science and experiment, his foreground in multinational industrial research planning in his 10 years as research director of Shell Chemicals. All the indications are that however strange the advice proffered by Lord Rothschild's think-tank it was genuinely independent. The change can only mean a reduction in the role of science in government, especially as the post of Chief Scientific Adviser has also lost status recently. Does the government really want informed, independent pacing of its



Last of the great independents?

performance 'within the system'?

Two years ago Lord Rothschild assessed the importance of the CPRS as follows: "Regional policy is the most intractable problem we have been faced with, but the most difficult and important is this analysis of the government's strategy". Perhaps today he would reassess the relative intractability of the subjects under study.

In general the subjects which the Cabinet has referred to the CPRS are confidential—as are the recommendations made. But it is known that apart from government research funding, inflation and energy it has studied Concorde, the British computer industry, the Post Office giro, whether there is an optimum population level for the nation, multinational companies and the bane of all governments, regional policy.

The think-tank has, under various circumstances, published three of its 'thinks'. There was (and is) the redistribution of

government research funds which threw Lord Rothschild himself into public prominence and is still somewhat controversial. He has continued to maintain that before the White Paper's adoption "the organisation of government science was wrong" but has subsequently admitted "the only real regret I have is that I did not explain the customer/contractor principle. I didn't explain it because I thought it self evident. But it evidently was not so." If he had, this would have made the report three times as long but perhaps "some of the scientists would have been less hostile".

The study on population has also been published and most recently the think-tank's long gestated views on energy were drilled out of the government machine by the Secretary of State for Energy, Mr Eric Varley. Nonetheless the think-tank published first and its emphatic reliance on saving energy rather than flirting with alternative energy sources was firmly lodged in the Department of Energy's paper which appeared a fortnight later. Particularly appealing features of the CPRS's *Energy Conservation* were the emphasis on an industrialists' 'annual energy audit' the possibility of saving substantially on electric light bulbs and the criticisms of large, fast uneconomic private cars. It would be a fitting memorial to Lord Rothschild's quirky reign in Whitehall if he could get public acceptance of a slow, small economical battery-driven urban runabout.

In the meantime, it seems that the CPRS is being downgraded with Rothschild's departure. Mr Wilson has already appointed a parallel unit of about eight advisers, currently untitled, but effectively an economics think-tank within No. 10 Downing Street itself headed by Dr Bernard Donoghue.

Those babies still pose problems

by Miranda Robertson

It has not been entirely clear from press reports over the past two weeks that no child in the immediately foreseeable future is likely to have been in any sense a test tube baby. The most that is contemplated is the occasional emergence—in the traditional manner, from a woman—of a baby which spent its first few days as a fertilised ovum in a Petri dish (test tubes are not used for this kind of thing). That much is in principle quite possible and even desirable, although there are good reasons for supposing, in spite of claims at the recent British Medical Association meeting in Hull, that it may not yet have happened.

Scepticism about Professor Bevis's announcement could only be abolished by the publication of a technique which proved more successful than those already reported. The existence of three children, even if they were born to couples who had taken part in artificial in ovulation trials, cannot vindicate the claim: where the wife is the egg donor and the husband the sperm donor, there is no way of telling whether the baby developed from the reimplanted egg, or whether it was naturally conceived in the course of the experiment, as can sometimes happen with presumed infertile couples.

The transfer of eggs fertilised outside the body into the uterus to complete development is already routine in laboratory animals used in genetic and developmental research. It is somewhat trickier to get eggs from humans than it is from mice or rabbits, but even so the techniques for obtaining viable fertilised human ova, developed over the past few years in Britain by Mr Patrick Steptoe of the Oldham and District General Hospital, and Dr Robert G. Edwards, of the University of Cambridge, are now extremely reliable. The problems which have prevented the animal success from being extended to humans lie not within the embryo but with the reproductive physiology of the mother in whose uterus the embryo must be able to implant.

It is not particularly surprising that the fourteen egg transfers so far reported by Mr Steptoe have failed. Even in mice, the failure rate for implantation of transferred eggs is something like 50%. There is every reason to suppose that some proportion of all fertilised eggs in normal women fail to implant and are flushed out, unnoticed, with the next menstrual flow. Patients whose infertility is an indication for artificial in ovulation are not normal women. The blocked oviducts which

makes the procedure necessary are likely to be the consequence of disease which may have had other effects on the reproductive apparatus. The very procedure of injecting the fertilised ovum into the womb may initiate contractions which militate against implantation.

Finally, the hormonal interaction between the mother and the embryo is both critical and ill understood. In order to control the timing of reimplantation of the ovum, the woman's cycle is induced artificially with hormones, and this may result in endocrine deficiencies which could account for the failures. Nor is it clear what is the optional point at which reimplantation should be attempted.

Clearly, it is only a matter of time before someone produces a baby which began its life in laboratory glassware. That is a realistic and human goal according to Dr Edwards: "To give a couple their own child obviously needs no justification" and fears about the possible biological hazards and social implications of the technique seem on the whole unwarranted.

The misgivings voiced by the Medical Research Council on the abnormalities in embryos fertilised *in vitro* do not seem to be substantiated by the considerable body of data on animals (*Nature*, **244**, 333; 1973). The essential point is that the embryo before implantation is undifferentiated and thus almost impervious to injury. If enough cells are killed during the manipulations, the embryo dies. If it does not die, the cells regulate to produce a normal organism. There is a slight danger of triploidy if the ovum is fertilised by more than one sperm, but this can be avoided by proper control of the number of sperm used to fertilise one egg.

Most of the social, legal and ethical issues raised in connection with fertilisation *in vitro* differ little from those which arise in connection with artificial insemination or with adoption. The more horrendous imaginings of determined doomwatchers can probably be dismissed under the general proposition that it does not follow that once a thing is feasible it will necessarily or even probably be turned to undesirable ends.

These arguments, both on the feasibility and on the desirability of the technology for growing embryos *in vitro*, apply only to preimplantation development. The human uterus is still irreplaceable between six days and 24 weeks of gestation, which includes the most critical developmental period—that of differentiation, when the foetus is at its most susceptible to teratogenic influences. Not only are the problems inherent in the creation of an artificial womb during this period enormous, but none of the stated aims of current

research on human embryos *in vitro* would be served by it. The immediate aims as seen by Mr Steptoe are to overcome infertility in the 2% of couples in which it is caused by blocked oviducts, to control sex-linked disease by selecting fertilised eggs of one sex only for reimplantation, to investigate the causes of abnormality in preimplantation embryos, and to test male contraceptive drugs which can only be evaluated on the basis of their ability to prevent sperm from fertilising eggs. None of these goals would be advanced by the development at vast expense of an elaborate surrogate uterus. Not only does Mr Steptoe see no medical justification for carrying development *in vitro* further than the first few days, but the danger of abnormalities in the foetus does become serious once differentiation begins. □

Crisis in science education

from Peter Lindon

AN unhappy conjunction of two events was the origin of a conference on The Crisis in Engineering and Science Education in the West held at The University of Manchester Institute of Science and Technology (UMIST) on July 16 and 17. The two events in question were the 150th anniversary of the Institute and the decline in applications for science and engineering in the universities.

A sense of gloom and impending disaster was created from the beginning as Lord Bowden the Principal of UMIST predicted bankruptcy within 18 months for universities in the absence of any change in government policies. With student applications having dramatically fallen, and classes containing an ever increasing proportion of foreign students he wondered where the engineers and scientists to drive our essential industries would be coming from. The mechanism of manpower supply and demand seemed to be seriously at fault. From the time a child made decisions affecting the future direction of his studies until the time when he qualified, some 8–10 years had elapsed: a lag which must be an inherent cause of instability in recruitment to industry and enrolment at universities. It is precisely at the time when the need for scientists is greatest that the numbers and quality of science school teachers are eroded, the children become disillusioned with their experience of science in the schools, and the manpower supply is shut off.

Some contributors did not consider this mechanistic explanation sufficient. The social influences upon student choice were also important and Sir

A DETAILED analysis of the surface waters of the Eastern Baltic and the Gulf of Finland has now provided a theoretical basis for an experiment begun with field trials in 1969—the use of these water masses as a source of irrigation.

The first trials took place on the island of Mukku (Estonian SSR). The irrigated land yielded the “hoped-for” results, and in the spring of 1970 the research programme was started by the Irrigation Section of the V.I. Lenin All-Union Academy of Agricultural Sciences. A systematic study was made of the salinity and mineral content of the coastal waters of northern and western Estonia and of the Kihiumaa and Sarremaa. In all, 63 coastal ‘points’ were tested, samples being taken from depths of 50 cm to 1 m. In all, 664 specimens of water were analysed.

According to the report published in *Pravda* (July 25, 1974), the average salinity for the area was 1.5–2.0 g l⁻¹ (“almost fit for drinking”—a not surprising result, since the low salinity of the Baltic is a well known phenomenon, particularly as regards the 30–50 cm and 50–60 cm layers). Also in 1970, field tests

Sea water for irrigation

by our Soviet Correspondent

were begun on three collective farms having contrasting soils (sandy, argillaceous and soddy-calcareous), using crops of perennial grass, barley, potatoes, swedes, early and late cabbage, table beet, cucumbers and so on. During the four years of the tests, good results have been obtained with pasture grasses, barley and a number of vegetables. Subsequent soil tests indicated the introduction of “a fairly considerable quantity” of dissolved mineral salts into the soil; in particular, after one summer of irrigation with sea water, the sodium content of the arable soil of the “Linnamee” state farm (of soddy-calcareous type) rose from 4–10 mg per 100 g soil to 30 mg per 100 g soil. In 1971–73, further tests were carried out to determine the effect of the increased salinity on the soil microflora.

According to the *Pravda* report, which is signed by no less a person than O. Valing, Chairman of the State Com-

mittee of the Council of Ministers of the Estonian SSR on Land Reclamation and Water Economy, the results indicate that, “with a proper choice of crops and agricultural technology”, the use of sea water in irrigation can prove an important adjunct to Estonian agriculture. Yet some facts, which he calls “curious” seem to indicate the need for caution in assessing the nutritional aspects of these results. Thus barley grows taller and gives a heavier grain yield, when grown on sea water, but the protein content is “somewhat” lower. In the case of cabbage and beet, the sugar content of the sea water crop is higher. And the presentation of the report in *Pravda*, on page 2, which is normally reserved for “party” news (science reports normally are found on page 3 or page 6) would suggest that the original decision to use sea water might well have been of political, rather than scientific, origin. When one recalls the disastrous effect on Soviet agriculture, and Soviet science as a whole, of another such political decision—Lysenkoism—one can only await future news from the fields of Estonia with a certain caution.

Frederick Dainton, chairman of the University Grants Commission, referred to the alienation from science caused by its unacceptable relationship with power (often misused), and its lack of concern for people. The exploitation of science in the military context and the connection between industrialisation and pollution were cited as examples. Sir Frederick believed that science should be taught in the universities as a humanity: not as an instrument of control over the environment, but as a subject of interest for enquiring minds.

It was reassuring to find that Britain is not alone in experiencing difficulties, the most frequently recurring theme being the effects of inflation which militate so strongly against educational interests. Relief from the prevailing atmosphere of doom was given by Dr W. B. Lewis in describing changes in science at Canadian universities. Here, one felt, was a country with security in its possession of large natural resources and confidence in its future. Despite earlier predictions that the need for scientists would decline, the numbers of graduates and PhDs had increased enormously in 1973 and all were now in relevant employment. Asked what had made such changes possible, Dr Lewis stressed the prime importance of cheap energy which in Canada was not simply derived from hydroelectric sources. The advanced energy programme was capable of producing electricity even more cheaply

than from water power.

Dr J. B. Adams of CRN believed the qualities required of graduates were job flexibility and the abilities to plan, budget and acquire new technologies. He praised the breadth of approach found in general applied physics degrees and made the interesting observation that 40% of the engineers in his organisation were in fact physics graduates. The point was taken up by Professor H. B. G. Casimir, the President of the Royal Netherlands Academy of Sciences and Letters who believed that the only purpose of specialisation was to inculcate the ability to specialise; the specific subject was only of secondary importance. The three year degree in England was too short to make specialisation within it a sound proposition and he suggested that industry in this country should be prepared to recruit far more PhDs.

Sir Frederick Dainton also had some harsh things to say about specialisation in the first degree. A curriculum based on professional requirements was the easy way out for university teachers who were subject specialists themselves. Yet the factual and professional content of a degree was ephemeral. What was needed was the ability to think logically and transmit ideas clearly. Science should be taught in a spirit of enquiry and more time should be devoted by faculty to reflective consideration of their subjects and the development of discovery-based learning techniques, instead of trivial research.

Crisis is Greek for decision. Predictably the conference did not produce one, though it did illuminate some of the underlying problems of university science. With issues of such complexity and gravity one wished the organisers had provided more opportunity for discussion in smaller groups. Half-hour discussions with 450 people all facing in the same direction rarely produce anything of substance. And what of the school-teachers, invited in large numbers yet unrepresented by speakers? They work amongst shifting sands; the science departments get poorer recruitment every year and the competition from what they see as undemanding soft options gets fiercer. At a time when even previously highly selective university departments are touting for undergraduates in the schools they needed some guidance on whether to try and stimulate schoolchildren to take up science or steer them away from it. None of the speakers, one felt, took this section of the audience very seriously.

What did emerge was the fundamental need to recover the ability to plan in the long term. Only in this way can there be orderly development and the maintaining of morale. Inflation has negated planning, and if it cannot be cured we must at least learn to live with it more successfully. Meanwhile we seem confined to the world of Kafka's aphorism: “There is an end but no way; what we call the way is shilly-shallying”. □

Patrick Blackett . . .

an appreciation by Sir Edward Bullard

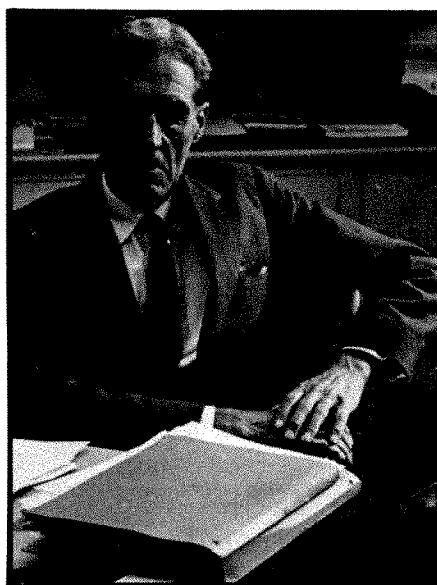
PATRICK BLACKETT, who died on July 13, was I suppose, the most versatile and the best loved physicist of his generation. His achievement was also without rival. Few of his friends had seen much of him during the past two years, but the sense of loss is, for me at any rate, unique. When J. J. Thomson and Rutherford died, it was sad and it was the end of an era, but their work was done and they had not, in the same degree, the wide ranging interest or the personal concern or kindness that were so characteristic of Blackett and which lasted unabated into old age.

Blackett was born in November 1897, the son of a stockbroker. At an early age he went to the Royal Naval College at Osborne and then to Dartmouth and to sea at the beginning of the 1914-18 war.

After the war he was sent to Cambridge by the Navy and soon decided that he did not want to return to the service. He once told me how, after this difficult decision, he felt as if a great weight had been lifted. He had all his life, a great affection for the Navy and a liking for an active life, but he felt he needed a wider field and one where the conventions as to what it was suitable to say and do were less restrictive.

I do not know exactly how he transformed himself from a naval officer to a research worker in the Cavendish, there is no mention in *Who's Who* of his taking a BA. He never attempted to take a PhD and when teased about accepting a peerage, replied that at any rate he had remained Mr Blackett until after he retired (this was not strictly true as he had a large number of honorary doctorates). He soon obtained striking results in the Cavendish planning of the postwar development of an automatic cloud chamber. C. T. R. Wilson, who first devised the cloud chamber before the war, was a slow-working perfectionist; Blackett was willing to take tens of thousands of photographs of hundreds of thousands of tracks if the problem needed that many. In 1925 he succeeded in photographing the emission of a proton from ^{14}N in a collision with an α particle. These famous photographs have adorned almost every text book of nuclear physics for the past fifty years.

The work with the cloud chamber led to the discovery of the positive electron in 1933 and to the study of cosmic rays by a counter-controlled chamber. This work brought out Blackett's remarkable facility in instrument



design. He could use physical theory to design a piece of equipment and then draw it, make it and get it to work. I have known nobody who possessed this combination of talents in the degree that he did. It is a major theme in his scientific work and can be seen in the bomb-sight he designed at Farnborough and in the magnetometer which he and his students used so effectively in the 1950s.

He was not entirely at ease in Cambridge or in the Cavendish. He felt that Rutherford gave a disproportionate share of support and funds to Kapitza and, perhaps, that his own left wing politics did not fit well in the still feudal-Victorian environment of Cambridge. He left for Birkbeck College in 1933, where he continued his work on cosmic rays, and then moved to Manchester in 1937. At this time he became involved in the controversies concerning the air defence of Great Britain and the relative importance of radar and other methods of detection. As a member of Tizard's Air Defence Committee he played an important part in ensuring that the essential stations were working when they were needed in 1940.

Soon after the outbreak of war he was at Farnborough; from there he moved to Coastal Command. There, and later at the Admiralty, he and E. J. Williams essentially invented operational research. He thought of it as the study of what actually happens as opposed to what is supposed or desired to happen. It was work that suited him; it involved not only intelligence and ingenuity but firmness,

persuasiveness and a commanding personality. The effect on the U-boat war was of great importance.

In the course of his work at the Admiralty he became convinced of the ineffectiveness of the area bombing of cities. After the war this argument became connected with questions of nuclear strategy. He, perhaps unwisely, said that the idea of winning a war by the wholesale destruction of cities and the slaughter of civilians was an Anglo-American innovation and was not shared by the Germans or the Russians. This argument culminated in his arrest by a sheriff in some city in the Southern United States where his plane had made a refuelling stop on the way from Mexico to Canada (the sheriff was very civil and took him to his own house and not to jail).

In the last four months of the war Blackett became deeply involved in the planning of the postwar development of science, the rapidity of the subsequent development is in large measure due to him. He returned to Manchester in 1945 and started on a completely new line of work. He investigated a possible relation between rotation and magnetisation and, when this failed as a theory of the Earth's magnetic field, turned to the study of rock magnetism. The part that this work played in the modern developments of geological theory cannot be described here.

In the Attlee administration he was regarded as too far to the left to be an acceptable advisor or member of the government. In Mr Wilson's first administration he played a large part in the setting up and conduct of the Ministry of Technology. He was a member of many official and unofficial committees, his work as Chairman of the Council of the Department of Scientific and Industrial Research (DSIR) in 1955-60 was of particular importance for the support of science in the universities.

The last time I saw Blackett I remarked that his portrait at the Royal Society made him look very serious. He replied: "But I am a very serious man". He was; he minded about the things he considered important, he thought deeply about them and held his views firmly. This could have made him tiresome and a bore, but it didn't: he was wonderfully intelligent, charming, fun to be with, dignified and handsome. There was no one like him. He had every kind of honour; the Presidency of the Royal Society, a Nobel Prize, a peerage, all the medals and honorary doctorates a man could ask for, membership of the American, French and Russian Academies and many more. To add to all this he was married to one of the most delightful women in the world who did much to prevent him from becoming too serious.

correspondence

Academics in Chile

SIR,—As signatories with other colleagues of a letter on academic life in Chile (*Nature*, May 29) we feel it necessary to comment on Dr Eyzaguirre's answer which appeared in the issue of July 5.

Dr Eyzaguirre says our letter was inaccurate, although he concedes that academic people were killed or persecuted because of "actively" opposing the military junta or because of "denunciation by neighbours or colleagues". We would like to point out the omissions and inaccuracies we find in Dr Eyzaguirre's letter.

(1) Dr Eyzaguirre depicts Dr Allende, the legally elected president of Chile, as a "corrupt" politician whose government was responsible for "chaos and anarchy". He omits to say however, that Dr Allende's government strictly respected freedom of speech and of the press and that nobody was killed, imprisoned or persecuted because of his ideas. Another omission concerns Dr Allende's respect for the independence of the university, exemplified by the election as Rector of the University of Chile of Professor Boeninger, well known to be an opponent of the government.

(2) Dr Eyzaguirre's eulogy of the military coup ("the military struck with vigour") is shocking. He admits that some "unpleasant" excesses were committed. But nothing is said about the killings in the working class quarters (*poblaciones*), the hunting of the foreigners, the tortures and the shootings at the stadiums, and the 'purification' of the libraries followed by bonfires of 'forbidden' books. The authenticity of these events is not in doubt since they have been repeatedly denounced by non-Marxist organisations including Amnesty International, the World Council of Churches and the Conference of Chilean Catholic Bishops.

(3) The comparison that Dr Eyzaguirre draws between the nomination of military chiefs as university rectors in Chile, with the nomination, for example, of General Eisenhower as President of Columbia University would be funny if it were not so sad. In the second case, the university chose to honour an outstanding military and political personality, whereas in the first a military group was imposed on the university to ensure the rigid political control of the faculty and the students.

(4) Dr Eyzaguirre states that "whole-

sale executions of faculty and scientists have not occurred" and that "very few fell either immediately after the coup because, allegedly, were caught either shooting at soldiers, carrying arms or actively participating in the organisation of revolutionary activities". Some examples may illustrate the inaccuracy of such statement. Professors Ramirez and Peña, after being sentenced by the military to only two months in prison, were shot in the city of La Serena. Leopoldo Benitez, an architect, of the Catholic University and Victor Jara, a musician, of the Technological University were assassinated in cold blood at the Stadium Chile. Enrique Paris and Luis Sanguinetti of the University of Chile at Santiago and Michael Woodward, of Valparaiso, died while being tortured. More than ten medical doctors were shot by the junta.

(5) Dr Eyzaguirre takes as an example of the university situation the Catholic University, where no student has been expelled. He says that Admiral Swett Madge, the new Rector, was nominated with the approval of Cardinal Silva Enríquez. He omits to say that the previous Rector, Professor Fernando Castillo Velasco, also approved by the Cardinal, was dismissed by the junta, had his house ransacked and decided to leave Chile. Dr Eyzaguirre also states that only 3.5% of the Faculty was dismissed by the Rector on political grounds. He omits to say that at least four institutes (among them the Institute of Economic History and the CEREN, a centre for Chilean studies) were dismantled and all their members dismissed. He also neglects to say that Father Mauricio Hebert and six other professors were expelled from the country.

Moreover the Catholic University enrolls only 10% of the student population. Dr Eyzaguirre does not quote the number of faculty members dismissed at the University of Chile. He only tells us that the only students expelled (no figure is given here either) were those with unsatisfactory academic records. It is difficult to accept, for instance, that more than half (141 out of 254) of the first-year students in the science school, who were not allowed to re-register, were agitators or professional politicians. A final remark is necessary. When we signed the letter to *Nature* asking for help for the jobless university teachers, and teachers who were persecuted by the junta or who refused to work under a totalitarian

regime, we did not state or imply that the scientists or academics who decided to stay in Chile (among them many respected friends) were fascists. Dr Eyzaguirre says that they are "labouring under difficult circumstances which are mainly economic". We think that this is an understatement in view of the present lack of freedom and disrespect of the human rights. It is because of this that we find it so unfair that Dr Eyzaguirre should dub those who left Chile as '*gauchistes de salon*', in particular because many of them still have families in Chile and are therefore unable to answer him publicly.

Yours faithfully,

H. M. GERSCHENFELD

J. P. CHANGEUX

Paris, France

Algae as fuel

SIR,—A few words may be in order about planktonic algae cultivation as a means of harnessing solar energy, which was not mentioned in your Energy Review (*Nature*, June 21, 1974).

Algal cultivation has many advantages over plant cultivation. Whereas land for afforestation is becoming scarce, vast areas of lagoons and inland lakes are available for algal cultivation. About 1% of sunlight that falls on an area is absorbed by the plants growing there, but sunlight can diffuse through water down to a few hundred feet and so more than 1% of the sunlight can ultimately be absorbed by algae in water. Algae can grow in a variety of aquatic environments and there will be added advantages if sewage is added to the medium because the oxygen liberated by algal photosynthesis can be used by the bacteria in the medium to break down the organic matter in the sewage.

Also algae multiply fast. In large experimental ponds yields as high as 36 tons of algae per acre of pond per year have been achieved, compared with the yield of about 1 ton per acre of dry organic matter which plants yield. Thus a lake of 100 square miles can yield more than 2,000,000 tons of algae per year, which can be used as fuel in conventional thermal power stations.

Yours faithfully,

N. UMAKANTHA

Karnatak University,
Dharwar, India

news and views

Symmetry in recognition sites for proteins

WHEN nucleotide sequence analysis on RNA was beginning to gather momentum in 1965, such endeavours with even the simplest of DNA molecules were limited to preliminary skirmishes from a few optimists; but it was at this time that Bernardi entertained a model for the interaction of spleen DNase with its substrate which is now widely invoked for the reaction of many proteins with DNA. From the nature of the 3' termini of the products generated by this enzyme, which contained two identical polypeptide chains, Bernardi (*Adv. Enzym.*, **31**, 13; 1968) proposed that the subunits of the enzyme are associated about an axis of two-fold rotational symmetry. Thus alignment of this axis with a corresponding axis between two opposed -G-C- pairs in a DNA duplex ensured that a catalytic site on each enzyme subunit is appropriately positioned to make equivalent breaks in each polynucleotide chain to leave -Gp.

Interactions based on this type of model proved very attractive for the more complex nucleotide sequences found at the positions of breakage of DNA by certain restriction enzymes. Following the demonstration by Kelly and Smith (*J. molec. biol.*, **51**, 393; 1970) that nucleotide sequences at the sites of breakage of DNA by a restriction enzyme (comprising four subunits) from *Haemophilus influenzae* were bisected by an axis of two-fold rotational symmetry, such rotationally symmetrical sequences, or palindromes as they are termed in some quarters, have been found at the interaction sites for several restriction and modification enzymes: the recognition sequences so far determined are tetra, penta, or hexanucleotides, becoming degenerate thereafter.

One would anticipate that some of the more important control functions would require larger nucleotide sequences to ensure their unique recognition. It was therefore of interest to note that in the first of the larger recognition sequences to be determined, that of the interaction site for the λ *ter* system, that is, the enzyme generating mature DNA molecules from a concatenated replicative intermediate form (Weigel, Englund, Murray and Old, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1151; 1973), rotational symmetry was again apparent, but this time the base pairs that were symmetrically arranged about the axis were not in continuous sequence, but showed a broken, or hyphenated symmetry. The *lac* operator provided a further, and rather more complex, example of a sequence of nucleotides with a discontinuous symmetry about an axis through a base pair rather than between two pairs, a feature of one of the recognition sites of the restriction enzyme (Gilbert and Maxam, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3587; 1973).

Recent and current work on the structure of DNA in areas concerned with the control of gene expression is providing more examples of structures of this sort, and a fascinating picture is emerging of the rather complex way in which the macromolecules may interact. Detailed study of regulation in phage λ has been made possible by exploiting the extensive background of genetics with this phage and the availability of many deletion and mutant strains. Expression of early genes in λ begins with transcription

from two promoters, P_L and P_R , located astride the immunity region. Interaction of RNA polymerase with these promoters is prevented by interaction of the λ repressor, the C_1 gene product, with the relevant operators, O_L and O_R . Maniatis and Ptashne (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1531; 1973) recently showed this to be a rather complex reaction involving at each promoter the stepwise addition of repressor molecules to six reiterated binding sites which were similar but not identical in nucleotide sequence. These sites, S_1 to S_6 at the two operators, ran in opposite directions towards the C_1 gene on the map of the λ chromosome. Other promoters mediate the expression of genes involved in late functions and yet another promoter dictates synthesis of repressor during the consolidation of lysogeny. The various promoters are accurately located on the map of the λ chromosome. This enabled Allet and Solem (*J. molec. Biol.*, **85**, 475; 1974), through a comparative analysis (by gel electrophoresis) of the fragments obtained by digestion of DNA from λ and a variety of deletion strains with several restriction endonucleases, to isolate fragments of DNA containing only one promoter; this work considerably enhances opportunities for biochemical studies of transcription *in vitro*.

Of more immediate interest, however, was their observation, made also by Maurer, Maniatis and Ptashne (*Nature*, **249**, 221; 1974), that DNA from the mutants *sex1* and *sex3* which are defective in the left promoters, P_L , when digested with restriction endonuclease R.HindII did not give the fragments that had been shown to result from breakage within O_L , the left operator, but instead gave a larger fragment equivalent in size to the two missing fragments. A corresponding mutant, *x13*, inactivating the right promoter, P_R , did not, however, affect the action of R.HindII at its site located within O_R . Both groups of workers then showed that the sites for R.HindII located within both O_L and O_R were protected from digestion by the binding of RNA polymerase, which established that operator and promoter sequences are identical or overlapping.

The occurrence of the R.HindII recognition sequence, -G-T-Y-R-A-C-, within the operators O_L and O_R has now been exploited by Maniatis, Ptashne, Barrell and Donelson, (see page 394 of this issue of *Nature*) for determination of a significant piece of nucleotide sequence in this important area of the λ DNA molecule. The R.HindII fragments flanking the break point in O_L , which occurs between sites S_1 and S_2 , are about 320 nucleotides long to the left and about 1,125 nucleotides long to the right. The longer of these two fragments was prepared in quantity (using gel electrophoresis), denatured, and then annealed to equimolar quantities of the purified *l*-strand of λ DNA. This gave a molecule that was both a primer and template for polymerase reactions, so that the 3' terminus of the annealed strand of 1,125 fragment could be extended by condensation of nucleoside 5'-triphosphates with DNA polymerase. Using Berg's adaptation of this reaction to substitute incorporation of a ribonucleotide for one of the deoxyribonucleotides, Maniatis *et al.* made products with rC or rG substituted (in separate reactions) to facilitate analysis of 32 P-labelled extensions of the 1,125 fragment.

• Analysis of the products of these syntheses were simplified by first cleaving with the HindII restriction enzyme and fractionation of the mixture of oligonucleotides (which had

a common 5' terminus but varied in their 3' terminus because of asynchronous synthesis) by the homochromotographic methods exploited so well by Sanger and his colleagues. Further cleavage of these oligonucleotides with pancreatic or ribonuclease T₁, followed by fractionation of the products and analysis of them by partial exonuclease digestion (to give further products that could be identified from their behaviour on two-dimensional fractionation systems), finally led to the proposal of a sequence of 59 nucleotides from the HindII site in *O_L* which extends into the region of *N* gene transcription. Since this sequence originated at the HindII site within *O_L* which is protected by RNA polymerase binding, it limits the distance separating bound RNA polymerase from the T-A-G with which *N* gene transcription begins to 33 base pairs and so reinforces the challenge to the suggestion made two years ago that the left promoter and start of transcription are appreciably separated.

Within this region of 33 base pairs to the left of the HindII site are three axes of two-fold rotational symmetry each involving hyphenated distribution of the symmetrically arranged 14 to 16 bases. All three blocks of symmetrical sequence seem to lie within the region covered by the repressor and it is intriguing to contemplate that these interdigitating symmetries are the recognition sequences for different regulatory proteins (including the *tof* (or *cro*) gene product to regulate leftward transcription). One can only agree with the authors that analysis of the structure of the remaining sites in the operators and localisation of regulatory mutations should provide a clearer understanding of the significance of these symmetries.

Of no less interest will be the elucidation of the structure of other promoters (in addition to that of the phage fd promoters determined recently by Seeburg and Schaller),

because Allet Roberts Gesteland and Solem (*Nature* **429**, 217; 1974) have shown that the HindII recognition sequence occurs in several, but not all, promoters in phages λ and T7 as well as in the mammalian viruses SV40 and adeno2. Extension of the work on the *lac* operator (Gilbert, Maizels and Maxam, *Cold Spring Harb. Symp. quant. Biol.*, **38**, 845; 1973) has now identified the position of several mutations in this sequence and these occur in both symmetrical and non-symmetrically arranged bases. With this system (and others) analyses of the regions of the enzyme involved in the interaction will also be relevant to the meaning of symmetry and here, production of active *lac* repressors that are covalently fused to β -galactosidase (Müller-Hill and Kania, *Nature*, **249**, 561; 1974) will help to define necessary regions of the repressor. Detailed analysis of the *L*-arabinose operon, which is under positive control, is also in prospect following the recent studies of araC protein binding to the ara operator by Wilcox, Clemetson, Cleary and Englesberg (*J. molec. Biol.*, **85**, 589; 1974). Much remains to interest those who do not believe that prokaryotic molecular biology is finished.

From a correspondent

Russian cosmology

It has been a recurring preoccupation among cosmologists to establish connections between the microscopic and macroscopic of the Universe. Eddington attempted to discover a fundamental relationship between atomic and cosmological systems, and other workers still continue to pursue this possibility. Among these workers is the nuclear physicist Andrei Sakharov, the same individual who has recently achieved world renown as a leading Soviet dissident. Sakharov and his colleagues, notably the eminent astro-

Sitting in a hot seat

THE spectacular nature of volcanic eruptions has long attracted scientific observers. As far back as 79 AD Pliny documented the eruption of Vesuvius and the accompanying destruction of Pompeii and Herculaneum, and by the beginning of this century, when Frank Perret was travelling the world compiling a chronicle of volcanic eruptions, volcanology had become well established as a science.

Today, there are volcanic observatories in a large number of places throughout the world. Many, including those on Vesuvius, and in Japan, Hawaii and Soufrière in the West Indies, use extremely sophisticated techniques. Seismic networks record tell-tale cycles of seismic activity, and geodetic measurements involve the use of lasers to detect the dilations of a volcano, which presage serious eruptions.

Volcanologists are not confined to predicting the onset of eruptions. By taking records during eruptions they can determine the source and nature of a lava, which often enables them to anticipate how a particular volcano is likely to behave during future periods of activity. The prognosis may be of use to communities who wish to prepare as best they can for likely contingencies. In that respect, the measurement of such parameters as the viscosity, effusion rates and temperature of a lava, and the detailed chemical and mineralogical analyses are all important. On page 385 of this edition of *Nature* an Anglo-Italian team of volcanologists report their observations of a recent eruption of Mount Etna in Sicily.

Most volcanologists are quick to admit that, unfortunately, there is very little that can be done to safeguard livelihoods and property. On Hawaii, always threatened by mobile basaltic magmas, there have been attempts to

chill advancing lavas, but these measures usually prove to be ineffective. The Hawaiians have, however, successfully diverted lava from its course with bombs; but elsewhere, in densely populated areas, that action may be a palliative which merely shifts the problem from one community to another.

There are few precautions that can be taken against the threat of ash falls, as evidenced on Heimaey in Iceland in 1973. Then, during the eruption of Eldfell, homes and land were completely blanketed by vast volumes of ash within a few days. And against the worst type of volcanic hazard—*nuées ardentes*—there is absolutely nothing that can be done. *Nuées ardentes* are hot avalanches of loose, explosive lava lubricated by constantly expanding gases and vapours. Because of their internal energy, they sweep down the flanks of volcanoes with tremendous speed, often flowing uphill and across barriers, burning all in their paths. In 1902 a *nuée ardente* swept down Mont Pele, on Martinique, and, within the space of a few minutes, annihilated the 30,000 inhabitants of the port of St Pierre.

Inevitably, volcanoes still retain many unknown characteristics, and continue to behave unpredictably. Volcanologists on Vesuvius are becoming "very worried". That volcano has a long history of particularly unpleasant eruptions, and all of the indications are that it should have erupted at least twice during the past 30 years or so. Geodetic measurements show that the flanks of Vesuvius are bulging to an alarming degree. Evidently, the Vesuvian volcanologists could do worse than consider their own counsel. They might also be wise to bear in mind recent events on Etna—in 1971 the volcanic observatory on that mountain was among the first obstacles to be swept away before the advancing lava.

ALLAN PIPER

physicist Zeldovich, have been developing a theory which attempts to link the phenomenon of gravitation, which governs the motion of large scale systems, with the quantum structure of the vacuum (*Dokl. Akad. Nauk S.S.S.R.*, **771**, 70; 1967).

The modern quantum field theorist has a conception of a vacuum which differs greatly from that implied by the popular usage of the word. The absence of particles does not mean just 'empty space'. Rather one must imagine a continual interchange of energy between different forms, as 'virtual' particles of all types appear and disappear in transient fluctuations of incredibly short duration. The origin of this vacuum energy is analogous to the energy which prevents the electrons in an atom from collapsing into the nucleus.

Also in gravitation theory, empty space is rich with structure. The presence of a gravitational field is interpreted in the general theory of relativity as a curvature of space, that is, a distortion of the geometry. The stronger the gravitational field, the more the space geometry becomes distorted. The precise amount of distortion produced by a given gravitating mass (for example the Sun) is determined by the value of Newton's gravitational constant G . Although this value may be measured, it has long been a dream among physicists that Newton's constant might be derived in some way, or connected in a relationship with other, perhaps microscopic, constants of nature.

Sakharov has proposed a derivation of G based on an analogy between the vacuum and an elastic medium. In the same way that distorting the shape of a piece of rubber leads to a change in its internal energy so, Sakharov argues, a distortion of empty space by a gravitating mass leads to a change in the energy of the vacuum, as interpreted by quantum field theory. G may then be calculated as an 'elastic constant of space' from the theory of elementary particles. The expression for G given by Sakharov contains an unknown mass which corresponds to the heaviest allowed elementary particle. To get the right value for G , this mass must be about 10^{-2} g, or 10^{19} times as heavy as the proton!

Whatever the merits of this particular piece of work, the idea of appealing to elementary particle physics and quantum field theory in investigations of large gravitating systems is a fascinating one which is occupying the attention of other Soviet scientists at this time. Zeldovich had previously explored a possible connection between the quantum fluctuations of the vacuum and the existence of a cosmological constant in Einstein's field equations of general relativity. A recent paper

(*Phys. Lett.*, **50B**, 340-342; 1974) by Kobsarev, Okun and Zeldovich presents new work in the same spirit. This time, these authors turn their attention to the subject of symmetry breaking in elementary particle physics. It is well known that certain elementary particle processes violate the so-called CP symmetry; that is, symmetry under charge reversal and space (mirror) reflection. The Russians have applied a simple model due to T. D. Lee for CP violation to the subject of cosmology. The essence of their article is that, because of CP violation the vacuum acquires a domain structure loosely similar to that of a ferromagnet, adjacent domains differing by the sign of the CP violation. These domains form during the early stages (at about 10^{-5} s) of the hot big-bang at the beginning of the Universe, and arise (at random) because of the existence of causally separated regions which always occur in this type of model universe. Roughly speaking, each domain wants to form with the same sign of CP violation throughout, but it cannot know the sign in adjacent domains. As the universe expands, so do the domains.

The behaviour of a universe filled with such domains is drastically different from the conventional models. The authors consider the dynamics of the system to be dominated by the behaviour of the domain walls, which are in a state of tension, and have the peculiar equation $p = -2/3 \epsilon$ connecting the pressure p and the energy density ϵ . This relation leads to a model universe which expands like t^2 , that is, accelerates to unlimited expansion, even in the case of the cosmological constant being zero, in contrast to the conventional models of this class which always decelerate as they expand.

As the authors point out, such a rapid expansion is not really consistent with present density observations of the Universe. More seriously, the gravitational field of the domain walls would be likely to destroy the observed isotropy of the cosmic microwave background radiation. For this reason, it is suggested that a mechanism must exist for removing the domain walls after about 10^{11} s. One possible such mechanism would be to connect the sign of CP violation with the expansion of the Universe, perhaps along the lines of the work by Ne'eman (*Int. J. theor. Phys.*, **3**, 1; 1970).

It is hard to know how seriously cosmological models of this type should be taken. As a general principle, however, it is important to appreciate that as accelerating machines become ever more expensive to build, the testing of theories in elementary particle physics by constructing cosmological models is becoming progressively more attractive.

P. C. W. DAVIES

Floating on high

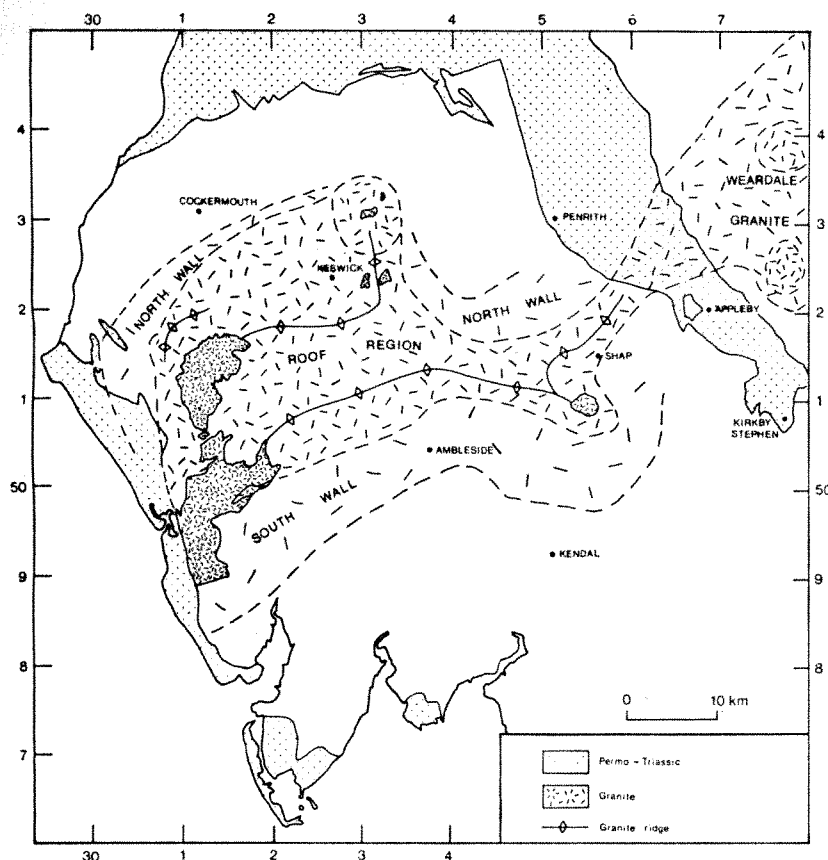
from J. Sutton

SEEN from the air—a journey between Glasgow and London on a fine day provides the opportunity—the mountainous Lake District contrasts strikingly with the surrounding subdued landscape. This contrast has its roots in a long geological history which Bott has recently investigated (*J. geol. Soc. Lond.*, **130**, 309-331; 1974).

The Lake District, like many other areas of high ground in Britain, is underlain by Palaeozoic metamorphic rocks; the surrounding lower ground is underlain by younger rocks, though these too rest on a buried Palaeozoic basement. This situation, a deep Palaeozoic basement below a younger cover, can be matched elsewhere in and around Britain—below the Cheshire Plain for example.

During the past 250 million years, much of the crust of the British Isles has been broken into a mosaic of blocks some tens of kilometres across. Some of these have moved upwards, and others have gone down. Why should this be? That is a question which Bott has made his speciality in recent years. He has already shown that south-west England and the northern Pennines are underlain by granites which are larger than their surface exposures alone suggest. He has now demonstrated that the Lake District is in the same condition. Bott has established that the Lake District is marked by a belt of relatively low Bouguer anomalies; in other words, some rock near the surface is unusually light compared with its neighbours. Particularly low gravity values occur over the isolated granites which reach the surface at Eskdale, Shap and Skiddaw. Bott attributes the anomaly to a concealed granite, connecting these outcrops at depth, and extending for some 80 km, at least as far east as Weardale. The granite lies high in the crust, it is suggested, within the top 10 km, and thus forms another example of a high level Caledonian intrusion—it was intruded in early Devonian times, nearly 400 Myr ago.

These results bear on two distinct, but related, geological questions. In the first place the work brings out the position of outline of another Caledonian granite, adding to the number known already in north-west Britain. As Shackleton pointed out in the discussion of the article, the nature of the upper surface of the Lakeland granite presents a problem. Why is it that the levels to which the granite rose were rather uniform over quite a wide area? As more geophysical evidence on the shape and depth of other buried Palaeozoic granites comes to hand this



Sketch map showing the inferred distribution of unexposed and exposed granite beneath north-west England. The map shows the roof and wall regions of the postulated Lake District batholith, the main granite ridges being indicated by the gravity anomalies and the postulated connection to the Weardale granite (from Bott, *J. geol. Soc. Lond.*, **130**, 319; 1974).

problem might become better understood.

Such information will help with the second question—does the distribution of low density granities, high in the crust, play a critical part in determining which blocks of Palaeozoic crust act as bouyant upward-moving blocks and which subside to be buried by sediments? So much of the wealth of this country in the form of coal or oil is present in sedimentary basins formed since Carboniferous times above a Palaeozoic floor, that an understanding of this question would have a practical as well as an academic value.

Bott shows that in the Lake District the density contrast between the granite and the country rocks would cause a gravity deficiency of magnitude $1 \times 10^{18} \text{g}$, and finds that this is of the same order as the total mass of the Lake District rocks above sea level, which he computes as $1.49 \times 10^{18} \text{g}$. These calculations support the hypothesis which Bott has already applied at Dartmoor and in the Northern Pennines, which proposes that low density granites high in the crust can account for blocks of elevated country. There is good stratigraphical evidence that the mountainous block seen in the Lake District today, has been rising and shedding detritus at least since the

Carboniferous. In other words, what is now the Lake District has been bouyant since the time the granite was intruded. An interesting experiment now would be to attempt to find out whether the depressed portions of Palaeozoic crust which underlie sedimentary basins lack low density intrusions. Such a discovery would give further support to Bott's hypothesis.

Radar mapping of the Moon

from David W. Hughes

WHY bother to look at the Moon with radar? The answer is quite simple. Whereas Earth-based telescopes can, with the best conditions of optical viewing, give a lunar surface resolution of a few hundred metres, returned radar echoes can yield the distribution of objects with sizes around the radar wavelength—typically in the 4–150 cm region. This degree of fine resolution can also be obtained using orbital and lunar surface photographic techniques but only at considerable expense.

Now a resolution of 1 arc s can be obtained at optical wavelengths, even using small telescopes. Using simple radar with currently available aerial beamwidths, and straightforward

attempts at angular resolution, a value of 4 arc min is obtained, which when compared with the 30 arc min diameter of the lunar disk shows that only very large scale features can be picked out.

The Delay-Doppler mapping technique gets over this problem. As the Moon is rigid, a known motion of the whole body will enable the motion of each surface feature to be calculated. This movement produces a Doppler shift in the echo, and by high resolution frequency analysis selection can be made of the component of the echo originating from a known strip on the lunar surface; this strip is parallel to the apparent axis of rotation. Range analysis enables a circular annulus, centred on the sub-radar point, to be selected. A combination of both techniques isolates two scattering areas, only 10^{-5} , or less, of the total visible lunar surface. The ambiguity produced by having two areas is overcome by making the separation between these areas greater than the angular half power beam width of the radar antenna.

The resolution problem can be thought of in another way. Consider the radar wavefront hitting the Earth. The apparent rotation of the Moon sweeps this wavefront past the antenna and so sequential sampling of the received signal produces results equivalent to sampling simultaneously the original waveform over the region swept out by the antenna during the time interval of observation. Thus, large observing apertures can be synthesised, producing greatly improved angular resolution; in fact the degree of resolution is at present only limited by system stability and data processing constraints.

The observed distribution of echo power with delay and Doppler frequency can be thought of as having two components. The first is coherent and quasispecular, caused by reflections from the relatively smooth undulating lunar surface. This component is polarised just like the incident radar beam. The second component is incoherent, diffuse and largely unpolarised, and originates from wavelength sized structures, thought to be mainly rocks on or near the surface. Careful normalisation of the returned echo enables surface tilt to be eliminated as a source of scattering difference between different regions of the lunar surface. The remaining enhancement of radar echoes is then a measure of the rock population (the rocks detected would have sizes between one quarter wavelength and about 10 wavelengths; rocks also give signals provided they are not buried deeper than about 20 wavelengths).

The results of high resolution radar mapping, at 3.8 cm and 70 cm, of the lunar surface facing the Earth are given in a series of three articles in a recent edition of *The Moon* (10, 1974). The

radar window is limited by the absorption by water and oxygen molecules in the Earth's atmosphere; 70 cm is in the centre of this window, and 3.8 cm is close to the edge. The 3.8 cm observations had a resolution of 2 km, and the 70 cm observations had an aerial resolution of 25 km², which is twenty times better than previous results. The results were obtained by Pettengill, Zisk and Catuna at the Haystack Observatory, Massachusetts Institute of Technology and by Thompson at the National Astronomy and Ionosphere Centre, Arecibo, Puerto Rico.

By comparing radar maps with Lunar Orbiter photographs of the same area three basic radar features can be distinguished. They are well defined, named craters, very small un-named craters and, finally, radar anomalies with no apparent optical counterpart. There seems to be an anomalously strong radar return from small craters, which is not necessarily correlated with their apparent size or optical albedo.

The authors conclude that the craters which give large echoes are young, with sharp contours and steep inner slopes. The rough inner surfaces are tilted steeply towards the Earth-based radar, leading to depolarisation of the returned signal. These craters also have excess rock populations caused by the excavation of large quantities of bedrock during formation and subsequent depositions around the crater in a strew field many kilometres across. Meteoric erosion slowly removes the hallow, grinds the rock into fine grain debris and produces a shallow subdued topography, thus reducing the echo strength to the average lunar value. Most of the enhanced echo craters are thought to be Copernican in age. It is concluded that spallation of rocks caused by impacts with sufficient energy to fracture centimetre sized and larger rocks, is a common lunar phenomenon.

One radar anomaly found in the Kepler region is thought to be formed by a large clod of material which was thrown out with the ray material when Kepler was formed. The 70 cm data, with the help of 'ground truth' observations at Apollo landing sites, has enabled the percentage roughness of the lunar surface to be calculated.

Not only are the youngest craters on the Moon strong radar scatterers; they also have enhanced eclipse temperatures (that is infrared anomalies—elevated temperatures which certain areas maintain during the umbral phase of an eclipse). Correlations between radar and infrared maps show that these 'hot spots' can be explained by the thermal behaviour of surface rocks, provided these are 10 cm or larger in size and are greater in number on the surface than at the Surveyor 1 and 3 landing sites.

Analysts celebrate

from T. S. West

THE celebrations of the centenary of the Society for Analytical Chemistry which were held from July 16 to 19, began with a special 'historical' meeting at the Royal Institution in London at which fraternal greetings from 30 kindred societies were presented—most of them from overseas. The scrolls and presentations are now on display for 6 months at the special exhibition "You and Your Analytical Chemist" at the Science Museum in South Kensington.

Sir Alan Hodgkin, president of the Royal Society, described the founding of the SAC on August 7, 1874 in the City Terminus Hotel, Cannon Street and traced the events that had been set in motion by the actions of Professor Theophilus Redwood and his group on that day. The society, which had formerly existed as The Society of Public Analysts (1874-1907), then as The Society of Public Analysts and

Other Analytical Chemists (1907-1953), had a membership of 2,000 in 1972 when it had undertaken a joint role with the new amalgamated Chemical Society as the kernel of the Chemical Society's Analytical Division with a membership, in 1974, of 5,000.

Dr G. W. C. Milner, the centennial president of the SAC, highlighted some of the events of the past hundred years and looked forward to the future. The society, with only 2,000 members in its hundredth year obviously stood at the crossroads and was faced with a decision to continue, as before, independently or to amalgamate fully with three other British sister chemical societies (the former Chemical Society, The Faraday Society and the Royal Institute of Chemistry) and become the Analytical Division of the (new) Chemical Society. The presence of more than 3,000 additional 'first choice' members in the Analytical Division of the Chemical Society had influenced the council's decision to recommend full unification to the members at a referendum to be held later this year.

Nitrogen aggregates in diamond

from John Walker

IT has been known for many years that some diamonds contain precipitates ('platelets') on (100) crystal planes which give rise to anomalous features ('spikes') in Laue X-ray photographs. When Kaiser and Bond (*Phys. Rev.*, **115**, 857; 1959) found by mass spectrometry that these diamonds also contained large quantities of nitrogen impurity, it was natural to assume that it was contained in the platelets. Shortly afterwards, in 1962, the platelets were observed in transmission electron microscopy. Everything fitted and everybody was happy.

Six years later Sobolev *et al.* (*Sov. Phys. Doklady*, **12**, 665; 1968) upset the applecart by discovering that the X-ray spikes did not correlate with the total nitrogen content of diamonds, which meant that the platelets contained little if any nitrogen. This raises two questions. Of what are the platelets made and where is the nitrogen? The first question was and is still open, but the second could be partially answered. Electron microscopy can detect features only a few Ångströms in diameter, and since none had been observed the nitrogen aggregates must be very small—effectively point defects. This deduction has now been confirmed by Turk and Klemens (*Phys. Rev. B*, **9**, 4422; 1974) using thermal conductivity.

Any imperfections in a crystal scatter the phonons (lattice vibrations) and decrease its thermal conductivity. The amount of scattering depends on how big the scattering centres are relative to the phonon wavelength, and since that changes with temperature, so does the scattering. Hence the variation of thermal conductivity with temperature contains information on the size and concentration of defects in a crystal. What Turk and Klemens have done is to calculate the scattering effect of platelets and point defects, and fit their results to the experimental thermal conductivity data of other workers. They deduce for one diamond that only 3% of the nitrogen it contains is in platelets: the rest is in small clusters of eight nitrogen atoms. They have therefore confirmed the conclusions of Sobolev *et al.* using a totally different technique, and the eight-atom cluster would probably be too small to see in the electron microscope, as was expected.

One would now hope that Turk and Klemens could extend their work to many more specimens: for example infrared and visible spectroscopy suggests that there are not one but at least three types of small nitrogen cluster (the so-called N₃, A and B defects) in some diamonds. It would be interesting to see if they could be distinguished and their sizes measured.

Hierarchy of behaviours in a gastropod

from Marian Dawkins
Animal Behaviour Correspondent

ANIMALS in their natural environments are often exposed to mixed, even contradictory sensory inputs. It may be impossible for an animal to react to all these inputs at once, as the behaviours involved may actually be mutually incompatible; for example, an animal cannot both approach and withdraw from an object at the same time. One of the interesting features of the evolution of the behaviour of animals has therefore been the development of a system of priorities whereby these conflicts are resolved in such a way that the survival and reproduction of the animal is most likely. This often takes the form of animals in conflict situations performing certain behavioural responses to the partial or complete exclusion of others, and in this sense the animals can be said to have a hierarchy of behaviours.

Marine molluscs such as *Pleurobranchaea* have already proved to be extremely fruitful material for understanding the neurophysiological bases of many behaviours and it is therefore promising for the discovery of the neural basis of such a behavioural priority system that their behaviour has been found to be organised into an explicit priority sequence. Davis, Mpitsos and Pinneo (*J. comp. Physiol.*, **90**, 207-224; 225-243; 1974) have found that in this species, which is a carnivorous gastropod, feeding is a relatively dominant behaviour and takes precedence over most other behaviours. They presented animals with stimuli which released two different behaviours: in one experiment they gave a food stimulus to animals which had been turned upside down. Instead of showing their usual almost immediate righting behaviour, the animals stayed on their backs and ate, often for up to an hour. Feeding also dominated head withdrawal and mating, and Davis *et al.* argue that the adaptive significance of this is that *Pleurobranchaea* is a carnivore with a sporadic and rather limited food supply and so opportunities for feeding being rather scarce, feeding requires immediate and decisive interruption of all other behaviours.

Interestingly, there are some situations in which feeding is not given top priority. Davis *et al.* found that this behaviour is hormonally suppressed during egg laying; presumably this is an adaptation to ensure that the animals do not eat their own eggs. Even higher in the hierarchy than egg laying

is the escape swimming response to avoid predators. This can interrupt any other behavioural act and so ensures that the animals survive to perform their other behaviours at times when no danger threatens.

The organisation of behaviours into hierarchies of adaptive priorities, Davis *et al.* suggest, may be a unifying theme for a diversity of complex behavioural phenomena in many animals, and to have discovered one in a mollusc with a simple analysable nervous system encourages the analysis of this phenomenon at the level of the single nerve cell.

Can partons be saved?

from David J. Miller

THE most eagerly anticipated parts of the International Conference on High Energy Physics, held in London during the first ten days of July, were the sessions devoted to electron-positron annihilation. Most delegates had already heard of the surprising results obtained by physicists at Stanford California, and at Cambridge Massachusetts, concerning the rate of production of strongly interacting particles (hadrons). The conference gave the Stanford group a chance to report their results in detail, and to be closely cross-questioned about them. It also gave theorists their first chance to try and explain what had been discovered.

The Stanford and Cambridge experiments involved the storage of electron and positron beams of up to 2.6 GeV. The stored beams were brought together, twice per circuit in the Stanford SPEAR ring, and allowed to make head-on collisions. There was an array of scintillation counters and spark chambers around each intersection region, and it was possible to recognise whether outgoing charged tracks were caused by electrons, muons or hadrons. Electrons produce characteristic showers when they pass through dense material; hadrons are totally absorbed by a foot or so of steel, because of their strong interactions; muons are more penetrating than any other charged particles. The American results confirmed findings, at Frascati in Italy, Orsay in France and at Novosibirsk in Russia, that electron-positron scattering has all the properties predicted for it by the well established theory of quantum-electrodynamics. Elastic scattering, the production of muon pairs and the production of photon pairs all behaved exactly according to theory. The surprise came in the rate of hadron production. Up to a beam energy of about 1.5 GeV at Frascati, this rate falls off in the same way as

the production of muon pairs. But in going from a total electron-positron energy of 3 GeV (that is 1.5 GeV electrons plus 1.5 GeV positrons, meeting head on) to the 5.2 GeV available at Stanford and Cambridge, the rate of hadron production levelled off and stayed roughly constant. This is not a contradiction of quantum electrodynamics, but it is very surprising.

Theorists had been quite happy with the behaviour of hadron production at the lower energies. They had compared it with muon production, and worked out a theory in which the electron and positron annihilated by first of all creating a massive single photon. This process is formally just the reverse of electron-positron pair production by a photon close to an atomic nucleus. The heavy photon could then generate a muon pair, one positive and one negative, or it might generate a 'parton' pair in just the same way. Partons are hypothetical objects which are supposed to be the building blocks from which the hadrons are made—just as the hadrons themselves are the building blocks for nuclei.

The parton theories have been supported by a number of recent experiments where leptons (electrons, muons or neutrinos) are scattered from nucleons (protons or neutrons, free or in nuclei). These so called deep inelastic scattering experiments have been done with electrons at a number of laboratories, including Stanford, and with neutrinos in the Gargamelle bubble chamber at CERN, Geneva. The results are in some ways similar to Geiger and Marsden's alpha particle scattering experiment, which led Rutherford in 1911 to postulate a small point nucleus in each atom. The angular distribution in deep inelastic lepton scattering from a proton is just what would be expected if protons had very small constituents. There is even evidence to suggest that these constituents or partons may have the same charge and spin as the quarks, which were postulated in 1973 by Gell-Mann and Zweig to explain the regularities in the spectrum of the resonances of the strongly interacting particles. The quarks are the most intuitively straightforward way of building up hadrons which fit into the 'SU(3)' symmetry scheme.

In 'parton pair production', by a single photon from electron-positron annihilation, it was suggested that the partons would first be produced, and would then 'clothe' themselves with other partons, spontaneously generated from the vacuum, forming hadrons of the kind which can be observed. Quarks, or any other form of parton, have never been observed, and perhaps cannot exist as free observable par-

ticles. Despite the complications of the 'clothing' process, the basic rate for hadron production by means of parton pair production should have the same dependence on the overall electron-positron energy as does muon pair production.

The new SPEAR and Cambridge results disagree with this. Muon production falls as the energy increases, but hadron production levels off. This directly contradicts simple parton theories. More than seventy theoretical papers were submitted to the conference, attempting to explain this effect in one way or another. Some suggest that the levelling off in hadron production is a temporary phenomenon at 'medium energies', and the high energy decrease has yet to begin. Others claim that massive 'charmed' particles are being produced (see *Nature*, **250**, 186; 1974 for a discussion of other contexts where 'charm' has been invoked), and the reaction rate is rising as more charmed final states become available. Other theorists have pointed out that the levelling off in hadron production is "more parton-like than partons". If the hadrons were not formed by production of pairs of point-like constituents, then the hadron production should fall faster than the rate of muon production. This suggests that some totally new phenomenon is being seen in electron-positron annihilation.

To test these theories it will be necessary to collect data at higher energies, and to make more detailed studies of the hadrons in the final state. The SPEAR ring is already being modified to give higher beam energies, but it seems likely that a much larger increase in energy will be needed to answer all the questions raised by the new data. British physicists could have a major part to play in the development of these studies. To reach the higher energies, new machines must be built, and Britain's long-term plans in this direction are as far advanced as those of any other nation. The Science Research Council should decide this year whether to build its EPIC storage rings which will have 14 GeV electrons colliding with 14 GeV positrons, a substantial step up from the energy of SPEAR. The Americans have similar plans but their new machine, called PEP, has also not been funded yet. If the United States does go ahead soon, PEP may be built a little more rapidly than EPIC since it can get its beams from the existing 20 GeV Stanford linear accelerator that now feeds SPEAR and other experimental facilities. But it takes a few years to do all the important experiments on a new accelerator, and there is plenty of room for two machines if they start work within a year or so of one another.

East African rift as membrane effect

from Peter J. Smith
Geomagnetism Correspondent

It is almost exactly a year since Turcotte and Oxburgh (*Nature*, **244**, 337; 1973) first sketched out their ideas on the nature of mid-plate tectonics. For some time before this (and even since), numerous workers had sought to explain the origin of major tectonic activity away from plate margins in the relatively *ad hoc* terms of hot spots, with or without mantle plumes. The Hawaiian-Emperor island chain, for example, was seen as the surface trace resulting from the motion of the Pacific plate over a stationary or near-stationary magma source—a not unreasonable explanation, although one difficult to substantiate in the face of older and equally plausible interpretations based on less contentious terrestrial phenomena. On the other hand interpretations of continental mid-plate tectonic features in terms of hot spots were much less successful. How, for example, could hot spots be held to account for the extension across graben and rift valleys?

In their original summary article, Turcotte and Oxburgh were able to abolish many such problems at a stroke, ironically by returning to explanations involving more traditional concepts. They argued that major mid-plate tectonic activity results from crustal extension due to tensional stresses. Clearly this is likely to offer a more satisfactory explanation for the finite extension of rift valleys, for extension would be required to relieve the stresses. In the case of the Hawaiian islands, it meant returning to the much older, though far from disproved, idea that the chain is a "propagating tensional fracture in the lithosphere which causes volcanic activity as it propagates".

But Turcotte and Oxburgh not only were to propose the mechanism (crustal extension), they were also able to suggest two plausible ways in which the required tensional stresses could be produced. The first type of stress, the theory of which was developed by Turcotte (*Geophys. J.*, **36**, 33; 1974), is the membrane stress arising from non-spherical plate tectonics. Because the Earth is not quite spherical, a lithospheric plate must deform as its latitude changes, producing regions of both compression and tension involving stresses of up to at least several kilobars. Second, there are thermal stresses, now discussed in greater detail by Turcotte (*J. geophys. Res.*, **79**, 257; 1974). As oceanic lithosphere moves away from the ridges, non-uniform cooling will lead to thermal stresses unless there is relief by plastic flow. In fact,

the upper lithosphere behaves elastically on geological time scales; Turcotte therefore concludes that thermal stresses up to 4 kbar (sufficiently large to fracture the lithosphere) may develop where the temperature is lower than about 300°C.

Presumably the forces giving rise to mid-plate tectonics generally arise from a complicated combination of membrane and thermal stresses, although the latter are clearly of particular relevance to the ocean floor. Indeed, in his most recent discussion in the *Journal of Geophysical Research*, Turcotte has suggested that even the transform faults and their associated fracture zones are contraction cracks which relieve the thermal stresses in the cooling lithosphere. In contrast to some other origins previously proposed for transform faults (for example, those involving small changes in spreading direction), thermal stress relief would explain the presence of such faults throughout the entire worldwide ridge system as well as in convecting lava pools (field) and freezing wax (laboratory). It would also explain why transform faults are apparently structurally similar to rift valleys. It would not explain directly why ridge segments are offset; but it is easy to envisage that once a thermal contraction crack appears in a ridge, small random variations in spreading rate on the two sides of the ridge will lead to offsetting.

Membrane stresses, on the other hand, are more obviously relevant to both ocean floors and continents; and it is to a plate comprising both continental and oceanic elements that Oxburgh and Turcotte (*Earth planet. Sci. Lett.*, **22**, 133; 1974) have now applied the concepts they have jointly and separately developed during the past year. The plate is the African plate; and the object of the exercise was to determine whether or not the formation and properties of the East African rift are explicable in terms of membrane tectonics.

The basic principle of membrane tectonics is that the two principal radii of curvature of a small plate at the Earth's surface will increase if the plate moves towards higher latitudes and, conversely, decrease if it moves towards lower latitudes. Thus an unstressed plate (regarded as a thin elastic shell) moving towards the poles will acquire a tension at the edge and a compression in the interior, whereas for a similar plate moving towards the equator the sites of tension and compression will be reversed. The difference between the Earth's polar and equatorial radii of curvature is such that a plate undergoing even a modest change in latitude (a few tens of degrees) will be subject to stresses comparable to the strength of the plate;

and so under the right circumstances large-scale lithospheric fractures may occur.

As interpreted by Oxburgh and Turcotte, the palaeomagnetic evidence suggests that, following a period of relative quiescence lasting most of the Mesozoic, the eastern part of Africa began to move northwards about 100 million years (Myr) ago at a rate of about 0.25° per million years. The western part of Africa, by contrast, began to move much more slowly. Taking into account the drift rate and the size of the African plate as extrapolated backwards throughout the past 100 Myr, Oxburgh and Turcotte calculate that the tensional stress in the centre of a circular plate the size of the African plate (and drifting towards the equator) would be about 135 bar, which seems to be of the same order as the stresses associated with current continental faulting. In other words, it is quite conceivable that the East African rift system was produced by membrane stresses in the lithosphere, developed in response to the rapid latitude change of eastern Africa during the late Cretaceous and early Tertiary—and that is precisely what Oxburgh and Turcotte propose happened.

It follows from this interpretation

that the East African rift system is probably a southward-propagating fracture system. There is little direct evidence on the age of the fracture itself; but it is implicit in a propagating fracture system that the onset of rift volcanism will migrate at the same rate as that at which the fracture extends. At most places along the East African rift, the volcanism, once started, has continued to the present; and so the onset of volcanism may be determined from the age of the oldest volcanic rocks available. Such age data from the eastern rift show that the locus of this onset has migrated southwards at a rate of about 0.23° per million years, which is in excellent agreement with the palaeomagnetically-determined northward drift rate of the eastern part of the plate. Numbers apart, however, the advantage of regarding the East African rift as a propagating fracture rather than the locus of a hot spot is that so much more can be explained. The tensional features of the rift are an obvious case in point. Equally, hot spots seem incapable of explaining why volcanism continues at any given point once the plate has passed over the spot; a propagating fracture, on the other hand, would imply continued dilation and thus continued volcanism.

The Oxburgh-Turcotte model is, of course, an oversimplification. The real African plate is not circular, does not remain the same size, and does not undergo a simple change of curvature. Moreover, the unstressed radii of curvature are not constant over the plate but vary from element to element within it. The oceanic lithosphere within the plate is subject to thermal stresses as well as membrane stresses; the location of fracturing within continental lithosphere may be governed partly by previous history of faulting; even mid-plate areas will be affected to some extent by interactions with neighbouring plates; and so on. But these are points of detail, rather than principle, which do not deflect Oxburgh and Turcotte from their view that membrane stress effects are substantially more plausible than hot spot processes.

Ribosome structure and function

from a Correspondent

THE EMBO meeting on ribosome structure (Göteborg, Sweden, June 27-30) was a field day for *Escherichia coli*. The most popular topic was the three-dimensional arrangement of the ribosomal proteins, where several different approaches are being used with considerable success. C. Cantor (Columbia University, New York) has used a fluorescence method in which reconstituted 30S ribosomes are labelled with two different fluorescent dyes on two specific proteins. By varying the proteins labelled, he has been able to classify many pairs of 30S proteins as being close, or far apart in the ribosome.

Experiments with bifunctional protein cross-linking reagents were reported by several delegates, the most impressive being those of R. Traut (University of California, Davis) who used the asymmetric reagent 4-mercapto-butyrimidate to establish nearly twenty pairs of cross-linkable proteins from both subparticles. This reagent reacts first with ϵ amino groups, leaving the thiol group free to react with protein sulphhydryl groups under oxidising conditions.

G. Stöffler and G. Tischendorf (Max-Planck-Institut, Berlin) have approached the topography question by electron microscopy. Since both subparticles have an asymmetric shape, it is possible to localise the positions of proteins by examining the complexes formed with the ribosome by individual protein-specific antibodies. So far twenty 50S and seven 30S proteins have been localised by this technique. H. Zeichhardt (Max-Planck-Institut, Berlin) used specific antibodies to examine the subparticle interface; although many

Internal magnetic field on Mercury?

from Peter J. Smith
Geomagnetism Correspondent

HITHERTO, it has not been considered likely that Mercury possesses an internal magnetic field. It is true that the planet has an unusually high density and thus by implication a large core; but the low rate of rotation would seem to preclude the generation of internal fields by dynamo action. Moreover, unlike Jupiter, Mercury has never produced any evidence of radio emissions which would indicate the presence of radiation belts.

The preliminary data from the Mariner 10 magnetic field survey near Mercury, now recorded by Ness *et al.* (*Science*, **185**, 151; 1974), are thus surprising. For on March 29, 1974 Mariner 10 passed within 704 km of Mercury's surface where it recorded a magnetic field of 98 γ , which is about five times higher than the average interplanetary field in the vicinity. Moreover, both a well developed bow shock wave and a magnetosphere-like region were observed — features comparable to those of the Earth, which has a global field, but completely alien to a body such as the Moon, which does not.

So do these results imply an in-

ternal Mercurian field? They certainly imply it, but do not prove it because there are other processes, such as field induction by solar wind interaction, which could just explain the limited data yet available. Another Mercury encounter will be required to settle the matter; but in the meantime Ness and his colleagues interpret the whole evidence as generally favouring the intrinsic field idea. Approximate figures then suggest that the Mercurian dipole moment is about 4×10^{-4} that of the Earth, that the dipole is close to the rotation axis and that it is offset by 0.47 Mercurian radii (not unacceptable in view of the large core).

A dynamo currently active within Mercury would indicate the viability of dynamo action under unexpected and unusual conditions. On the other hand, if the Mercurian field is due to remanent magnetism left from a dynamo now extinct, the implication must be that the planet once rotated much faster than it does now. In any event, internal planetary magnetic fields are rare enough to make the discovery of one within Mercury (if confirmed) a major advance in the exploration of the Solar System.

Fabs would inhibit the reassociation of subparticles, only one protein (L19) was inaccessible to antibodies in the 70S particle itself. H. Roth (Max-Planck-Institut, Berlin) has isolated more than twenty ribonucleoprotein fragments from LiCl cores of the 50S subparticle by digestion with nuclease. The fragments, separated on sucrose gradients, contained from two to twenty-eight proteins. With a few notable exceptions, the data from these various sources were in good agreement, and two topographical models of the 30S proteins were presented by R. Traut and A. Bollen (Université de Bruxelles).

On the RNA front, C. Ehresmann (Institut de Biologie Moléculaire, Strasbourg) reported the latest improvements on the primary sequence of 16S RNA, and C. Branlant (Institut de Biologie Moléculaire, Strasbourg) described some binding sites of 50S proteins on the 23S RNA, in particular the complex formed between 5S RNA (with L5, L18 and L25) and 23S RNA in the presence of L2. As far as primary structure of individual proteins is concerned, it seems that L7 and L12 have been strongly conserved during evolution; two acidic proteins from rat liver were shown to be related immunologically to L7/L12 (G. Stöffler and I. Wool, Max-Planck-Institut, Berlin), and the corresponding proteins from the halophile *Halobacterium cutirubrum* showed large homologous sequences (A. Matheson, National Research Council of Canada, Ottawa). S5 has again turned up as an altered protein in several ribosomal mutants, notably in revertants from valyl-tRNA synthetase mutants described by H. Wittman (Max-Planck-Institut, Berlin). K. Isono (Max-Planck-Institut, Berlin) also found alterations in the *Bacillus stearothermophilus* protein corresponding to S5 (as well as S12) in streptomycin-resistant mutants.

On the functional side, S12 was shown by A. Spirin (Academy of Sciences of the USSR, Poustchino) to exert a negative control over non-enzymatic translocation, since particles reconstituted without S12 showed increased activity in this assay. With the 50S particle, K. Nierhaus (Max-Planck-Institut, Berlin) found that L11 and L16 are the proteins responsible for chloramphenicol binding. L6 is also important for the chloramphenicol effect, but does not actually bind the antibiotic. L11 binds thiostrepton (J. Gordon and J. Highland, Friedrich Miescher Institut, Basel), and is also involved together with L18 at the peptidyl transferase centre, as was shown by C. Cantor with a peptidyl tRNA photo-affinity label. E. Küchler (University of Vienna) also used a photo-affinity label to demonstrate that peptidyl tRNA can be

bound to 23S RNA near to the binding site of the 5S RNA-protein complex. Evidence for conformational changes during protein synthesis came from D. Vasquez (Instituto de Biología Celular, Madrid), who found that the antibiotic anisomycin binds to eukaryotic ribosomes with different affinities according to the functional state of the particles.

Looking at the more intimate details of tRNA binding, M. Sprinzl (Max-Planck-Institut, Göttingen) used suitably modified tRNA analogues to demonstrate that tRNA synthetases attach the amino acids to the 2'-hydroxyl position of the terminal ribose. Elongation factor T_u also recognises only a 2'-acylated tRNA. Peptidyl transfer can be effected to either a 2'- or 3'-acylated tRNA at the A site, but only from a 3'-acylated moiety at the P site. V. Erdmann (Max-Planck-Institut, Berlin) showed that the oligonucleotide T ψ CG can be used instead of a complete de-acylated tRNA to stimulate magic spot synthesis.

Last but by no means least, M. Nomura (University of Wisconsin, Madison) reported some elegant experiments with λ phages containing various amounts of the genetic material in the *aroEstrA* region of the *E. coli* genome. Using a DNA-dependent protein-synthesising system, he found that at least ten 30S and fourteen 50S proteins are encoded within this segment of DNA. The technique offers the possibility of detailed mapping of the ribosomal protein genes.

How microorganisms damage plants

from a Correspondent

In plant, as in animal pathology much research effort has been directed towards investigating the mechanisms by which pathogens damage their hosts. One of the most important advances of recent years has been the realisation that in a small, although by no means unimportant group of plant pathogens, the ability to produce toxin is the major factor determining virulence towards specific hosts. The most convincing experiment to demonstrate this relationship was performed by Scheffer and his associates (*Phytopathology*, **57**, 1288; 1967). They crossed two closely related toxin-producing fungi *Cochliobolus victoriae* and *C. carbonum*, pathogens, respectively of oats and maize. F₁ offspring segregated in a 1:1:1:1 ratio for the abilities to produce *C. victoriae* toxin, *C. carbonum* toxin, both toxins or no toxin. These progeny were, respectively pathogenic on oats, maize, both oats and maize or neither host.

Attempts to elucidate the mechanism

of action of these host-specific toxins have been stimulated by the knowledge that these toxins comprise some of the most potent biologically active substances known. Root growth of susceptible oat seedlings treated with *C. victoriae* toxin is prevented by as little as 0.0002 μ g toxin ml⁻¹, a potency approaching that of the more active clostridial toxins. In susceptible plants almost any metabolic system that one chooses to examine is deranged by toxin treatment. Many of these changes are undoubtedly secondary effects and evidence is accumulating that the primary site of action of at least some toxins resides in the plasmalemma. Supporting evidence for this hypothesis comes from the findings that toxin treatment of susceptible host cells leads to a rapid leakage of intracellular contents (Scheffer and Samaddar, *Rec. Adv. Phytochem.*, **3**, 123; 1970; Keck and Hodges, *Phytopathology*, **63**, 226; 1973), to a rapid depolarisation of the plasmamembrane potential (Novacky and Hanchey, *Physiol. Pl. Path.*, **4**, 161; 1974) and also results in an inhibition of solute uptake and failure of the protoplasts to plasmolyse in hypertonic solutions.

Even more convincing evidence has come from the studies of Strobel and his associates (*J. biol. Chem.*, **248**, 1321; 1973; *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1693; 1973; *ibid.*, **71**, 1413; 1974) on the eyespot disease of sugar cane caused by *Helminthosporium sacchari*. The toxin produced by this fungus seems to be a primary determinant of disease and has been characterised as 2-hydroxy-cyclopropyl- α -D-galactopyranoside. Strobel has found that sensitivity to the toxin is correlated with the presence of a specific toxin-binding receptor protein in the plasmalemma of susceptible cells. Those cells resistant to the toxin possess an immunologically identical protein which does not bind the toxin. The specific proteins from resistant and susceptible cells have similar molecular weights, both consist of four subunits but differ slightly in electrophoretic mobility and with respect to four different amino acid residues.

This is the first positive identification of a specific chemical factor associated with disease susceptibility in a higher plant, an association long suspected on theoretical and genetical grounds. Although these reports establish the presence of a toxin-binding protein within the plasmalemma, much remains to be discovered as to the mechanism by which the toxin-protein interaction destroys the normal functioning of the membrane. These reports also illustrate another basic similarity between the functioning of animal and plant cells and suggest that the study of plant cell surfaces should prove useful.

The ageing, growth and death of cells

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The ageing and death of cells in higher plants and higher animals is discussed in relation to cellular rejuvenation by growth and division.

No cell is immortal. If a cell grows and divides, it becomes two cells; if it does not divide, sooner or later it dies. Multicellular organisms are not aggregates of cells in a state of exponential growth and division. Some cells divide, but most differentiate and do not undergo further division. Here I shall discuss the ageing and death of cells in vascular plants and vertebrate animals in an attempt to explore the significance of these processes in relation to growth and development, both normal and abnormal.

It is often convenient to think of cells or living organisms as maintaining a more or less steady state; but it is also easy to forget that this is an approximation, an abstraction which, if realised, would confer on cells and on organisms the doubtful blessings of eternal life and eternal youth. The realities of growth, development, ageing and death cannot be understood in terms of steady state concepts. They are directional and irreversible changes in time.

Some cells die as they differentiate, for example xylem cells in plants and keratinised epidermal cells in animals; some, such as phloem sieve tubes in plants and red blood cells in mammals, lose their nuclei; others which retain their nuclei may lose their ability to divide—as do most nerve cells. But even cells which retain their ability to divide will die if they do not do so; they senesce.

Any general hypothesis of cellular ageing must not only explain cellular ageing itself, but also the way in which some cell lines do not senesce and die out. The germ cell line is continuous from generation to generation (also many plants can be propagated vegetatively indefinitely) and some cell lines derived from plant or animal tissues can be propagated *in vitro* for an indefinite number of generations.

General hypotheses of ageing based on genetic mutation face two difficulties. First, in explaining the universality of the processes of ageing in non dividing cells in terms of a lethal accumulation of harmful mutations and, second, in accounting for the facts of sexual and vegetative reproduction, which show that mutations do not accumulate to a lethal extent in all cells. The alternative to a genetic-mutation hypothesis of ageing is some sort of 'cytoplasmic' hypothesis, the most recent and best known of which is Orgel's 'error catastrophe' hypothesis, which postulates that an accumulation of errors in protein synthesis leads to a positive feedback of error as the enzymes involved in protein synthesis themselves develop errors and thus produce more defective proteins¹. For this hypothesis to account for the continuity of the germ line and the indefinite propagation of 'permanent cell lines' *in vitro* it is necessary to postulate a process of 'cellular selection' whereby error-containing cells are selected out².

Neither the genetic-mutation hypothesis³ nor the protein synthesis 'error catastrophe' hypothesis² of ageing are supported by sufficient evidence to rule out the possibility that cellular ageing may be explicable in terms of the accumu-

lation of cytoplasmic breakdown products, some of which might be deleterious to the cell if they accumulated sufficiently. In all actively metabolising cells, there is a turnover of cytoplasmic constituents such as proteins and membrane lipids. More is known about their synthesis than about their breakdown *in vivo*; while some of them may be broken down completely, others may be broken down only partially or not at all. They must therefore accumulate.

Lipid peroxidation

One example of such an accumulation is provided by the 'age pigment' or lipofuscin granules which accumulate in an age-dependent way in the cells of many mammalian tissues⁴. The lipofuscin material contains lipid and protein and may be formed in autophagosomal vesicles, for example during the digestion of mitochondria; haem groups, released as the cytochromes are degraded, may catalyse the peroxidation of unsaturated lipids in the degenerating mitochondrial membranes⁵ which may cross link with each other and with denatured proteins⁶. The cells seem to be unable to destroy these cross-linked polymers. Lipofuscin granules do not seem to damage cells directly except when they accumulate, as they do in certain diseases, to such an extent that they mechanically interfere with the structure and functions of the cell⁷.

Lipid peroxidation does not always result in the formation of microscopically visible lipofuscin granules, nor is it confined to autophagosomal vesicles; it occurs in all functional cell membranes, including the surface membrane. Once the peroxidation of unsaturated lipids is initiated, by haem groups, Fe²⁺ ions and other simple catalysts in the presence of oxygen, it takes place by a free radical chain reaction. It can be inhibited by lipid-soluble antioxidants such as vitamin E and accelerated by vitamin E deficiency, ionising radiation, chloroform and ethanol poisoning and hyperbaric treatments^{8,9}, which can cause irreversible damage to cells.

The peroxidation of lipids within cell membranes is occurring *in vivo* all the time. Some peroxidised lipids may be metabolised⁹ but others, perhaps those which are cross linked to other lipids and lipoproteins may not be. The chain reaction of lipid peroxidation may be terminated by the oxidation of other substances which may themselves be damaged and accumulate¹⁰. Such substances formed within the surface membrane, for example, may accumulate *in situ*; if they are removed from the surface membrane as the membrane is recycled by the invagination of membrane vesicles¹¹⁻¹⁴ or by other means, some of them might find their way into residual bodies, but they might also be incorporated into intracellular membranes. The formation and accumulation of such substances within the outer and intracellular membranes, for example in the Golgi apparatus, endoplasmic reticulum nuclear membrane and lysosomal membranes, could well be deleterious to normal membrane functioning and could also lead to a positive feedback of damage by further lipid peroxidation, and thus to the senescence and death of the cell. The rate of ageing would be temperature dependent and would also depend on the composition, structure and functions of the cellular membranes, the extra- and intracellular environments, antioxidant levels and so on. Thus, different types of cells would

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age at different rates but, according to this hypothesis, all cells would be ageing to a greater or lesser extent all the time; all cells would be heading towards senescence and death.

The elimination of membranous material from cells might enable the ageing process to be retarded and there are a few examples of the shedding of membranes by cells which I will discuss further. But, in general, the only way in which cells could avoid their otherwise inevitable mortality would be by growing and dividing, thus diluting the accumulated breakdown products. Although lipid peroxidation may be the most important cause of the formation of such substances, the following general considerations could apply to any deleterious substances which accumulate with age.

Growth and division of cells

An artificially simple case is provided by cells dividing symmetrically with a fixed generation time if these accumulate deleterious breakdown products linearly with time, an amount, x , being formed per cell generation time (Fig. 1). Successive generations contain more of the accumulated breakdown products but the increments become smaller and smaller. If the rate of accumulation is not linear, but proportional to the amount already accumulated, the content per cell will increase exponentially; and if there is a progressive lengthening of the cell generation time, there will be a greater accumulation within individual cells in succeeding generations. With either or both of these assumptions, it can be seen that the whole population will undergo senescence and sooner or later die out.

But another type of cell division is possible, an asymmetrical division in which one of the daughter cells receives all or most of the accumulated breakdown products (becoming more 'mortal') while the other is rejuvenated, receiving little or none. The more 'mortal' of the daughter cells might die or differentiate directly, or it might divide again unequally, producing a rejuvenated cell and a cell even more 'mortal' than itself, or it might undergo one or more sequential symmetrical divisions (as discussed above) to produce a population of cells which sooner or later die (unless they can undergo further asymmetrical divisions to produce rejuvenated cells).

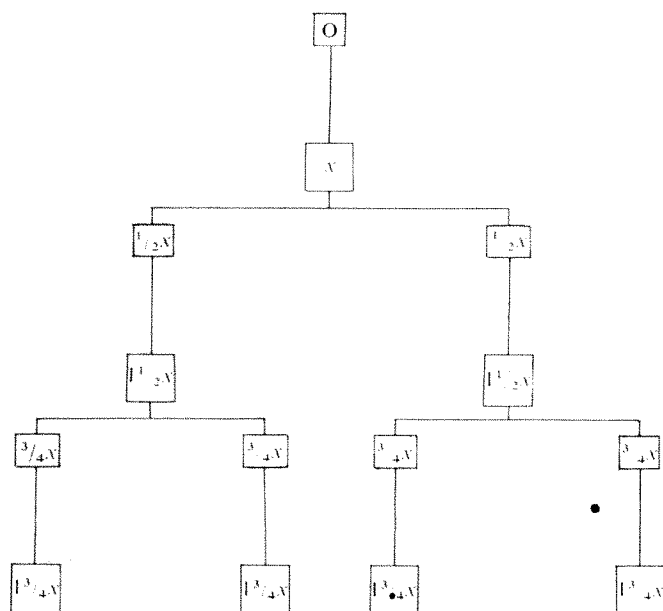


Fig. 1 Cells dividing symmetrically with a fixed generation time showing the accumulation of deleterious breakdown products linearly with time, an amount, x , being formed per cell generation time.

I shall now consider a few aspects of the growth and development of higher plants and higher animals in the light of these ideas. Dicotyledonous trees illustrate the pattern of indefinite growth that is characteristic of plants. (There are of course plants, such as herbaceous annuals, which die after they have flowered. But annuals are capable of growing for much longer than their normal life-span if they are prevented from flowering, indicating that they die because they flower and not because of an innate inability to go on growing¹⁵.) The life span of trees is limited by a variety of mechanical factors, but cuttings taken from old trees can give rise to healthy young trees, and this process can be repeated indefinitely. The growing points of the tree, the apical meristems, remain perpetually young.

Cell divisions within the apical meristems of the shoots give rise to daughter cells with different fates: some remain meristematic, others give rise to the differentiated structures of the stems and the leaves. Some of these cells die as they differentiate into vascular tissues and fibres, others, for example the leaf mesophyll and pith parenchyma, remain alive for some time, but, unless they are stimulated to divide again in a regenerative response to wounding or damage, they eventually die. The leaves senesce and fall from the tree; the pith breaks down. The root meristems give rise to the primary tissues of the root which, apart from those which divide to produce further root meristems, sooner or later die. In secondarily thickening stems the divisions of the cambial cells give rise to cells which die as they differentiate into xylem or undergo further asymmetrical divisions to produce phloem companion cells and sieve tubes. These cells eventually die and are sloughed off in the bark. Cell divisions in the cork cambium give rise to cork cells which die as they differentiate; divisions of the root cap initials give rise to root cap cells which die and are sloughed off. Thus, in the various meristems of the plant the continued growth and continued rejuvenation of the meristems is associated with the production of cells which die during or after differentiation.

Vertebrates

Vertebrates, unlike trees, do not go on growing indefinitely, nor can they be propagated vegetatively. At first, fertilised eggs undergo cleavages which rapidly increase the number of cells, but this rate of increase of cell number declines progressively as the animal develops, and as cells and tissues differentiate¹⁶. Throughout the development of the embryo many tissues and groups of cells regress and die^{17,18}. Some of these cell deaths are associated with tissue differentiation¹⁹, some occur during morphogenetic processes²⁰, and others may represent the regression of phylogenetically vestigial structures¹⁷, but the significance of other cell deaths is obscure¹⁷. As the animal develops, the cells of some tissues, such as nerve and muscle, differentiate and to a large extent lose the ability to undergo further division. Some of these cells die as the animal grows older and are not replaced^{21,22} but in the adult animal a number of other tissues continue to grow, for example the epidermis, the intestinal lining, the liver and blood cells continue to be formed. In all these examples the production of new cells is offset by cell death. Cell divisions in the basal layers of the mammalian epidermis give rise to daughter cells which remain in the basal layers and divide again, and other daughter cells which differentiate and keratinise, dying as they do so. Cell divisions in the crypts of the intestinal villi replenish the population of crypt cells capable of further division and produce other daughter cells which move up the villi where they die and are sloughed off²³. Asymmetrical divisions of the early precursors of all cells of the blood occur throughout life and give rise to further precursor cells as well as to the maturing and mature cells of the blood, all of which have a limited life span. During

the formation of red blood cells²¹ and granulocytes²⁵ in the bone marrow, and lymphocytes in the thymus²⁶, considerable numbers of cells die *in situ* soon after they are formed. The reasons for this 'ineffective' erythropoiesis, granulopoiesis and lymphopoiesis are unknown.

The mortality of at least some of the cells which die in developing animal embryos and in mature animals may represent the price that is paid for the rejuvenation of other cells which continue to grow and divide. But unfortunately too little is known about cell lineages in animals, especially in embryos, for it to be possible to decide how general is the phenomenon of asymmetrical cell divisions giving rise to daughter cells of unequal mortality. The recognition of this pattern is complicated by the fact that by no means all cell death takes place as a result of cellular senescence. Some cells die as they differentiate and others may die because they find themselves in the wrong places at the wrong times¹⁹. Cell deaths may be controlled chemically, for example by steroid hormones: the injection of glucocorticoids can cause large numbers of lymphocytes to die²⁷, the regression of Mullerian and Wolffian ducts is controlled by androgens and oestrogens^{19,28} and the regression of the lining of the female genital tract is under the control of oestrogens²⁸. But, under the hypothesis that asymmetrical cell divisions lead to a rejuvenation of 'meristematic' daughter cells at the price of the increased mortality of their sister cells, it does not matter whether the latter die as a result of senescence, or whether they die as they differentiate or for any other reason.

Sexual reproduction

In the sexual reproduction of both higher plants and higher animals almost all the cytoplasm from which the embryo and the new organism develops is provided by the egg. In both cases, the egg cells are formed as a result of asymmetrical divisions of the egg mother cell. In the great majority of higher plants, the meiotic divisions of the egg mother cell produce four cells, three of which die. The fourth undergoes further divisions to produce the cells of the embryo sac, most of which die before or shortly after fertilisation. In some species, one or more of the three sister cells of the cell which gives rise to the egg may undergo further division to produce short-lived embryo sac cells²⁹. In animals the first and second meiotic divisions of the egg mother cell give rise to the first and second polar bodies, which regress and die^{30,31}.

It is particularly striking that in both plants and animals, only one of the progeny of the egg mother cell gives rise to an egg while the sister cells die (or if they divide give rise to short-lived progeny). By contrast, there is no comparable cell loss in male gametogenesis associated with the meiotic divisions of the pollen mother cells and spermatogonia.

The many examples in both higher plants and higher animals (and many more can be found in the lower plants and lower animals) of the production of rejuvenated meristematic, stem or egg cells by asymmetrical divisions do not of course prove that these divisions involve an asymmetrical distribution of deleterious breakdown products; but the available facts appear to be consistent with this hypothesis.

Loss of membranous material by animal cells

If the accumulation of deleterious breakdown products of membrane lipids is one of the causes of cellular senescence, the loss of membranous material might be of considerable importance in enabling cells to rid themselves of such substances. The shedding of membranous material by living cells does not seem to be of common occurrence but can take place in mammalian cells as follows.

First, in apocrine secretions part of the cell membrane is

lost. The best example, and the only one for which conclusive ultrastructural evidence exists, is in the secretion of lipid droplets by the cells of lactating mammary glands. The secreted lipid droplets are surrounded by a unit membrane derived in part from the surface membrane and in part from Golgi vesicle membranes³².

Second, membrane-bounded vesicles of cytoplasm can break away from mammalian macrophages both *in vitro* and *in vivo*³³. This process, known as clasmotosis, is of unknown significance. Lymphocytes which are activated in immunological reactions or as a result of phytohaemagglutinin stimulation form 'tails' (uropods) which can bleb off vesiculated buds *in vivo* and *in vitro*³⁴. Again, the significance of this process is unknown. Clasmotosis is also frequently observed in cultures of fibroblasts.

Third, many types of animal viruses are budded off from host cells in membrane-bounded vesicles. The protein in the membrane of the vesicles is largely viral, at least in the case of RNA tumour viruses, but the lipids are derived from the host cell membrane³⁵. Viral particles bounded by membrane are also budded off from the cells of a number of spontaneously cancerous tissues³⁶ and from many of the cell strains and permanent cell lines which are commonly cultured in laboratories³⁷.

Tissue cultures

Many plant callus cultures can be grown indefinitely *in vitro*. During the early stages of the growth of some calluses, an exponential increase in cell number takes place at a rate which suggests that many of the cells may undergo a limited number of sequential symmetrical divisions before the growth rate declines^{38,39} but in most plant tissue cultures the rate of increase of cell number is more or less linear for most of the growth period^{39,40}. Linear growth characteristics would be compatible with a meristematic pattern of cell division such that some daughter cells continue to grow and divide while their sister cells age and sooner or later die. Unfortunately nothing is known in detail about cell lineages within these cultures, nor are there any quantitative data on cell death. Nevertheless, dead and dying cells are by no means uncommon.

'Permanent' mammalian cell lines capable of indefinite propagation *in vitro* can be derived from cancerous tissues and also from cells which have undergone a spontaneous 'transformation' during culture. Diploid fibroblast cultures can be propagated, however, only for a finite number of subculturings, more (up to about 60) if the cells are derived from embryonic tissues, fewer if they are derived from mature organisms⁴¹. The number of generations through which the cells can be passed before the population senesces and dies out is reduced if the period of time between the subculturings is increased⁴². Fibroblasts of the mouse L strain have been observed to divide symmetrically over six to seven cell generations with a more or less constant generation time⁴³; if the cells in the diploid fibroblast cultures also divide symmetrically, deleterious breakdown products might accumulate in the cells of succeeding generations, as discussed above, and account for the senescence of these cultures. It is impossible, however, to make any detailed interpretation of the senescence of these cultures in the absence of quantitative information about the proportions of dividing and nondividing cells, the incidence of cell death, and the extent and significance of clasmotosis within these cultures—or indeed with cultures of 'transformed' and 'permanent' cell lines.

Cancer

Malignancy must not only involve the freeing of cells from the normal controls on their proliferation, but also the avoidance of senescence by at least a part of the cell population. Many animal tumours contain a stem cell or

'meristematic' population which gives rise to daughter cells which may or may not differentiate, but which sooner or later die⁴⁴. There are numerous examples of cell death within cancerous tissues⁴⁵⁻⁴⁸. Some of the cell deaths can be explained in terms of an inadequate vascularisation of the tumour tissue, but in most tumours this is by no means the only cause and does not apply at all to leukaemias; many of the cells may die as a result of ageing⁴⁴.

Little attention has been paid to the incidence of cell death within cultures of cancerous cells and it is therefore at present impossible to know to what extent the patterns of cell division, ageing and death within these cultures resemble those within *in vivo* cancers. It is sometimes assumed, if only implicitly, that overall exponential growth characteristics of cell cultures mean that there is a homogeneous population of symmetrically dividing cells. This assumption is not justified: a heterogeneous population containing proliferating, nonproliferating and dying cells can also grow exponentially if the proportion of cells that die is constant with time^{49,50}.

It is conceivable that the loss of membranous material either spontaneously, as in certain types of mammary gland tumours⁵¹, or as a result of the budding off of viruses (such as RNA tumour viruses) could play a significant role in the retardation of cellular senescence in certain types of cancer.

Effects of cell death

Very little is known about the biochemistry of dying cells. Such cells probably release all sorts of proteins, glycoproteins, peptides, amino acids, amino acid breakdown products, nucleic acids and nucleic acid breakdown products, lipids and lipid breakdown products as well as salts and other substances which were sequestered inside the cells.

It has recently been found that in higher plants the hormone auxin (indole-3-acetic acid) is formed as a consequence of cell death as tryptophan, released by proteolysis, is broken down. Dying cells in differentiating vascular tissue, regressing nutritive tissues and so on, are probably the major source of this hormone within the plant⁵². Other plant hormones may also be produced by damaged and dying cells: ethylene from the breakdown of methionine and cytokinins by the hydrolysis of transfer RNA⁵². In higher plants the normal production of hormones as a consequence of cell death and the production of 'wound hormones' by damaged cells can be seen as two aspects of the same phenomenon⁵².

Wound and regenerative responses in vertebrates cannot be explained simply in terms of wound hormones, but there is evidence that dying cells release substances that stimulate phagocytosis⁵³, and affect growth and development in both normal^{54,55} and cancerous tissues⁵⁶. And at least some of the cell deaths which occur during normal embryonic development may well result in the production or release of substances involved in the control of differentiation and development.

Dying cells may not only have a chemical effect on neighbouring cells but also a physical effect as cell to cell contacts are broken. Cell deaths within a tissue may also affect the functioning of the tissue as a whole: for example, the death of nerve cells within the brain²² seems likely to affect pathways or patterns of nervous conduction, perhaps leading to the formation of new pathways or patterns. Such cell deaths could act as a source of random change within the nervous system that might not always be deleterious⁵⁷.

So little attention has been paid to the ageing and death of cells during growth and development, both normal and abnormal, that detailed information about these processes is scarce. Where facts are few, speculation can flourish. Most of the speculations advanced in this article could be

opposed by alternative speculations, but they illustrate the view that growth and development cannot be understood in isolation from ageing and death. This is by no means an original concept, but at the cellular level it provides a perspective in which many familiar facts take on a new significance and suggests a new approach to familiar problems.

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- ¹ Orgel, L. E., *Proc. natn. Acad. Sci. U.S.A.*, **49**, 517-521 (1963).
- ² Orgel, L. E., *Nature*, **243**, 441-445 (1973).
- ³ Smith, J. M., *Proc. R. Soc. B.*, **157**, 115-127 (1963).
- ⁴ Strehler, B. L., *Adv. gerontol. Res.*, **1**, 343-384 (1964).
- ⁵ Goldfisher, S., Villaverde, H., and Forschirm, R., *J. Histochem. Cytochem.*, **14**, 641-652 (1966).
- ⁶ Bjorkend, S., *Adv. gerontol. Res.*, **1**, 257-288 (1964).
- ⁷ Zeman, W., *Adv. gerontol. Res.*, **3**, 147-170 (1971).
- ⁸ Barber, A. A., and Bernheim, F., *Adv. gerontol. Res.*, **2**, 355-403 (1967).
- ⁹ Slater, T. F., *Free Radical Mechanisms in Tissue Injury* (Pion Ltd, London, 1972).
- ¹⁰ Dormandy, T. L., *Lancet*, **ii**, 684-688 (1969).
- ¹¹ Abraham, S. J., and Holtzman, E., *J. Cell Biol.*, **56**, 540-558 (1973).
- ¹² Geuze, J. G., and Pont, C., *J. Cell Biol.*, **57**, 159-174 (1973).
- ¹³ Heuser, J. E., and Reese, T. S., *J. Cell Biol.*, **57**, 315-344 (1973).
- ¹⁴ Orci, L., Malisse-Lagae, F., Ravazzola, M., and Amherdt, M., *Science*, **181**, 561-562 (1973).
- ¹⁵ Priestley, J. H., and Scott, L. I., *An Introduction to Botany* (Longmans, Green and Co., London, 1938).
- ¹⁶ Minot, C. S., *The Problem of Age, Growth and Death* (John Murray, London, 1908).
- ¹⁷ Glücksmann, A., *Biol. Rev.*, **26**, 59-86 (1951).
- ¹⁸ Menkes, B., Sandor, S., and Ilies, A., *Adv. Teratol.*, **4**, 169-215 (1970).
- ¹⁹ Glücksmann, A., *Archs Biol.*, **76**, 419-437 (1965).
- ²⁰ Saunders, J. W., *Science*, **154**, 604-612 (1966).
- ²¹ Broun, W. F., *J. Neurol. Neurosurg. Psychiat.*, **35**, 845-852 (1972).
- ²² Brody, H., *J. comp. Neurol.*, **102**, 511-556 (1955).
- ²³ Quastler, H., and Sherman, F. G., *Expl Cell Res.*, **17**, 420-438 (1959).
- ²⁴ Harris, J. W., and Kellermeyer, R. W., *The Red Cell* (Harvard University Press, Cambridge, Mass., 1970).
- ²⁵ Maloney, M. A., Patt, H. M., and Lund, J. E., *Cell Tissue Kinet.*, **4**, 201-209 (1971).
- ²⁶ Metcalf, D., in *The Thymus* (edit. by Wolstenholme, G. E. W., and Porter, R.), 242-263 (Churchill, London, 1966).
- ²⁷ Pushin, R. W., and Harven, E., *J. Cell Biol.*, **50**, 583-597 (1971).
- ²⁸ Biggers, J. D., in *Cellular Injury* (edit. by de Reuck, A. V. S., and Knight, J.), 329-349 (Churchill, London, 1964).
- ²⁹ Maheshwari, P., *An Introduction to the Embryology of the Angiosperms* (McGraw Hill, New York, 1950).
- ³⁰ Odor, D. L., *Am. J. Anat.*, **97**, 461-492 (1955).
- ³¹ Odor, D. L., and Renninger, D. F., *Anat. Rec.*, **137**, 13-23 (1960).
- ³² Wooding, F. B. P., *J. Cell. Sci.*, **9**, 805-821 (1971).
- ³³ Jacoby, F., in *Cells and Tissues in Culture*, **II** (edit. by Willmer, E. N.), 1-93 (Academic Press, London, 1965).
- ³⁴ Biberfeld, P., *Expl Cell Res.*, **66**, 433-445 (1971).
- ³⁵ Blough, H. A., and Tiffany, J. M., *Adv. Lipid Res.*, **11**, 267-239 (1973).
- ³⁶ Gross, L., *Oncogenic Viruses* 2nd ed. (Pergamon Press, Oxford, 1970).
- ³⁷ Lieber, M. M., Benveniste, R. E., Livingston, D. M., and Todaro, G. J., *Science*, **182**, 56-59 (1973).
- ³⁸ Sunderland, N., *Ann. Bot.*, **31**, 573-591 (1967).
- ³⁹ Street, H. E., King, P. J., and Mansfield, K. J., in *Les Cultures de Tissus des Plantes* (Colloques Internationaux du Centre National de la Recherche Scientifique No. 193), 17-40 (CNRS, Paris, 1971).
- ⁴⁰ Short, K. C., and Torrey, J. G., *J. exp. Bot.*, **23**, 1099-1105 (1972).

- ⁴¹ Hayflick, L., *Expl Cell Res.*, **37**, 614-636 (1965).
⁴² McHale, J. S., Mouton, M. L., and McHale, J. T., *Expl. Geront.*, **6**, 89-93 (1971).
⁴³ Miyamoto, H., Zeuthen, E., and Rasmussen, L., *J. Cell Sci.*, **13**, 879-888 (1973).
⁴⁴ Bullough, W. S., and Deol, J. U. R., *Symp. Soc. exp. Biol.*, **25**, 255-275 (1971).
⁴⁵ Glücksmann, A., in *Recent Advances in Clinical Pathology* (edit. by Dyke, S. C.), 338-349 (Churchill, London, 1947).
⁴⁶ Cooper, E. H., *Cell Tissue Kinet.*, **6**, 87-95 (1973).
⁴⁷ Kerr, J. F. R., and Searle, J., *J. Path.*, **107**, 41-44 (1972).
⁴⁸ Kerr, J. F. R., Wyllie, A. H., and Currie, A. R., *Br. J. Cancer*, **26**, 239-257 (1972).
⁴⁹ Steel, G. G., *Cell Tissue Kinet.*, **1**, 193-207 (1968).
⁵⁰ Gavosto, F., and Pileri, A., in *The Cell Cycle and Cancer* (edit. by Baserga, R.) (Dekker, New York, 1971).
⁵¹ Tarin, D., *Br. J. Cancer*, **23**, 417-425 (1969).
⁵² Sheldrake, A. R., *Biol. Rev.*, **48**, 508-559 (1973).
⁵³ Bessis, M., in *Cellular Injury* (edit. by de Reuck, A. V. S., and Knight, J.), 287-316 (Churchill, London, 1964).
⁵⁴ Majno, G., in *Cellular Injury* (edit. by de Reuck, A. V. S., and Knight, J.), 87-98 (Churchill, London, 1964).
⁵⁵ Teir, H., Lahtiharju, A., Alho, A., and Forsell, K.-J., in *Control of Cellular Growth in Adult Organisms* (edit. by Tier, H., and Rytömaa, T.), 67-82 (Academic Press, London, 1967).
⁵⁶ Vasiliev, J. M., and Guelstein, V. I., *Prog. exp. Tumor Res.*, **8**, 26-65 (1966).
⁵⁷ Sheldrake, A. R., *Theoria to Theory*, **7**, (3) 31-38 (1973).

Recent eruption of Mount Etna

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Mount Etna erupted for the first time in 31 months on January 30, 1974. The eruption, which continued until February 16, was watched closely between January 30 and February 15 by Italian and British members of the Etna Research Programme. This article summarises their observations.

On January 30, 1974 Mount Etna erupted for the first time since the middle of 1971 (ref. 1). Between these eruptions, activity on the volcano had been restricted to strombolian explosions and the extrusion of lava at the bottom of two deep pits within the summit crater. The centre of the new eruption lay 5.75 km to the west of the summit crater, at an altitude of about 1,680 m (grid ref. 938776 on the Monte Minardo 1:25,000 map sheet).

The eruption was first noticed at about dusk on January 30, but it probably started during the afternoon. The first observed activity was the occurrence of strong, almost continuous (30-50 a minute), strombolian explosions which threw bombs to a maximum of 700 m, and rapidly built up a cone. The first erupted lavas (Flow 1) were observed on January 31, as 2-3 m thick aa flows (Fig. 1). At this time the flow front was at the foot of Mt Nuovo (which erupted in 1763). During the next four days, two more lava streams (Flows 2 and 3) were emitted, forming parallel flows which extended between the new cone and that of Mt Nuovo. After three (?) days the explosive activity had decreased in rate to about 25 explosions a minute.

On February 2 (?), two short, thick, viscous flows (Flows 4) erupted from boccas on the eastern side of the cone. They were more than 20 m thick and short lived, and later became largely covered by ash from the cone. Another viscous lava, observed on February 6, probably started erupting on the previous day from a bocca on the northern side of the cone. This flow (Flow 5), an aa flow with a maximum thickness of about 10 m at its front, was advancing on a 70-80 metre front, at a maximum flow rate of 1 m h⁻¹. This rate was, however, evidently reduced, implying that earlier rates must have been much higher to give rise to this flow. The flow stopped on February 7, by which time there was no flowing lava, although the explosion

rate in the crater continued at about 14-16 explosions a minute, with maximum projectile heights of about 500 m. On February 8, strong blizzards prevented any observation but by February 9 the violent strombolian activity had changed to occasional explosions which ejected bombs to about 100 m above the crater lip, and there were rumblings within the crater. We considered the magma level in the cone to be very low at this time. On the same day a new aa flow about 800 m long (Flow 7) was discovered emanating from a bocca at the foot of the cone on the south-eastern side of the volcano. The bocca was in a hollow 120 m across with a flat floor containing a small lake of

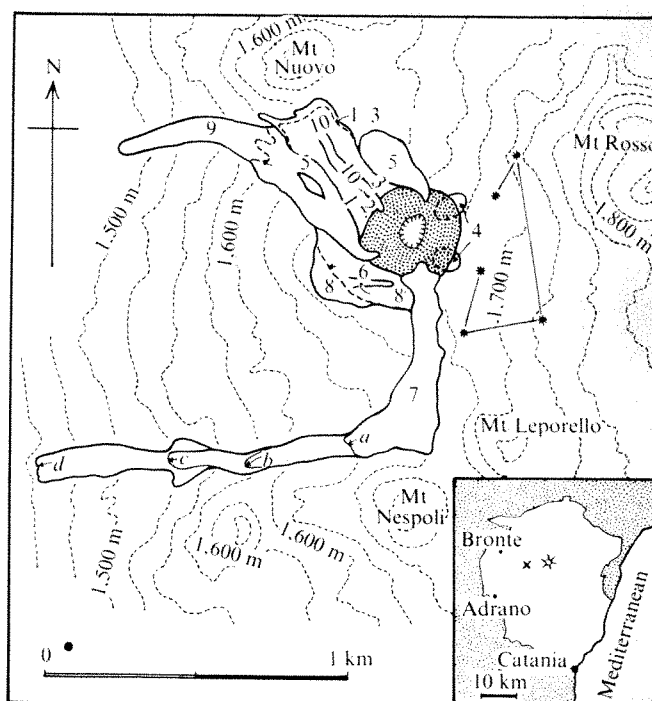


Fig. 1 The area around the eruption site of Mt Etna, Sicily, showing the course of the eruption of January 30 to February 16, 1974. 1-10, Flow numbers (see text); a, the extent of Flow 7 on February 9; b, February 10; c, February 11; d, February 15; *, e.d.m. station. Inset: x, January 1974 eruption, *, summit of Mt Etna.

viscous lava. Lava from this lake was pouring down a short 40° slope towards Mt Leporello, where it turned westwards. The active flow front was about 80 m across and was moving slowly, except on a slope of about 9° down a dirt track, where a flow 3 m across and 1.5 m high advanced at a rate of about 33 cm min⁻¹. The rate of eruption at the bocca was variable and apparently stopped late on February 9, only to restart a few hours later, early the following morning. An examination on February 9 had shown that before this flow, an earlier flow (Flow 6) from the same bocca had flowed around the side of the cone and towards Mt Nuovo, probably on February 8. This flow was about 2–5 m thick.

On February 10, the explosive activity was still discontinuous, with occasional periods of stronger activity (12 explosions every minute) throwing bombs about 150 m above the lip of the cone. The extrusion of lava was also irregular and late in the day the flow front was hardly moving, although several new lobes had developed along the southern side. By the morning of February 11, the lava had moved another 250 m and there were only occasional explosions in the crater. Most of the ejected material was derived from collapses of the crater walls. A gas jet formed at the bocca of Flow 7 and sublimates were deposited. The flow front was advancing at about 30 cm min⁻¹, but it had slowed down to 1 m h⁻¹ on an 80-m front by February 13. On that day it was discovered that a bocca had opened on the south side of the cone. Lava (Flow 8) from this bocca was flowing around the foot of the cone, towards the west. By 1700h LT, Flow 7 had stopped and Flow 8 was flowing at a rate of 0.3 m min⁻¹. A new bocca (1–2 m across) had opened to the west of the cone, extruding lava (Flow 9) which flowed at a rate of 3 m min⁻¹. Another bocca (Flow 10) to the north-north-west of the cone had also apparently opened but we did not visit this until the next day. Weak explosions continued in the crater, and there were strong rumblings with gas explosions which ejected a little pyroclastic material. Ground shocks could be felt up to 900 m from the cone. By February 14, Flow 8 had stopped, but Flow 9 was still erupting, flowing at about 2.5 m min⁻¹ in a flow 3–5 m wide. On February 14 and 15, the inner walls of the cone suffered major collapses. On the latter day, we observed radial fractures on the southern and western flanks of the cone. The rate of flow in Flow 9 was about 4 m min⁻¹ and Flow 10 was erupting at a rate of 2 m min⁻¹ and was 1.5 m wide. By February 17, the activity had stopped. Twenty-three days later a further period of activity began in the same area, about 200 m to the south-west of the centre of activity which we had been observing.

We computed the amounts of lava which were erupted during the February period of activity: between January 30 and February 7, 1.0×10^6 m³; February 8 and 9, 1.0×10^6 m³; February 10–15, 0.35×10^6 m³. This is a total of 2.35×10^6 m³, giving an average rate of eruption of about 1.5 m³ s⁻¹, although on February 8 and 9 the rate may have been more than 6 m³ s⁻¹ for a short period, thus lowering the level of magma in the cone and reducing the strength of the explosions.

Viscosities inferred from the rate of flow in channels of Flow 6 were about 1.8×10^3 poise 50 m downstream from the bocca, where lava surface temperatures were about 980° C (measured with an optical pyrometer), and about 1.0×10^6 poise near the flow front, where lava surface temperatures of 962° C were measured (using an optical pyrometer and a thermocouple). In the boccas of Flow 6 and later flows, maximum temperatures of 1,060° C were measured with a thermocouple inserted to 20 cm.

Activity at the central crater

We visited the Central Crater at the summit of Etna (3,200 m) on February 12. There are two pits within the summit

Table 1 Analyses of lavas and bombs*

	JG/E1†	JG/E6‡
SiO ₂	47.75	47.74
TiO ₂	1.81	1.79
Al ₂ O ₃	17.25	17.25
Fe ₂ O ₃	5.24	5.16
FeO	5.54	5.62
MnO	0.22	0.21
MgO	5.51	5.49
CaO	10.45	10.46
Na ₂ O	3.17	3.17
K ₂ O	1.65	1.65
P ₂ O ₅	0.34	0.37
CO ₂	0.16	0.16
H ₂ O (total)	0.48	0.39
Total	99.57	99.46

* Analysis by A. C. S. Smith (University College, London). Both analyses are the mean of duplicate analyses using the atomic absorption method.

† Fresh bomb ejected on February 7, and partially quenched in snow.

‡ Quenched lava from the front of Flow 7 (February 11).

crater², but only one, the Bocca Nuova, showed any significant activity—the spasmodic expulsion of clouds of very fine, brown ash, accompanied by muffled explosions, which were estimated to originate from a depth of several hundred metres. Quiet emission of fume was occurring at both the Chasm and the 1964 Crater, but the NE Crater remained inactive.

The vigorous strombolian activity which characterised the Chasm and the Bocca Nuova during parts of 1973 left a deposit of ash and bombs near the Chasm. This activity, which reached its culmination in November, seems to have raised the floor of the Chasm from about 150 m to about 75 m below its lowest lip. Since then, however, there has been a lowering of the Chasm floor. It now falls in a series of steps produced by arcuate fractures, towards a deep, central pit, 50 m across, from which most of the gas emerges.

This seems to indicate that there has been a drop in magma level or pressure on the Chasm–Bocca Nuova conduit system, possibly connected with a movement of magma away from there, and into the western side of the volcano.

Electromagnetic distance measurements (e.d.m.), on the southern side of the volcano, at a height of 2,000 m, indicate a significant amount of deflation of the volcano since July 1973. A quantitative assessment of the role played by the central magma conduit system during the eruption described here must, however, wait till the summit e.d.m. network can be measured later in 1974.

A small e.d.m. network was set up on the eastern side of the eruptive site (Fig. 1) and repeatedly measured from February 10 to February 15. There were no movements recorded above the level of error of the measurements, (± 8 mm, Hewlett-Packard 3800 B).

Eruption type

Compared with known Etnaean eruptions, this eruption had lower rates of effusion, a high viscosity, greater explosivity, possibly lower temperatures and a higher crystallinity of the lavas on extrusion. Ancient eruptive cones with small lava flows nearby suggest that, locally, this type of activity has been common.

Rittmann³ has classified types of eruption on Etna to include: lateral eruptions, which occur because magma migrates from the central conduit below the summit crater to fissures on the lower flanks; and eccentric eruptions, supplied independently of the central magma conduit. The differences between the eruption described here and the clearly lateral eruptions (for example, the 1971 eruption),

considered together with the seismic activity and the differences in petrology between the new lavas and those present in the Central Crater a few months before the eruption, suggest that this new eruption is fed by a conduit that is not related to the central conduit (eccentric eruption). On the other hand, Guest¹ noted a rise in fumarole temperatures on the western side of the summit crater in 1972 (and in 1973), and predicted a westward migration of magma from the central conduit. Furthermore, the summit pits were active up to a few days before the latest eruption, but they became inactive after the new outbreak, except for deep explosions in the Bocca Nuova. This would be expected for a lateral eruption, although it does not necessarily preclude an eccentric eruption. Unfortunately, heavy snow and bad weather conditions prevented distance measuring at the summit.

Further work on the petrology and chemistry (Table 1) of the lavas, together with geodetic studies, will help to determine the origin of the eruption.

Drs R. Romano and L. Villari of the Instituto Internazionale di Vulcanologia, Catania made available their observations and provided help in the field.

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¹ *Phil. Trans. R. Soc.*, **A274**, 1-179 (1973).

² Guest, J. E., *Phil. Trans. R. Soc.*, **A274**, 63-78 (1973).

³ Rittmann, A., *Volcanoes and their activity*, second ed. (Wiley, New York, 1962).

⁴ Guest, J. E., *J. geol. Soc. Lond.*, **129**, 311-315 (1973).

Androgen transport and receptor mechanisms in testis and epididymis

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To influence spermatogenesis and sperm maturation, androgen has to be transported from the Leydig cells to the germinal epithelium and epididymis. This article describes extracellular transport and intracellular receptor mechanisms for androgen.

SPERMATOGENESIS is an androgen dependent process which can be maintained in hypophysectomised rats by the administration of either testosterone¹ or 5 α -dihydrotestosterone². Gonadotrophins may exert their effects on spermatogenesis indirectly by way of androgens. The sole action of luteinising hormone (LH) seems to result from the stimulation of testosterone synthesis by the interstitial Leydig cells. Follicle stimulating hormone (FSH) is necessary for the full maintenance of spermatogenesis, but its effects can be mimicked by androgens and blocked by the 'anti-androgen', cyproterone, indicating that its action is serially linked with androgen¹ or that androgen is the mediator of FSH action.

Androgen binding protein

An androgen binding protein (ABP) with high affinity for 5 α -dihydrotestosterone (DHT) ($K_a = 1.25 \times 10^9 \text{ M}^{-1}$) and testosterone ($K_a = 0.5 \times 10^9 \text{ M}^{-1}$) has been demonstrated in 105,000g supernatants of rat testis and epididymis³⁻¹⁰. ABP is produced in the testis, secreted into testicular fluid and carried to the epididymis by the efferent ducts^{9,10}. The concentration of ABP in efferent duct fluid (EDF) ($4-8 \times 10^{-8} \text{ M}$) is sufficient to bind testosterone and (or) DHT in a concentration of 13-26 ng ml⁻¹, assuming one binding site per molecule ABP (ref. 10). Ligation of efferent ducts

causes ABP to disappear completely from caput epididymidis supernatant within 3 d, suggesting that the testis is the only source of ABP in the epididymis⁹. ABP must be produced within the seminiferous tubules, since it is present in EDF and is absent from testicular lymph; also, the presence of ABP in testis supernatants following complete destruction of the germ cells by X-radiation or chemicals, like nitrofurazon, suggests that ABP is formed by Sertoli cells (Hagenäs *et al.*, unpublished observations). It seems that ABP might have an important function in the transport of testicular androgen to the germinal epithelium as well as to androgen-dependent epithelial cells in the epididymis.

ABP in efferent duct fluid and supernatants of testis and epididymis

Concentrations of ABP were measured in testis, efferent duct fluid (EDF) and segments of the epididymis from adult rats. Testis and epididymis segments were homogenised separately at 4° C in three volumes of 50 mM Tris HCl, pH 7.4, containing 1.5 mM EDTA, 0.5 mM 2-mercaptoethanol, and 10% glycerol. Homogenates were centrifuged at 105,000g for 60 min. Supernatants and EDF were extracted with charcoal (1 mg per mg protein) overnight at 0° C, to remove endogenous steroids and the charcoal removed by centrifugation. Total protein was measured in each sample by the method of Lowry *et al.*¹¹.

Binding capacity of ABP was measured by 'steady state' polyacrylamide gel electrophoresis (SS-PAGE)¹². By this method, 1,2-³H-5 α -dihydrotestosterone (³H-DHT) is dissolved in the acrylamide solution before it polymerises. ³H-DHT is thus distributed uniformly in the polymerised gel and remains stationary in the electrophoretic field until

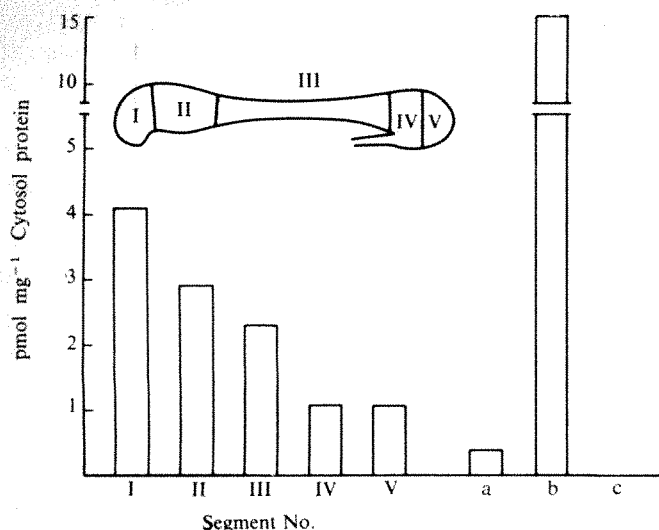


Fig. 1 Concentrations of ABP in 105,000g supernatants prepared from segments of rat epididymis in comparison with rat testis (a), efferent duct fluid (EDF) (b), and serum (c). ABP was measured by steady state polyacrylamide gel electrophoresis (SS-PAGE). Samples were adsorbed overnight with dextran coated charcoal at 0°C (1 mg Norit A mg⁻¹ protein). Charcoal was removed by centrifugation twice at 10,000g in a Beckman microfuge, and samples of 100 µl were layered onto 6.5% acrylamide gels (5 × 70 mm) containing 10% glycerol, to which 2 nM ³H-DHT had been added before polymerisation. Electrophoresis was run at 0°C for 2 h, and 2 mAmp per tube was applied. Following electrophoresis, the gels were transversely sliced into 2.3 mm segments which are placed directly into counting vials with 10 ml toluene scintillation fluid. After standing overnight at room temperature, more than 98% of the radioactivity was extracted into the toluene. Steady state between bound and free ³H-DHT is obtained when the level of radioactivity in front of the ABP peak is identical to that behind the peak. At steady state the amount of ³H-DHT in the peak is a linear function of the total amount of binding protein (ABP) present in the sample: Total ABP = Peak ABP($K_D/[H] + 1$) where K_D is the equilibrium constant of dissociation and $[H]$ is the concentration of the ³H-DHT in the gel.

it is bound by protein moving through the gel. After electrophoresis, the gels are sliced and the radioactivity is measured in each slice. A steady state between bound and free radioactivity is reached when the level of free radioactivity behind the binding protein peak equals the level in front. The concentration of binding protein can then be determined (assuming one binding site per protein molecule).

The concentration of ABP in EDF expressed as ³H-DHT binding capacity at saturation (pmol per mg protein) was much higher than in testicular and epididymal supernatants (Fig. 1). There was sufficient ABP in EDF to account for all the ABP measured in supernatants of testis and epididymis. Since the primary fluid from the seminiferous tubules becomes diluted before reaching the rete testis¹³, the concentration of ABP surrounding the germinal epithelium might be even higher than in EDF. The relatively large amount of ABP in caput epididymidis supernatant compared with testis supernatant, probably results from absorption of water as the testicular fluid passes through the efferent ducts and the first segment of caput¹⁴, and may also reflect a larger proportion of luminal fluid per unit of tissue weight. ABP concentration in epididymis supernatants decreases from segments I to V (Fig. 1). Further, the amount of ABP leaving the epididymis through the vas deferens is much less than that which enters into the caput, suggesting that the ³H-DHT binding activity of ABP is destroyed during passage through the epididymis. Although the exact role of ABP in the caput epididymidis is not yet known, it probably concentrates large amounts of androgen in the lumen close to the spermatozoa and surrounding epithelial cells. The concentration of endogenous DHT in

the caput epididymidis of rats (40–60 ng per g tissue)¹⁵ is several times higher than in any other body tissues so far examined, and is very similar to the concentration of ABP.

ABP production is stimulated by FSH

To establish the relationship between the production of ABP and maturational changes in the testis, ABP was measured in rats of various ages. At 1 and 2 weeks of age, testis and epididymis contained negligible amounts of ABP; at 3–4 weeks, however, increasing amounts of ABP were detected. After 4 weeks, ABP levels increased rapidly in the caput, reaching a peak at about the time of puberty (8 weeks) and decreasing in the adult to about 50% of the pubertal value¹⁶. Since the appearance of ABP corresponded with the onset of gonadotrophin secretion in the immature rat¹⁷, the effect of hypophysectomy on the levels of ABP was examined in rats at 5 weeks of age. Three days after hypophysectomy, the concentration of ABP in caput epididymidis supernatants was only about 40% of that in intact littermates, and by the 10th day, ABP had completely disappeared from both testis and epididymis. Evidence that this action of the pituitary is mediated through gonadotrophins, and not some other pituitary factor, was obtained by the injection of a human gonadotrophin preparation, containing both FSH and LH, to adult hypophysectomised animals. FSH-LH administration for 5 d caused a distinct increase of ABP in the testis supernatant (Fig. 2). The ABP concentration was more than 10 times higher than in the hypophysectomised controls and almost twice as high as in the intact littermates. These findings strongly indicate that ABP production is under pituitary control both in immature and adult rats.

Highly purified preparations of human FSH and LH were then used to examine their separate effects on ABP production. In 5-week-old intact rats, FSH injected subcutaneously for 10 d markedly increased the concentration of ABP both in the testis and the caput epididymidis, while LH treatment had no significant effect¹⁶. Similar results were obtained when rats were hypophysectomised at 5 weeks and started on daily injections of FSH 10 d later. After treatment for 10 d, FSH restored ABP levels in testis and epididymis supernatants¹⁶. When increasing doses of highly purified FSH were injected intramuscularly to immature rats, there was a linear increase in ABP concentration in caput epididymidis at doses between 2–10 µg of FSH

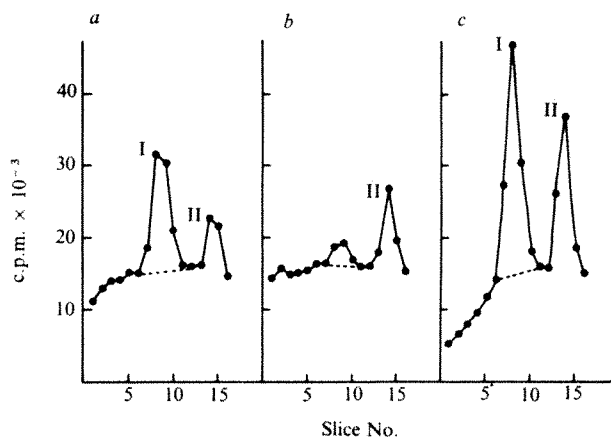


Fig. 2 Effect of FSH + LH treatment on ABP levels in rat testis. Adult rats in groups of four were hypophysectomised for 19 d. From day 20 after hypophysectomy, each animal was injected daily with 75 IU FSH + 25 IU LH or saline only. After treatment for 5 d, the testes were examined for ABP as described in Fig. 1. a, Sham operated; b, hypophysectomy for 19 d and saline for 5 d; c, hypophysectomy for 19 d and FSH + LH for 5 d. I, ABP; II, albumin. ABP, 100% in a, 14% in b and 157% in c.

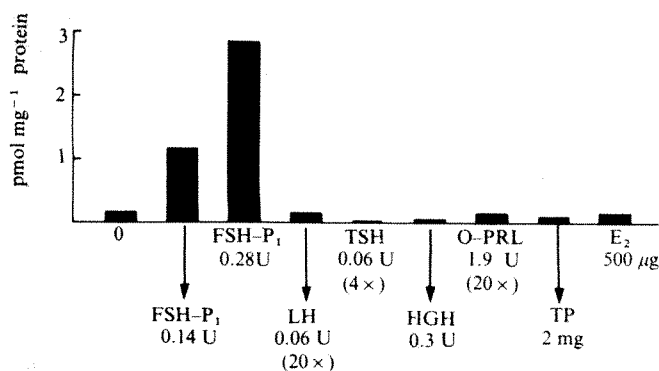


Fig. 3 Specific stimulation of ABP by FSH. Rats in groups of four were hypophysectomised at 28 d of age and injected with 0.14 or 0.28 NIH units FSH-P₁ (total dose). The injections were started on day 30. Parallel groups of animals were injected with high doses of LH, thyroid-stimulating hormone (TSH), ovine prolactin (O-PRL), human growth hormone (HGH), testosterone propionate (TP) and oestradiol-17 β (E₂). Twenty times the maximum contamination of LH or O-PRL in 0.28 units FSH-P₁; (4 \times), four times the maximum contamination of TSH in 0.28 units FSH-P₁.

(ref. 18). Administration of testosterone propionate (TP) in increasing doses (10–100 μ g per 90 g body weight) to intact 50-d-old ('pubertal') rats caused a dose-dependent decrease in ABP concentrations in both testis and epididymis, indicating that ABP is sensitive to feedback suppression of pituitary gonadotrophins¹⁹.

FSH stimulation of ABP both in intact and hypophysectomised animals is specific, since no increase in ABP could be obtained using other pituitary hormones or sex steroids (Fig. 3). The sensitivity of the ABP response to FSH is comparable with that of the ovarian augmentation test^{20,21}. Significant stimulation of ABP in hypophysectomised animals was obtained with as little as 2 IU of FSH (equivalent to about 0.08 NIH units). The ABP response diminished with time after hypophysectomy but was restored by pretreatment with high doses of TP (0.5–16 mg TP per 90 g body weight) (unpublished observations). Similar augmentation of the ABP response to FSH was obtained by pretreatment with LH. The enhanced response to FSH following androgen priming is reflected in increased concentrations of ABP both in testicular and epididymal supernatants; however, it remains to be determined whether TP priming increases Sertoli cell production of ABP or decreases the rate of ABP degradation.

Androgen receptors in epididymal epithelium

After testosterone is taken up by epithelial cells of the epididymis, it is rapidly converted to several metabolites, with DHT and 5 α -androstane-3 α ,17 β -diol as major products^{22,23}. DHT is bound to intracellular androgen receptors in the cytoplasm^{24,25}, subsequently transported into the nucleus and bound to nuclear chromatin^{22,26}. DHT-receptor complexes can be extracted from epididymal nuclei in 0.4 M KCl and have a sedimentation constant of 3–3.5S (refs 22, 25 and 26).

Epididymal cytoplasmic receptor (CR) has physical properties and steroid specificity very similar to the ventral prostate receptor^{24,25,27}; both are highly specific for DHT. They also have similar electrophoretic mobilities by PAGE ($R_x = 0.4$), similar sedimentation rates by sucrose gradient centrifugation (8S and 4S) and similar elution volumes by Sephadex G200 gel filtration^{24–27}. Both are destroyed by heating at 50 $^{\circ}$ C for 30 min and lose binding activity after treatment with sulphydryl blocking agents, such as PCMPS (1 mM)^{24,25}. A characteristic feature of ³H-DHT-CR complexes of epididymis and prostate is the very slow rate of dissociation at 0 $^{\circ}$ C ($t_{1/2}$ 0 $^{\circ}$ C > 4 d) (refs 25, 27). This property clearly distinguishes ³H-DHT-CR from ³H-DHT-ABP,

which dissociates much more rapidly ($t_{1/2}$ 0 $^{\circ}$ C = 6 min) (ref. 25). The slow rate of dissociation of ³H-DHT-CR is consistent with a receptor function of CR. During the process of translocation from cytoplasm to nucleus, androgen is firmly bound to the receptor and cannot be exchanged by excess of free DHT (ref. 26). The rapid dissociation of DHT from ABP, however, suggests that ABP acts as a carrier protein capable of releasing its androgen to surrounding epithelial cells. A striking difference between ABP and CR was also found in their affinity for cyproterone²⁵. Cyproterone acetate inhibited the binding of ³H-DHT to epididymal CR to the same extent as it reduced uptake and binding by epididymal nuclei²⁵. Binding of ³H-DHT to ABP on the other hand was not affected by this treatment. These studies indicate that nuclear uptake and binding of DHT are dependent on CR and independent of ABP.

Androgen receptors in the seminiferous tubules

The evidence for androgen action on spermatogenesis prompted studies to discover androgen receptors in rat seminiferous tubules. Immature Sprague-Dawley rats hypophysectomised at 25 or 35 d of age were used. Three to twenty-four days after hypophysectomy, animals in groups of 10 or 20 were eviscerated, functionally hepatectomised²² and injected intravenously with ³H-testosterone. Whole testes or washed seminiferous tubules were homogenised in three volumes of 50 mM Tris HCl buffer, pH 7.4, containing 0.32 M sucrose and 3 mM MgCl₂ (TSM buffer); and 105,000g supernatants prepared similarly from epididymis and prostate. Binding was examined by PAGE (refs 6 and 8), Sephadex G200 gel filtration^{6,24}, and by sucrose gradient centrifugation^{6,24}.

Testicular cytosol fractions contained an androgen-protein complex moving as a symmetrical peak of protein bound radioactivity with an R_x of 0.4 (Fig. 4). The mobility of this androgen-protein complex was different from that

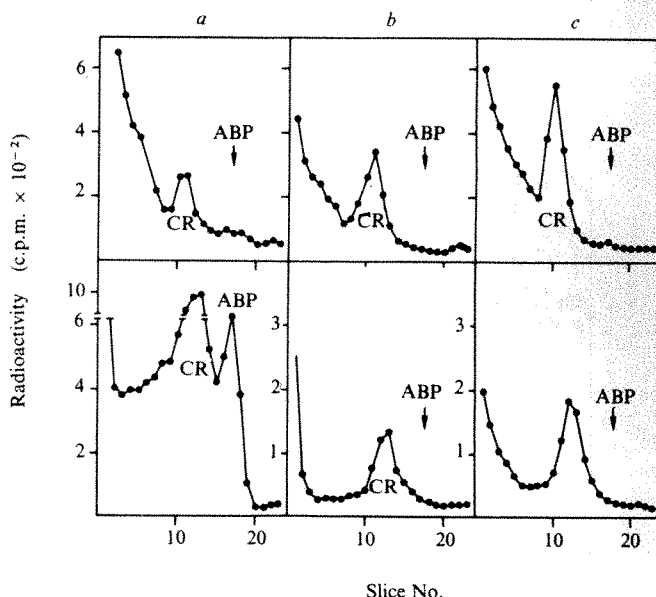


Fig. 4 Cytoplasmic receptors (CR) in testis (upper) and epididymis (lower) different from testicular androgen binding protein (ABP). Sprague-Dawley rats were hypophysectomised at 35 d of age. At different intervals afterwards (a, 3; b, 10 and c, 24 d) animals in groups of 10 or 20 were eviscerated and functionally hepatectomised²². Five ml of 5% glucose in water and 200 μ g cortisol were given subcutaneously 1 h before the operation. ³H-testosterone (91 Ci mmol⁻¹, 50 μ Ci) was then injected into the femoral vein, and the animals were killed after 3 h. Testis and epididymis 105,000g supernatants (100 μ l) were layered over 3.25% acrylamide gels (5 \times 60 mm) containing 0.5% agarose²⁵. Electrophoresis was run for 2 h at 0 $^{\circ}$ C and 1.5 mAmp per tube. After electrophoresis, the gels were sliced and counted as in Fig. 1.

of ABP ($R_s = 0.71$), but very similar to the CR of the epididymis and ventral prostate, which were run simultaneously for comparison. When the seminiferous tubules were separated from interstitial tissue, CR was present in the seminiferous tubular fraction. No androgen binding was detected in cytosol fractions of the interstitial tissue. Radioactivity bound to CR was eluted from the gel and identified by thin layer chromatography (TLC) and crystallisation of the isolated metabolites to constant specific activity. Only testosterone (60%) and DHT (40%) were bound to testicular CR, though these androgens were relatively minor fractions of the total labelled metabolites. Most of the radioactivity in the testis supernatant chromatographed with 5α -androstane- $3\alpha,17\beta$ -diol and more polar metabolites. In the same animals, the radioactivity bound to the epididymal and prostate CR was more than 90% ^3H -DHT.

Gel filtration chromatography of a similar aliquot of testicular supernatant yielded one peak of bound radioactivity in or close to the void volume of the Sephadex G200 column. The elution position of the steroid-receptor complex was similar to that of receptors in epididymis and prostate supernatants^{24,27}, but different from the ^3H -DHT-ABP complex which has a Stokes radius of 47 Å (refs 6, 7, 24). The bound radioactivity was extracted and again identified by TLC and crystallisation as testosterone (55%) and DHT (45%). Centrifugation of these supernatants through 5–20% linear sucrose gradients (sucrose solution in 50 mM Tris HCl, pH 7.4, containing 1 mM EDTA, 0.5 mM 2-mercaptoethanol and 10% glycerol) gave one nondisplaceable peak of bound radioactivity sedimenting as 6–8S.

To establish the receptor identity of CR in testis supernatant, the temperature stability and the dissociation of the androgen-protein complex were compared with cytoplasmic receptor in epididymis. Twenty animals, 25-d-old, were hypophysectomised and injected with ^3H -testosterone 3 d later. Testicular and epididymal 105,000g supernatants were heated at either 25° C or 50° C for 30 min, and binding analysed by PAGE. Androgen-CR complexes in

both testis and epididymis were stable after heating at 25° C, but were destroyed by heating at 50° C. Similar heat stability was found for the androgen-protein complex excluded from Sephadex G200 and the 6–8 S complex obtained by sucrose gradient centrifugation. This heat sensitivity was identical to that of the prostate cytoplasmic receptor^{24,25,27}, but different from that of ABP which was stable at 50° C (refs 6, 8, 24, 25). Dissociation of the labelled androgen-CR complexes was examined by PAGE after incubation of *in vivo* labelled supernatant with a large excess (10 µg) of unlabelled DHT for 2 h at 0° C. Dissociation of the androgen-CR complex in testis was negligible as was the dissociation of the androgen-receptor complex in epididymis. This extremely tight binding of androgen by the testis CR at 0° C ($t_{1/2}$ 0° C = 35 h) is similar to the binding by androgenic receptors in the epididymis and prostate, giving further support to a receptor function of testicular CR. Evidence for translocation of cytoplasmic androgen-receptor complexes into testis nuclei was obtained from the demonstration of androgen binding macromolecules in purified nuclei with steroid specificity identical to CR. These androgen-protein complexes could be extracted with 0.4–1 M KCl following injection of ^3H -testosterone and had a sedimentation of 3–4S.

Our results support the view that the germinal epithelium of rat testis is an androgen-dependent target tissue. The intracellular receptors for androgen demonstrated here probably mediate the androgenic stimulus to spermatogenesis. It is not yet clear which cells within the seminiferous tubules contain CR. Spermatocytes show typical 'target tissue' metabolism of ^{14}C -testosterone to DHT and 5α -androstane- $3\alpha,17\beta$ -diol²⁸. Also the formation of these metabolites by the rabbit testis is greatly reduced after selective destruction of the germinal cells of the seminiferous tubules by heat²⁹. The persistence of CR for as long as 24 d after hypophysectomy, however, indicates that CR is present in either Sertoli cells, spermatogonia or primary spermatocytes, since these were the only cell types remaining. To what extent the germinal epithelium cells are capable of retaining the CR-androgen complexes in their nuclei remains to be determined. Further studies are necessary to determine whether androgen acts directly on proliferating germ cells or stimulates spermatogenesis by an indirect action on the Sertoli cells.

The demonstration of androgen-receptor complexes in cytoplasmic and nuclear fractions of seminiferous tubules supports the concept that spermatogenesis is an androgen-dependent process. LH stimulates spermatogenesis by increasing androgen production by the Leydig cells. FSH stimulates production of ABP, presumably by the Sertoli cells, thereby increasing the binding and accumulation of androgen within the seminiferous tubules close to androgen-dependent cells of the epithelium (Fig. 5). ABP might, therefore, act as an essential androgen concentrating factor, which seems to be under control of FSH. In addition, FSH stimulation of ABP secretion into testicular fluid increases the amount of ABP (and androgen) transported to the caput epididymidis by the efferent ducts. Testosterone, which is taken up by epididymal cells both from the lumen and the circulating blood is 'activated' by 5α -reduction to form DHT. DHT bound to cytoplasmic receptors is transported into the cell nuclei where the androgen-receptor complex binds to chromatin and initiates metabolic processes which are necessary for sperm maturation. After releasing its bound testosterone to epithelial cells, ABP is free to bind testosterone or DHT which diffuses into the lumen from the blood or epithelium.

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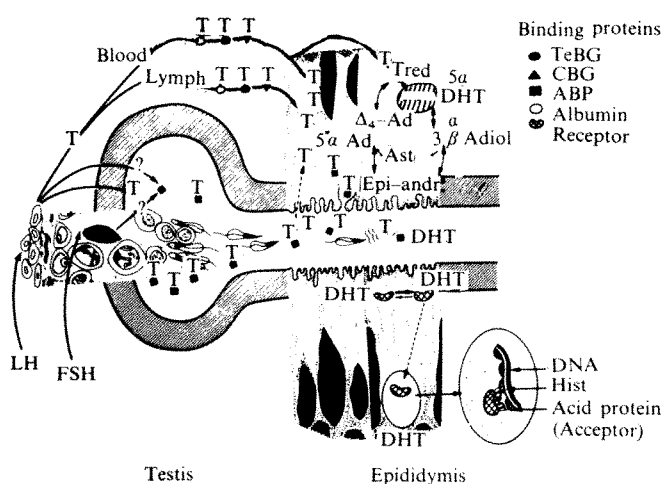


Fig. 5 Schematic drawing of androgen action in the testis and epididymis. Testosterone (T) is secreted from the Leydig cells due to LH stimulation. Most of the testosterone is removed from the testis bound to different binding proteins in blood and lymph. ABP is produced by the Sertoli cells due to FSH stimulation and secreted into the seminiferous tubules. There, ABP generates a diffusion potential causing a net inflow of testosterone and accumulation of androgens. FSH also causes an increased secretion of ABP (and androgen) from the testis into the epididymis, by the way of the efferent duct fluid. Testosterone which enters the epididymis is activated by the formation of DHT. DHT bound to receptors is translocated to the cell nucleus, initiating the different metabolic processes necessary for sperm maturation.

Δ_4 Ad = Δ_4 -adione; 5α Ad = 5α adione; Ast = Asterone; 3β Ad = 3β adiol; Epi-andr = epiandrosterone

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- ¹ Steinberger, E., Steinberger, A., and Ficher, M., *Recent Prog. Horm. Res.*, **26**, 547 (1970).
- ² Ahmad, N., Haltmeyer, G. C., and Eik-Nes, K., *Biol. Reprod.*, **8**, 411 (1973).
- ³ Blaquier, J. A., Cameo, M. S., and Burgos, M. H., *Endocrinology*, **90**, 839 (1972).
- ⁴ Dyson, A. L. M. B., Orgebin-Crist, M. C., *Endocrinology*, **93**, 391 (1973).
- ⁵ Ritzén, E. M., Dobbins, M. C., French, F. S., and Nayfeh, S. N., *Excerpta Medica Int. Congr. Ser.*, No. 256, 79 (1972).
- ⁶ Hansson, V., *Steroids*, **20**, 575 (1972).
- ⁷ Hansson, V., Djoseand, O., Reusch, E., Attramadal, A., and Torgersen, O., *Steroids*, **21**, 457 (1973).
- ⁸ Ritzén, E. M., Dobbins, M. C., Tindall, D. J., French, F. S., and Nayfeh, S. N., *Steroids*, **21**, 593 (1973).
- ⁹ French, F. S., and Ritzén, E. M., *Endocrinology*, **93**, 88 (1973).
- ¹⁰ French, F. S., and Ritzén, E. M., *J. Reprod. Fert.*, **32**, 479 (1973).
- ¹¹ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265 (1951).
- ¹² Ritzén, E. M., French, F. S., Weddington, S. C., Nayfeh, S. N., and Hansson, V., *J. biol. Chem.* (in the press).
- ¹³ Tuck, R. R., Setchell, B. P., Waites, G. M. H., and Young, J. A., *Pflügers Arch. ges. Physiol.*, **318**, 225 (1970).
- ¹⁴ Crabo, F., *Acta vet. scand.*, **6**, suppl. 5 (1965).
- ¹⁵ Aafjes, J. H., and Vreeburg, T. M., *J. Endocr.*, **53**, 85 (1972).
- ¹⁶ Hansson, V., Reusch, E., Ritzén, E. M., French, F. S., Trygstad, O., and Torgersen, O., *Nature new Biol.*, **246**, 56 (1973).
- ¹⁷ Swerdloff, R. S., Walsh, P. C., Jacobs, H. S., and Odell, W. D., *Endocrinology*, **88**, 120 (1971).
- ¹⁸ French, F. S., McLean, W. S., Smith, A. A., Tindall, D. J., Weddington, S. C., Petrusz, P., Sar, M., Stumpf, W. E., Nayfeh, S. N., Hansson, V., Trygstad, O., and Ritzén, E. M., in *Proc. Conf. on hormone binding and activation in testis*, Houston, Texas, February 1974, (in the press).
- ¹⁹ Hansson, V., Ritzén, E. M., and French, F. S., *Proc. ninth Acta Endocrinologica Congress, Oslo, June 1973*, (in the press).
- ²⁰ Steelman, S. L., and Pohley, F. M., *Endocrinology*, **53**, 604 (1953).
- ²¹ Petrusz, P., Robyn, C., and Diczfalusy, E., *Acta endocr., Copenh.*, **63**, 454 (1970).
- ²² Tindall, D. J., French, F. S., and Nayfeh, S. N., *Biochem. biophys. Res. Commun.*, **49**, 1391 (1972).
- ²³ Djoseand, O., Hansson, V., and Haugen, H., *Steroids*, **21**, 773 (1973).
- ²⁴ Hansson, V., Djoseand, O., Reusch, E., Attramadal, A., and Torgersen, O., *Steroids*, **22**, 19 (1973).
- ²⁵ Tindall, D. J., Hansson, V., McLean, W. S., Nayfeh, S. N., and French, F. S., *Biochemistry*, (in the press).
- ²⁶ Blaquier, J. A., and Calandra, R. S., *Endocrinology*, **93**, 51 (1973).
- ²⁷ Fang, S., and Liao, S., *J. biol. Chem.*, **246**, 16 (1971).
- ²⁸ Dorrington, J. H., and Fritz, I. B., *Biochem. biophys. Res. Commun.*, **54**, 1425 (1973).
- ²⁹ Ewing, L., and Brown, B., *Biol. Reprod.*, **9**, 92 (1973).

Nucleotide pyrophosphatase, a sialoglycoprotein located on the hepatocyte surface

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A sialoglycoprotein enzyme hydrolysing nucleotide pyrophosphate bonds is a major externally located component on the hepatocyte surface membrane.

MEMBRANE-BOUND enzymes play a paramount role in the control of cell metabolism. Enzymes located on the surface membrane occupy a crucial position for control of the interaction of cells with the external environment. To assess the role of cell surface membrane enzymes in metabolic regulation and cell surface phenomena, knowledge of the location of these enzymes, especially with regard to the outer or inner faces of the membrane is desirable. By using an enzymic technique¹ to label the surface membrane of isolated cells and a sub-cellular fraction derived from the liver cell surface, I show that nucleotide pyrophosphatase, an enzymic marker in plasma membrane isolation studies^{2,3}, is externally located on the hepatocyte.

Nucleotide pyrophosphatase was purified from liver plasma membrane as a sialoglycoprotein of molecular weight 130,000 and is a major constituent of liver plasma membranes⁴. The external location of the nucleotide pyrophosphatase accounts for the failure of nucleotides to enter the liver in metabolic experiments⁵⁻⁷. The fact that the nucleotide pyrophosphatase is on the outside of the cell, whereas the sugar transferases are on the Golgi apparatus^{8,9}, strongly suggests that mechanisms proposing a role for membrane transferases in intercellular adhesion^{10,11}, and the uptake of serum glycoproteins¹² are unlikely to be generally operative.

Iodination of liver plasma membranes

Plasma membranes were prepared from liver homogenates as a population of vesicular membrane profiles derived from the sinusoidal, canalicular and contiguous faces of the hepatocyte^{2,13,14}. They were labelled with ¹²⁵I by using the lactoperoxidase-catalysed iodination procedure in which H₂O₂ was generated by a glucose-glucose oxidase system¹. This iodination technique was shown to label specifically and covalently the external face only of erythrocytes, erythrocyte ghosts¹, liver 'microsomal' vesicles¹⁵, and lymphocytes¹⁶. Iodinated, washed liver plasma membranes were dissolved in sodium dodecyl sulphate, and the polypeptides separated, mainly on a molecular weight basis, by electrophoresis in flat sheets of polyacrylamide¹⁷. Figure 1a shows liver plasma membranes to consist of at least 30 polypeptides, of which about six of the components in the higher molecular weight range are glycosylated. Iodinated plasma membranes contained two major peaks of ¹²⁵I-radioactivity corresponding to glycosylated polypeptide bands of apparent molecular weight of 130,000 and 100,000 (Fig. 1b).

The enzymic identity of one of the major iodinated polypeptides in liver plasma membranes was established by copurification of ¹²⁵I-radioactivity and nucleotide pyrophosphatase activity peaks. Since the enzyme also hydrolysed phosphodiester linkages⁴, it was more convenient to monitor purification of the enzyme by measuring alkaline phosphodiesterase activity by hydrolysis of the artificial substrate thymidine 5'-monophosphate *p*-nitrophenol ester. Iodinated plasma membranes,

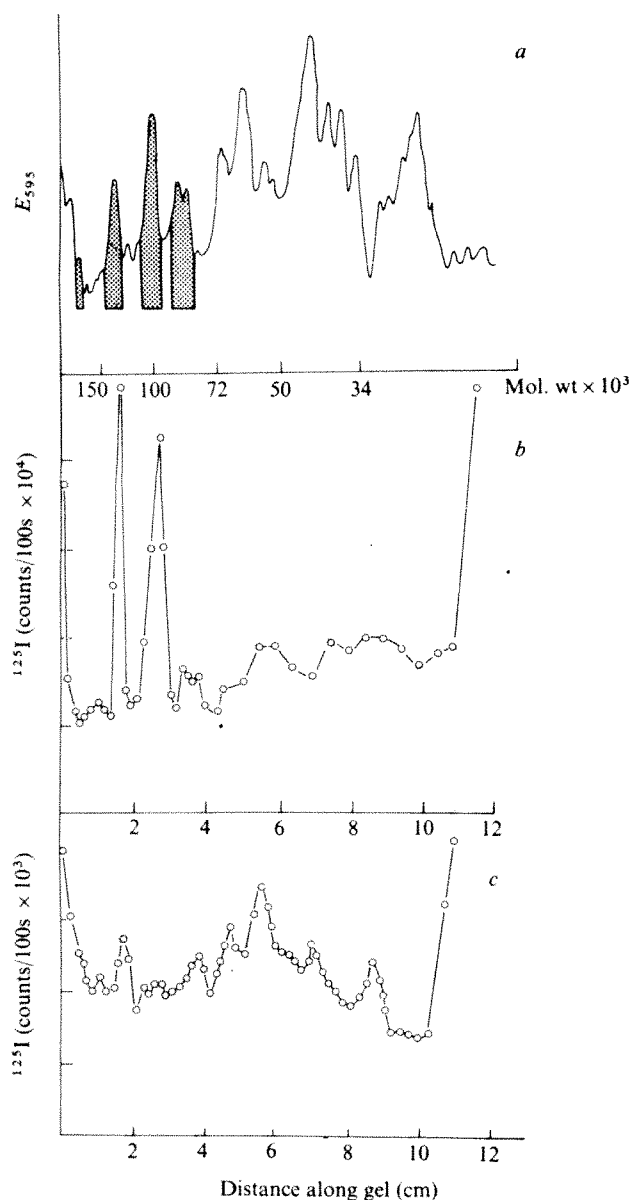


Fig. 1 Electrophoretic separation on polyacrylamide gels of iodinated liver plasma membranes (*a*, *b*) and isolated hepatocytes (*c*). Liver plasma membranes were prepared from 'nuclear' pellets by a rate zonal procedure¹³, or from a 'microsomal' fraction by density gradient centrifugation^{2,14}. Livers were dissociated by perfusion through the portal vein with hyaluronidase and collagenase²¹. Membranes (13 mg protein) or hepatocytes (1×10^7 cells) suspended in 0.8 ml phosphate buffered saline containing 5 mM glucose were iodinated with 0.4 to 0.6 mCi carrier-free ^{125}I for 15–30 min at 20°C in the presence of 100 μg lactoperoxidase (Sigma) and 24 μg glucose oxidase (Worthington Biochemicals)¹. Iodinated membranes (*a*, *b*) were washed six times by centrifugation in water, and then dialysed against water until the dialysate was free of radioactivity, before dissolving by heating in 2% sodium dodecyl sulphate–4 M urea–1% mercaptoethanol. Iodinated hepatocytes (*c*) were washed six times in phosphate buffered saline by centrifugation at 50g for 2 min and then suspended for 15 min at 37°C in 1 ml phosphate-buffered saline containing 1% deoxycholate and 25 μg DNase. The supernatant, containing 90% of ^{125}I radioactivity attached to the cells, was mixed with sodium dodecyl sulphate–urea–mercaptoethanol. Membrane polypeptides and extracts of cells were resolved by flat plate electrophoresis in 8.5% polyacrylamide gels equilibrated in 0.1% sodium dodecyl sulphate¹⁷. Plasma membrane polypeptides were stained for protein with Coomassie Blue and the bands densitometrically traced at E_{595} (*a*). Glycoproteins (hatched areas) were identified by using the Schiff periodate reagent¹⁸. Gels were then cut into thin slices of equal length with a blade and ^{125}I -radioactivity determined on a γ -counter (*b*, *c*). In the absence of lactoperoxidase, fewer than 5% of the counts were incorporated and no peaks of radioactivity associated with protein were present in gel separation of membranes or cells.

solubilised in the detergent N-lauryl sarcosinate¹⁸, were added to a Sepharose 6B column, and the resolution of ^{125}I -radioactivity, protein and two plasma membrane marker enzyme activities—phosphodiesterase and 5'-nucleotidase followed (Fig. 2). A close coincidence between a peak of radioactivity and of phosphodiesterase activity was observed. Column fractions corresponding to the phosphodiesterase and 5'-nucleotidase peaks were pooled, concentrated and analysed by polyacrylamide gel electrophoresis. Figure 3*a* indicates that the phosphodiesterase activity peak corresponds to the ^{125}I -radioactivity peak of molecular weight 130,000 present in plasma membranes (Fig. 1*a*, *b*). The 5'-nucleotidase activity peak from the column that overlapped the trailing edge of the phosphodiesterase activity peak (Fig. 2) also contained a small ^{125}I -radioactivity peak of molecular weight 130,000 but the main peak of ^{125}I -radioactivity was at molecular weight 100,000 (Fig. 3*c*).

A sialoglycoprotein with dual specificity towards nucleotide pyrophosphate and phosphodiester bonds was purified from liver plasma membrane as a single band on polyacrylamide gels of apparent molecular weight 130,000 (ref. 4). The above results, therefore, suggest that the enzyme was iodinated. But to examine more directly the relationship between the enzyme activities separated by gel filtration and peaks of radioactivity resolved on polyacrylamide gels, the two enzyme activity peaks collected from the Sepharose column were also electrophoresed in polyacrylamide gels equilibrated in buffers containing deoxycholate (Fig. 3*b* and *d*). Although the resolution of membrane components in gels equilibrated in deoxycholate-buffer was inferior to that obtained in sodium dodecyl sulphate-buffer as judged by protein staining, the major ^{125}I -radioactivity and phosphodiesterase peaks were coincident (Fig. 3*b*). But the major 5'-nucleotidase activity peak did not coincide with the ^{125}I -radioactivity peak of molecular weight 100,000 (Fig. 3*d*).

The overall results show that the alkaline phosphodiesterase-nucleotide pyrophosphatase of liver plasma membranes was iodinated by the adopted procedure, since enzyme activity, ^{125}I -radioactivity and the molecular weight determined independently on purified enzyme were coincident. The liver plasma membrane fraction prepared consisted of closed vesicles. Studies with similar vesicles of a liver microsomal fraction have shown that they are impermeable to lactoperoxidase, and only proteins on the external face of the microsomal membrane were iodinated¹⁵. One may conclude, therefore, that the sialoglycoprotein functioning as a phosphodiesterase-nucleotide pyrophosphatase is located on the outer surface of the liver plasma

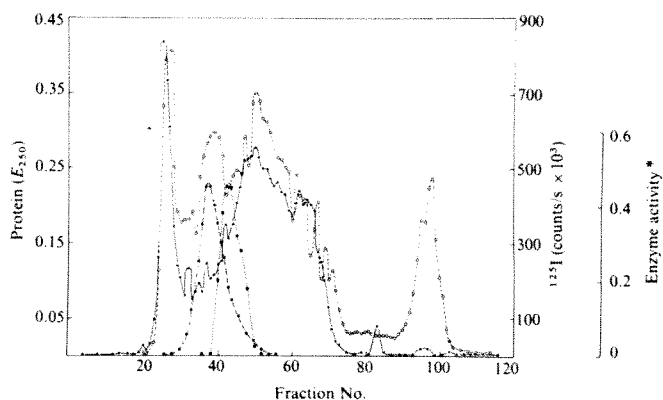


Fig. 2 Gel filtration on a Sepharose 6B column of a sarcosyl extract of iodinated liver plasma membranes. Iodinated membranes were extracted with a 4% sarcosyl, 2.1% Tris buffer pH 7.9^{18,19}, and the extract, after concentration by pressure filtration across Diaflow XM50 filters, was resolved in a column equilibrated with 0.2% sarcosyl buffer. Column fractions were assayed for protein at E_{280} (\blacktriangle), ^{125}I (\circ), 5'-nucleotidase (\bullet) and alkaline phosphodiesterase (\blacksquare) activities. The activity peaks of phosphodiesterase (tubes 35–39) and 5'-nucleotidase (tubes 42–48) were concentrated by pressure filtration across Diaflow UM50 filters.

*Phosphodiesterase or 5'-nucleotidase (arbitrary units).

membrane vesicles. Carbohydrate chains of glycoproteins are externally orientated on surface membranes¹⁹, and the liver plasma membrane contains sialic acid on its outer surface²⁰. It is highly likely that the plasma membrane vesicles used in the present study were of a normal 'right side out' orientation, but to obtain further evidence for the external location on the liver surface membrane of the labelled sialoglycoprotein enzyme, free hepatocytes were prepared and iodinated in the same manner.

Iodination of isolated hepatocytes

Perfusion *in situ* of rat liver through the portal vein with a Krebs-Ringer medium containing collagenase and hyaluronidase dissociates the lobules into cells²¹. Hepatocytes, separated from the Kupffer, endothelial and other cells by repeated low-speed centrifugation, were iodinated as described for the plasma membrane fraction¹. The iodinated cells were briefly incubated in a medium containing deoxycholate and the extracts then analysed by polyacrylamide gel electrophoresis in sodium dodecyl sulphate-buffer (Fig. 1c). Many peaks of ¹²⁵I-radioactivity were obtained, and one major peak corresponded in its molecular weight position to one of the two major iodinated peaks obtained with isolated plasma membranes. The coincidence in molecular weight of a ¹²⁵I-radioactivity peak of apparent molecular weight 130,000 both in a plasma membrane subfraction and cells supports the conclusion that the phosphodiesterase-nucleotide pyrophosphatase is located on the surface of the hepatocyte. The presence of a greater number of labelled components on hepatocytes compared with isolated plasma membranes contrasts with the results obtained with iodinated erythrocytes¹. It may be caused by damage to the outer cell surface of the hepatocyte occurring during the enzymic treatment used to dissociate the tissue, or to the presence on the cells of tightly-adsorbed nonmembrane proteins.

Implications for membrane functioning

To resolve the molecular basis of events occurring at the cell surface, a number of chemical²² and enzymically catalysed labelling reagents^{1,23} have been used to identify molecules externally located on the surface membrane. These studies, initially made on erythrocytes²³ and later extended to include platelets²⁴, fibroblasts²⁵, lymphocytes^{16,26} and cultured cells^{27,28}, in general identify glycoproteins of high molecular weight among the labelled components. For example, lymphocyte and sarcoma ascites tumour cell plasma membranes possess a major protein of molecular weight 130,000 that was labelled by iodination^{26,29}. These studies do not, however, assign a direct functional role to the iodine-labelled cell surface membrane components. My experiments show that a glycoprotein component located on the outer face of the hepatocyte surface membrane possesses nucleotide pyrophosphatase activity.

The rapid degradation of nucleotides, for example, NAD, ATP, UDP-galactose by perfused livers^{5,7} is now explained. Hydrolysis by the blood sinusoidal surface membrane of sugar nucleotides yields nucleotide monophosphates which may be further degraded by nucleotide monophosphatases, for example, 5'-nucleotidase which, although not significantly labelled in the present experiments, was also shown by the use of antisera³⁰ and by cytochemical studies³¹ to be externally located on liver surface membranes. These enzymes may be components of a metabolic network of enzymes integrated to varying degrees into the surface membrane and functioning in the transport of nucleotides across the cell surface.

Cell surface galactosyl transferase activity transferring galactose from sugar nucleotides to galactosyl acceptors has featured in proposed intercellular contact mechanisms^{10,11} and the enzyme was also implicated as a binding site on liver plasma membranes for asialoglycoproteins¹². But subcellular fractionation studies of the liver have clearly distinguished between

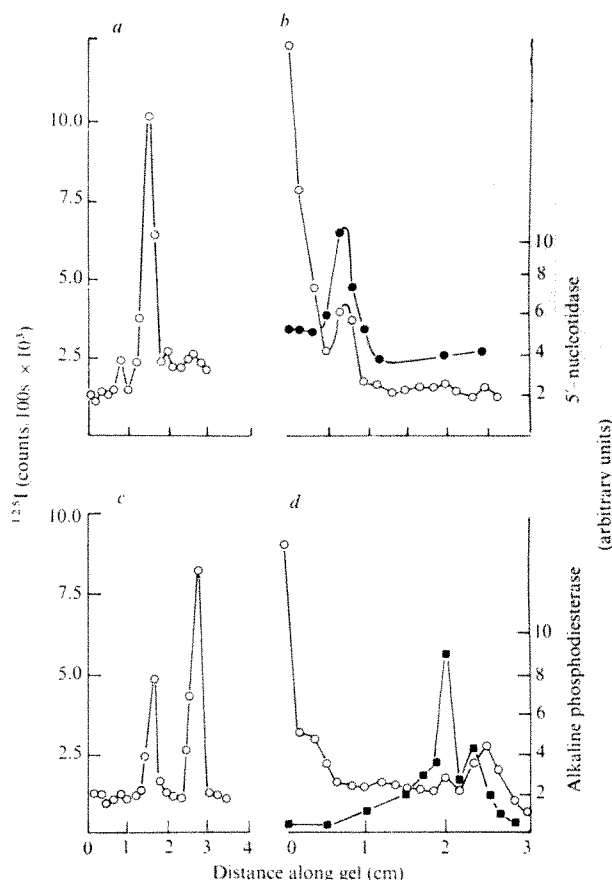


Fig. 3 Polyacrylamide gel electrophoresis of the alkaline phosphodiesterase (a, b) and 5'-nucleotidase (c, d) activity peaks separated by gel filtration (Fig. 2). The concentrated samples were electrophoresed in gels equilibrated in Tris-glycine buffers containing either sodium dodecyl sulphate (a, c) or deoxycholate (b, d)⁴. Enzyme activities (expressed as arbitrary units) were determined on dispersed gel fragments after determination of ¹²⁵I-radioactivity (○).

Golgi and plasma membrane fragments^{8,9} and shown that galactosyl transferases are Golgi enzymes and are not significantly located on the plasma membrane. Therefore in liver, and probably other cells, galactosyl transferases are not directly involved in cell surface phenomena. My results reinforce this conclusion, since even if some transferases were on the cell surface, the presence of the nucleotide-phosphatase would ensure a rapid disappearance of the transferase substrates, unless a lateral topographical separation of enzymic activities was invoked. Nucleotide pyrophosphatases-alkaline phosphodiesterases have been detected on a number of mammalian surface membranes^{3,32,33} and such an enzyme is probably one of the surface components of approximate molecular weight 100,000 shown to be iodinated on various cells. But the generalisation that this enzyme is always located externally on the cell surface membrane as currently shown for hepatocytes, must await copurification of enzymic and radioactivities in other cells and tissues. Cultured hamster cell lines were shown indirectly to contain an externally located surface membrane nucleotide pyrophosphatase, and the enzymic activity was absent in virally transformed cells^{31,34}. The present identification thus focuses on a molecular difference between normal and virally transformed cell lines of a cell surface sialoglycoprotein enzyme³⁵.

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¹ Hubbard, A. L., and Cohn, Z. A., *J. Cell Biol.*, **55**, 390-405 (1973).

² Touster, O., Aronson, N. N., Dulaney, J. J., and Hendrickson, H., *J. Cell Biol.*, **47**, 604-618 (1970).

- ³ Sandford, J. B., and Rosenberg, M. D., *Biochim. biophys. Acta*, **288**, 333-346 (1972).
- ⁴ Evans, W. H., Hood, D. O., and Gurd, J. W., *Biochem. J.*, **135**, 819-826 (1973).
- ⁵ Bischoff, E., Liersch, M., Keppler, D., and Decker, K., *Hoppe-Seyler's Z. Physiol. Chem.*, **351**, 729-736 (1970).
- ⁶ Domschke, W., Liersch, M., and Decker, K., *Hoppe-Seyler's Z. Physiol. Chem.*, **352**, 85-88 (1971).
- ⁷ Bischoff, E., Wilkening, J., and Decker, K., *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 1112-1114 (1973).
- ⁸ Fleischer, B., and Fleischer, S., *Biochim. biophys. Acta*, **219**, 301-319 (1970).
- ⁹ Bergeron, J. J. M., Ehrenreich, J. H., Siekevitz, P., and Palade, G. E., *J. Cell Biol.*, **59**, 73-88 (1973).
- ¹⁰ Roth, S., McGuire, E. J., and Roseman, S., *J. Cell Biol.*, **51**, 536-547 (1971).
- ¹¹ Roth, S., and White, D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 485-489 (1972).
- ¹² Aronson, N. A., Tan, L. Y., and Peters, B. P., *Biochem. biophys. Res. Commun.*, **53**, 112-118 (1973).
- ¹³ Evans, W. H., *Biochem. J.*, **116**, 833-842 (1970).
- ¹⁴ Wisher, M., and Evans, W. H., *Biochem. Soc. Trans.* (in the press).
- ¹⁵ Kreibich, G., and Sabatini, D. D., *Fedn Proc.*, **32**, 2133-2138 (1973).
- ¹⁶ Uhr, W. J., and Vitetta, E. S., *Fedn Proc.*, **32**, 35-40 (1973).
- ¹⁷ Evans, W. H., Bergeron, J. J. M., and Geschwind, I., *FEBS Lett.*, **34**, 259-262 (1973).
- ¹⁸ Evans, W. H., and Gurd, J. W., *Biochem. J.*, **126**, 681-700 (1972).
- ¹⁹ Benedetti, E. L., Dunia, I., and Diawara, A., *Eur. J. Cancer*, **4**, 263-272 (1973).
- ²⁰ Benedetti, E. L., and Emmelot, P., *J. Cell Sci.*, **2**, 499-512 (1967).
- ²¹ Berry, M. N., and Friend, D. S., *J. Cell Biol.*, **43**, 506-520 (1969).
- ²² Bretscher, M. S., *J. molec. Biol.*, **58**, 775-781 (1971).
- ²³ Phillips, D. R., and Morrison, M., *Biochem. biophys. Res. Commun.*, **40**, 284-289 (1970).
- ²⁴ Nachman, R. L., Hubbard, A., and Ferris, B., *J. biol. Chem.*, **248**, 2928-2936 (1973).
- ²⁵ Podulso, J. F., Greenberg, C. S., and Glick, M. C., *Biochemistry*, **11**, 2616-2621 (1972).
- ²⁶ Ladoulis, C. T., Misra, D. N., and Gill, T. J., in *Protides of the Biological Fluids* (edit. by Peeters, H.), **21**, 67-71 (1974).
- ²⁷ Gahmberg, C. G., and Hakomori, S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3329-3333 (1973).
- ²⁸ Hynes, R. O., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3170-3174 (1973).
- ²⁹ Shin, B. C., and Carraway, K. L., *Biochim. biophys. Acta*, **330**, 254-268 (1973).
- ³⁰ Gurd, J. W., and Evans, W. H., *Archs Biochem. Biophys.* (in the press).
- ³¹ Farquhar, M. G., Bergeron, J. J. M., and Palade, G. E., *J. Cell Biol.*, **60**, 8-25 (1974).
- ³² Sela, B., Lis, H., and Sachs, L., *J. biol. Chem.*, **247**, 7575-7590 (1972).
- ³³ Hubbard, A. L., and Cohn, Z. L., *J. Cell Biol.*, **59**, 152a (1973).
- ³⁴ Sela, B., and Sachs, L., *FEBS Lett.*, **30**, 100-104 (1973).
- ³⁵ Kalckar, H. M., and Hakomori, S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1637a (1972).

Sequence of a repressor-binding site in the DNA of bacteriophage λ

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The sequence of 33 nucleotides immediately preceding the start point of transcription of an operon in bacteriophage lambda has been determined. This region includes recognition sites for lambda repressor and, probably, for other proteins. The sequence contains interdigitating symmetries.

Two loci on the DNA of bacteriophage λ —the operators—contain multiple repressor binding sites. Superimposed on each operator are sites recognised by a restriction endonuclease (Hin) and by *E. coli* RNA polymerase¹⁻⁵. At each operator (Fig. 1), repressor preferentially binds to a terminal site and the remaining sites are filled in the direction opposite to that of transcription of the adjacent controlled operon. Operator O_L , for example, controls leftward transcription of gene *N*. Repressor (probably a dimer)⁶ first binds to a 30-35 base pair site (S_1) proximal to *N*, and additional molecules (probably monomers) add sequentially to the right, filling five 15-base pair sites of lower repressor affinity. Each operator contains a single Hin target between the first and second repressor binding sites. At O_L , this site is located immediately to the right of S_1 . The region of each operator recognised by RNA polymerase includes or is immediately adjacent to the Hin site^{3,4}. O_L is not transcribed *in vivo* (ref. 7 and H. Lozeron, personal communication); the start-point of leftward transcription is to the left of S_1 .

We report here the nucleotide sequence of 33 residues between the Hin site in O_L and the start point of leftward transcription. We also confirm the sequence of the first 26 residues to the left of the transcription start point, originally determined by analysis of an RNA transcript (J. E. Dahlberg and F. F. Blattner, in preparation). We refer to this latter sequence as the

5' end of *N*, although it is not known where translation of *N* begins. We present here the essentials of our arguments and our conclusions; details of the sequence determination will be presented elsewhere.

Experiments and results

When λ DNA is digested with Hin, about 50 different double-stranded fragments are generated. One of these, which is about 1,125 bases long (Hin 1125) contains S_2 - S_6 , and another contains S_1 (Fig. 2)^{2,3}. We purified microgram quantities of Hin 1125 by gel electrophoresis, and used it to prime *de novo* synthesis of one strand of S_1 as follows. Denatured Hin 1125 was reannealed in the presence of approximately equimolar quantities of purified λ I strand DNA. The *r* strand of the annealed Hin 1125 was then extended into S_1 by incubating the mixture with *E. coli* DNA polymerase I in the presence of Mn^{2+} , ribo-CTP or ribo-GTP and the appropriate three deoxynucleoside triphosphates,

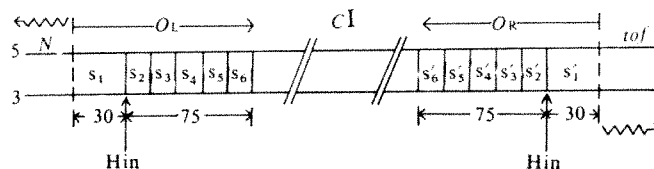


Fig. 1 Schematic representation of the λ operators and adjacent genes. The wavy arrows show the direction of transcription of the repressor-controlled genes *N* and *tof*. Two arrows indicate the order of repressor binding from the first sites (S_1 and S'_1) to the final sites (S_6 and S'_5) in O_L and O_R . The Hin cutting sites in O_L and O_R are indicated along with the number of base pairs within the operators on either side of each Hin cut. The repressor gene is *cl*.

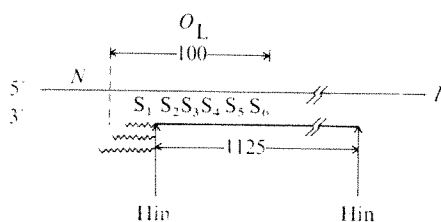


Fig. 2 Schematic representation of primed DNA synthesis at the leftward operator O_L . The lines labelled l and r represent, respectively, the l strand of λ DNA and the r strand of the restriction endonuclease fragment Hin 1125. Repressor binding sites within O_L are designated S_1 – S_6 . The boundary between O_L and the start-point of transcription of gene N is indicated by the dashed vertical line. The varying lengths of *in vitro* synthesised DNA resulting from asynchronous chain growth are represented by the wavy lines. The restriction endonuclease (Hin) cleavage sites at the extremities of Hin 1125 are indicated.

one of which was labelled in the α position with ^{32}P . The *in vitro* product of interest was then released from the template-primer complex by Hin digestion followed by denaturation. In some cases the product was digested with RNase without Hin digestion. The labelled oligonucleotides were fractionated and

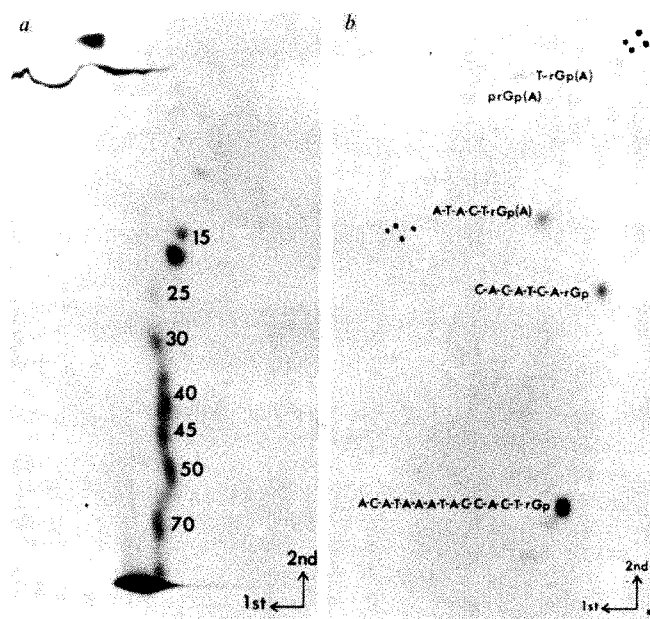


Fig. 3 *a*, Two-dimensional fractionation of the products of a restriction-fragment-primed DNA polymerase I reaction. A total volume of 250 μl contained: 12 μg purified λ l strand DNA¹⁸, 1 μg Hin 1125 DNA², 67 mM Tris-HCl, pH 7.4, 0.67 mM MnCl_2 , 1 mM 2-mercaptoethanol, 1 mM ribo-GTP, 0.025 mCi α - ^{32}P -dATP (100 Ci mmol⁻¹), 0.05 mM dTTP, 0.05 mM dCTP, 20 μl DNA polymerase I (approximately 1–5 units). The reaction was started by the addition of DNA polymerase and incubated at 8° C. After 30 min the reaction mixture was extracted with phenol, and residual phenol removed by several ether extractions. The products were dialysed overnight against Hin buffer^{2,3}. To release the newly synthesised DNA from the restriction fragment, the sample was digested for 2 h with exonuclease-free Hin prepared as described previously³. The sample was precipitated by the addition of 1/10 volume 3 M sodium acetate and two volumes of ethanol. The precipitate was taken up in 5 μl of H_2O , sealed in a capillary, heated to 100° C and cooled in an ice bath. The denatured sample was fractionated in the first dimension by electrophoresis on cellulose acetate in a pH 3.5 buffer containing 5 mM EDTA and 7 M urea. The second dimension was homochromatography using a 7% unhydrolysed RNA mixture. The approximate chain lengths of the products are shown. *b*, Two-dimensional fractionation of an RNase digest of a ribo-G-substituted oligonucleotide. The sequences of the various products are indicated on the figure. The primary product, about 40 bases long, was eluted from the homochromatography plate of (a) and digested overnight with 5 μl of a solution containing 10 mg ml⁻¹ RNase T_1 in 0.05 M Tris-HCl, pH 7.4, and 0.005M EDTA. The digest was fractionated as in (a) except that a 3% RNA mixture hydrolysed for 30 min was used in the second dimension.

their sequence was determined using methods described by Sanger *et al.*⁸.

A typical experiment was as follows. The product of a Hin 1125-primed synthesis, polymerised from rGTP, dTTP, dCTP and ^{32}P -labelled dATP, was separated from the template primer complex by Hin digestion and subjected to two dimensional fractionation (Fig. 3). The homochromatography mixture used in the second dimension separates oligonucleotides of lengths about 1–80 bases. The products seen in Fig. 3a have a common 5' end (produced by Hin digestion) but differ in the 3' ends because of asynchronous growth of the chains. Each fractionated product was eluted, digested with ribonuclease T_1 , and again subjected to two-dimensional fractionation. Figure 3b shows a fingerprint of the oligonucleotides generated by RNase T_1

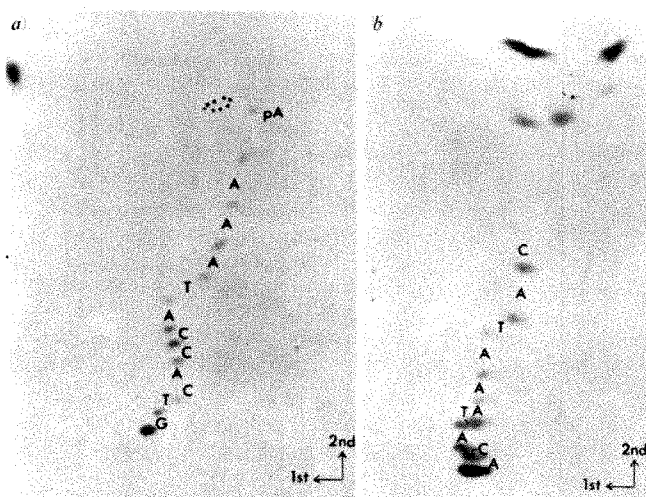


Fig. 4 Two-dimensional fractionations of snake venom exonuclease (a) and spleen exonuclease digests (b) of the 15-base long ribo-G terminated product, A-C-A-T-A-A-T-A-C-C-A-C-T-rGp. The digests were fractionated as described in the legend to Fig. 3. From the distance and angle of shift between a digestion product and that product with one base removed the identity of the removed base can be determined⁸. The nucleotide removed is shown between each product. Thus the venom exonuclease digestion gives a 3' sequence of A-A-A-T-A-C-C-A-C-T-G and the spleen exonuclease gives a 5' sequence of A-C-A-T-A-A-T-A-C. These two sequences contain the common sequence A-A-A-T-A-C. Digestion conditions were as follows. For venom exonuclease the sample was incubated first with 3 μl of a solution containing 10 mg ml⁻¹ bacterial alkaline phosphatase, 0.05 M Tris-HCl, pH 8.9, and 0.005 M MgCl_2 for 30 min at 37° C, to remove 3' phosphate. 4 μl of a solution containing 0.1 mg ml⁻¹ snake venom phosphodiesterase, 0.05 M Tris-HCl, pH 8.9, 0.05 M MgCl_2 was then added and the sample incubated at 37° C, and 1 μl aliquots were taken at 0, 15, 30, 45, 60, 90, and 120 min, frozen, and then pooled. For spleen exonuclease digestion was with 7 μl of a solution containing 0.1 mg ml⁻¹ spleen phosphodiesterase, 0.05 M citrate buffer pH 6.0. The reaction was treated as in (a).

digestion of one of these products, about 40 residues long. In this case the homochromatography mix used in the second dimension separates oligonucleotides of lengths about 1–20 bases. By comparing the fingerprints generated in this way from each of the Hin digestion products seen in Fig. 3a, the order of synthesis of various ribo-G-containing oligonucleotides, labelled with ^{32}P -dATP was determined. Finally, the sequence of the various ribonuclease T_1 products was determined by partial exonuclease digestion and by nearest neighbour analysis. Figure 4a shows a two-dimensional fractionation of a partial venom phosphodiesterase digest of an RNase T_1 product identified in the experiment of Fig. 3b. Figure 4b shows a partial spleen phosphodiesterase digest of the same product. (Venom and spleen phosphodiesterase attack, respectively, from the 3' and 5' ends of polynucleotide chains.) Inspection of these fingerprints reveals the following sequence, which was confirmed by nearest neighbour analysis (not shown):

5' G/ACATAAATACCACTG/G. 3'

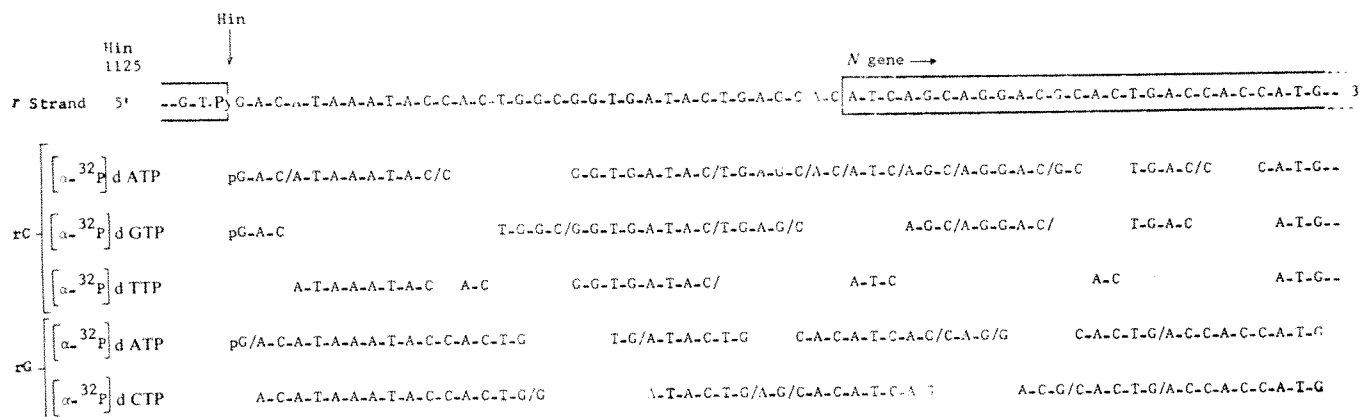


Fig. 5 The sequence from the Hin site of O_L to residue 26 beyond the startpoint of transcription of N . The sequences and order of synthesis from the Hin site of various ribo-C and ribo-G containing oligonucleotides are shown. The ribonucleotide triphosphate and the labelled deoxynucleotide triphosphate used in each reaction are indicated on the figure. Note that the orientation of the sequence is the reverse of that in Figs 2 and 6. See text for explanation.

The order of appearance and nucleotide sequence of each RNase digestion product are presented in Fig. 5. Note that in this figure the orientation of the sequence is the opposite of that in Fig. 6. This maintains the convention that oligonucleotides are written in the orientation 5'-3'. The complete sequence from the Hin site in O_L to N as well as that of the first 26 residues of N , deduced from this information, is also shown in the figure. The sequence GT Py (Py=pyrimidine, Pu=purine) to the left of the Hin cut in Fig. 5 has been added by inference from the fact that Hin II, one of the activities in our Hin preparation, is reported to recognise the sequence 5' GTPyPuAC 3' (ref. 10, and K. Murray, personal communication).

The sequence of a mutant operator was determined as follows. Experiments were performed using as primer wild type Hin 1125 and as template / strands isolated from λ 101. This phage bears a mutation that decreases the affinity of S_1 for repressor^{2,3}. We found a single base difference between the wild type and λ 101 sequences. The sequence ATAAATAC in wild type DNA is replaced in λ 101 DNA with ATAAATGC.

Interesting aspects of sequence

The complete double stranded sequence of the region between the O_L Hin site and the beginning of N is shown in Fig. 6. Because RNA polymerase binds to the Hin site³⁻⁵, the maximum number of base pairs separating bound polymerase from the transcription start point is 33. This value is consistent with our

previous estimate³ and is considerably less than that suggested by Blattner *et al.*¹⁰. A revised estimate from Blattner *et al.* (personal communication) agrees with our data. DNA-bound polymerase is reported to cover 40 base pairs¹¹, and so it is possible that polymerase bound to the Hin site extends to the transcriptional start point.

There are three axes of two-fold rotational symmetry in the sequence in Fig. 6: at nucleotide 17 (Fig. 6a), between residues 15 and 16 (Fig. 6b), and at residue 14. In *a*, 16 of 24 residues are perfectly symmetric and 6 of the remaining 8 show pyrimidine-pyrimidine or purine-purine symmetry. In *b*, 14 of 26 residues are perfectly symmetrical and 6 of the remaining 12 show Py-Py or Pu-Pu symmetry. In *c*, 14 (or 16) of 30 are perfectly symmetrical while 12 of the remaining 16 (or 10 of the remaining 14) show Py-Py or Pu-Pu symmetry. We calculate the probability that a region containing the symmetry properties of configuration *a* or *c* appears by chance in a sequence of 35 base pairs is less than 1%; for configuration *b* the probability is perhaps a few percent. Analysis of the pyrimidine tracts of the smallest fragment protected from nuclease digestion by repressor¹ suggests that the minimal region covered by repressor extends from about five residues to the right of the Hin target to residue 30. All three symmetries lie within this region. The O^c mutation at position 10 lies in two of the symmetry regions, but we cannot say which of these symmetries (if either) is recognised by repressor. In the *lac* operator O^c mutations have been found in bases

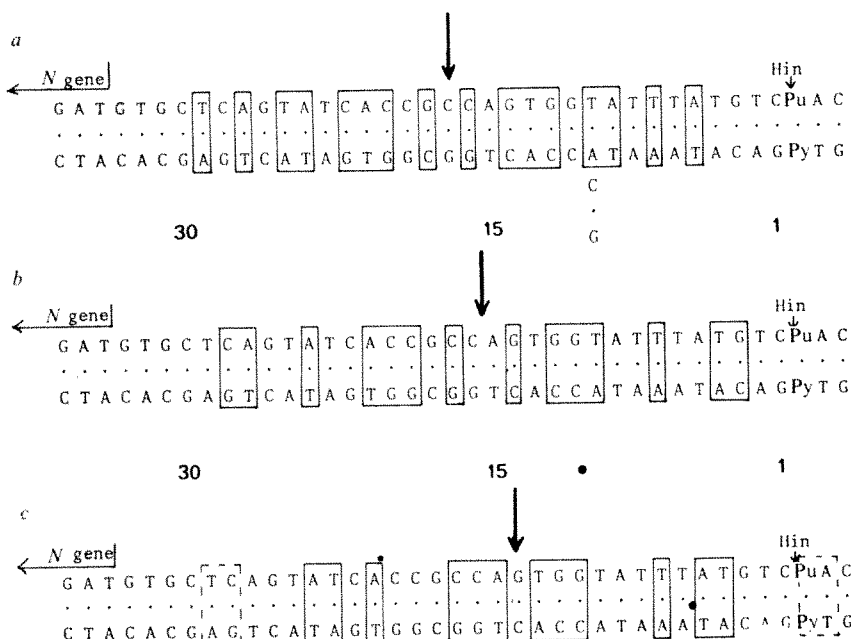


Fig. 6 Symmetries in the region between the Hin site in O_L and gene N . The bases in boxes are symmetric about two-fold rotational axes indicated by vertical arrows. An operator mutation at position 10 is indicated.

comprising a symmetric sequence, as well as in interspersed bases which do not contribute to the symmetry¹²⁻¹⁴.

Why (other than fortuitously) should there be three overlapping (interdigitating) symmetries between the O_L Hin site and N ? One intriguing possibility is that these symmetries are recognised by different regulatory proteins—'multiplexing', as R. Pollack calls it. For example, the *tof* gene product is believed to act at O_L because a mutation which reduces the affinity of O_L for repressor also decreases the effect of *tof* product on leftward transcription¹⁵. RNA polymerase and N -product are two other proteins which might recognise these symmetries^{16,17}. We note that it is much more difficult, if not impossible, to construct interdigitating symmetries using perfect as opposed to hyphenated symmetries such as those seen in Fig. 6. Sequence analysis of the remaining sites in the operators and localisation of various regulatory mutations should provide a clearer understanding of the significance of these symmetries.

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- ¹ Maniatis, T., and Ptashne, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1531 (1973).
- ² Maniatis, T., and Ptashne, M., *Nature*, **246**, 133 (1973).
- ³ Maniatis, T., Ptashne, M., and Maurer, R., *Cold Spring Harbor Symp. quant. Biol.*, **38**, 857 (1973).
- ⁴ Maurer, R., Maniatis, T., and Ptashne, M., *Nature*, **249**, 221 (1974).
- ⁵ Allet, R., and Solem, R., *J. molec. Biol.*, **85**, 475 (1974).
- ⁶ Chadwick, P., Pirrotta, V., Steinberg, R., Hopkins, N., and Ptashne, M., *Cold Spring Harbor Symp. quant. Biol.*, **35**, 283 (1970).
- ⁷ Blattner, F. R., and Dahlberg, J. E., *Nature new Biol.*, **237**, 227 (1972).
- ⁸ Sanger, F., Donelson, J. E., Coulson, A. R., Kossel, H., and Fischer, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1209 (1973).
- ⁹ Kelly, T. J., and Smith, H. O., *J. molec. Biol.*, **51**, 393 (1970).
- ¹⁰ Blattner, F. R., Dahlberg, J. E., Boettiger, J. K., Fiandt, M., and Szybalski, W., *Nature new Biol.*, **237**, 232 (1972).
- ¹¹ Heyden, B., Nusslein, C., and Schaller, H., *Nature new Biol.*, **240**, 9 (1972).
- ¹² Gilbert, W., and Maxam, A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3581 (1973).
- ¹³ Maizels, N. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3585 (1973).
- ¹⁴ Gilbert, W., Maizels, N. M., and Maxam, A., *Cold Spring Harbor Symp. quant. Biol.*, **38**, 845 (1973).
- ¹⁵ Sly, W. S., Rabideau, K., and Kolber, A., in *The bacteriophage lambda* (edit. by Hershey, A.), 221-231 (Cold Spring Harbor Laboratory, New York, 1971).
- ¹⁶ Franklin, N., *J. molec. Biol.* (in the press).
- ¹⁷ Adhya, S., Gottesman, M., de Crombrughe, B., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ¹⁸ Szybalski, W., Kubinski, H., Hradecna, Z., and Summers, W. C., in *Methods in enzymology*, **21**, Part D (edit. by Grossman, L., and Moldave, K.), 383 (Academic Press, New York, 1971).

letters to nature

High velocities and cocoon stars

LARGE scale gas movements with velocities of the order of 50 km s^{-1} giving rise to split, asymmetric or broadened emission line profiles have been observed in many H II regions¹⁻⁴. Proposed explanations for these motions include supernova explosions within or near to the H II regions⁵, and the acceleration of their ionised material by relativistic particles⁶ or by massive stellar winds⁷⁻⁹. I suggest that the breakup of the neutral gas and dust shell around a cocoon star could conceivably also produce high velocities. The existence of cocoon stars seems fairly well established from observations of infrared point sources^{10,11} and these are frequently associated with compact H II regions¹² and OH masers. Kahn¹³ investigated the evolution of a cocoon star and found that a star which accretes matter will form a false photosphere with a radius of the order 10^{13} cm and no appreciable H II region, provided that the rate of accretion exceeds approximately 10^{20} g s^{-1} . Around the false photosphere is a dust-free region with a radius of about 10^{16} cm , which corresponds to the radius at which refractory particles melt. At accretion rates of less than 10^{22} g s^{-1} the radiation pressure on the dust becomes powerful enough to stop the infall of matter and to drive a shock wave into the accreting gas flow outside a radius of 10^{16} cm . This situation is Rayleigh-Taylor unstable, and the shell will break up into globules. Consider a portion of neutral material with a density of $M \text{ g cm}^{-2}$, at a distance, R , from a star with mass, M_* , and luminosity L_* ($M_* \approx 6 \times 10^{34} \text{ g}$, $L_* \approx 6 \times 10^{38} \text{ erg s}^{-1}$). If the radiation force just balances the gravitational force exerted by the star, then

$$M = L_* (4\pi c G M_*)^{-1} \quad (1)$$

Thus, $M \approx 0.7 \text{ g cm}^{-2}$ independent of distance. This is very close to the surface density required by the models of Dyson¹⁴ ($M \approx 2 \text{ g cm}^{-2}$) for which a great deal of observational

evidence^{15,16} has been collected, particularly in the case of M8 and M42. It is thus possible that some of the globules represent the material that was just balanced by the radiation—gravitational forces, at the time of the breakup of the cocoon.

Next, consider the period of the onset of instability, when the accretion rate drops below 10^{22} g s^{-1} . The false photosphere will break up in certain directions according to a time scale given approximately by the free-fall time from a radius, R ($\approx 10^{16} \text{ cm}$) to the star. That is about 10^{10} s . Thus, an H II region is formed very soon after the onset of the instability which quickly spreads through the dust-free cavity around the star. An estimate of the properties of the H II region can be obtained.

The density distribution of a freely falling gas cloud in steady flow is given by

$$\rho(R) = \dot{M} (32\pi^2 G M_*)^{-1/2} R^{-3/2} \quad (2)$$

where \dot{M} is the accretion rate. Putting $\dot{M} = 10^{22} \text{ g s}^{-1}$ and integrating out to $R = 10^{16} \text{ cm}$, the density in the initially formed H II region will be of the order of 10^8 cm^{-3} and the total mass involved will be of the order of $2 \times 10^{32} \text{ g}$. This 'ultra-compact' H II region could not be contained by the overlying neutral material, and so would punch a hole (or holes) through those parts of the cloud with the lowest density, finally allowing the ionised material to escape more or less unimpeded.

The expansion of the ionised gas can be regarded as virtually isothermal, and so the ratio of the velocity, V , attained by a mass element and the sound speed, V_s , in the gas will be approximately given by:

$$V/V_s = (2 \ln(\rho_i/\rho_f)/3)^{1/2} \quad (3)$$

Where ρ_i is of the order 10^8 cm^{-3} and ρ_f is the final density, which is put equal to the density of a typical H II region—about 10^3 cm^{-3} . Thus, velocities of the order of three times the speed of sound should be attained along rather directional

streams. This is sufficient to explain the high velocities observed in a small fraction of the nebular material.

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- ¹ Sheglov, P. V., *Astrophys. Lett.*, **1**, 145 (1968).
- ² Meaburn, J., *Astrophys. Space Sci.*, **13**, 110 (1971).
- ³ Smith, M. G., and Weedman, D. W., *Astrophys. J.*, **169**, 271 (1971).
- ⁴ Dopita, M. A., Gibbons, A. H., Meaburn, J., and Taylor, K., *Astrophys. Lett.*, **13**, 515 (1973).
- ⁵ Gershberg, R. E. and Sheglov, P. V., *Soviet Astron.*, **8**, 337 (1964).
- ⁶ Kahn, F. D., *Q. Jl R. ast. Soc.*, **12**, 384, (1971).
- ⁷ Pikelner, S. B., *Astrophys. Lett.*, **2**, 97 (1968).
- ⁸ Dyson, J. E., and de Vries, J., *Astr. Astrophys.*, **20**, 223 (1972).
- ⁹ Dyson, J. E., *ibid.*, **23**, 381 (1973).
- ¹⁰ Becklin, E. E., and Neugebauer, G., *Astrophys. J.*, **147**, 799 (1967).
- ¹¹ Wynn-Williams, C. G., Becklin, E. E., and Neugebauer, G., *Mon. Not. R. ast. Soc.*, **160**, 1 (1972).
- ¹² Habing, H. J., *The Interstellar Medium* (edit. by Pinkau K.), (Reidel, 1974).
- ¹³ Kahn, F. D., *ibid.*
- ¹⁴ Dyson, J. E., *Astrophys. Space Sci.*, **1**, 388 (1968).
- ¹⁵ Dopita, M. A., Dyson, J. E., and Meaburn, J., *ibid.* (in the press).
- ¹⁶ Elliott, K. H., and Meaburn, J., *ibid.* (in the press).

Long term periodicities in the sunspot cycle

THE existence of a long period undulation (~ 179 yr) in the solar cycle (Fig. 1, based on data from Waldmeier^{1,2}) is suggested by the planetary theory of sunspots. Jose³, for example, postulated an association between the 178.8-yr periodicity in orbital positions of the planets and the phase of the solar cycle. Wood⁴ used the presumed existence of a cycle of 170–180 yr in solar activity to predict the peak dates of future sunspot cycles. Our work indicates that although a 179-yr periodicity does exist in the sunspot cycle, it is not caused by a primary long term excitation function but is, instead, a beat phenomenon. This can be seen by computing the spectrum for the data shown in Fig. 1.

Burg (unpublished; see also ref. 5) suggested a method for estimating the spectrum of a time series that is based on an autoregressive description of the data to be analysed. This method, 'maximum entropy spectral analysis' (MESA), yields a result consistent with the information of the data and nothing more. We used this method in our initial analyses of the sunspot cycle.

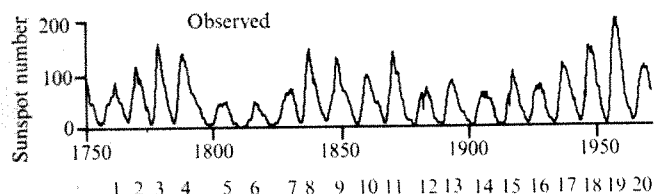


Fig. 1 Twelve-month running mean sunspot numbers (1750–1971), based on data given by Waldmeier^{1,2}

Because the quality of early sunspot data is considered suspect by Waldmeier¹, we first analysed only data acquired after 1800. Specifically, for reasons related to computational procedures, we computed the spectrum for the 12-month running mean sunspot numbers from 1844 to 1971. The

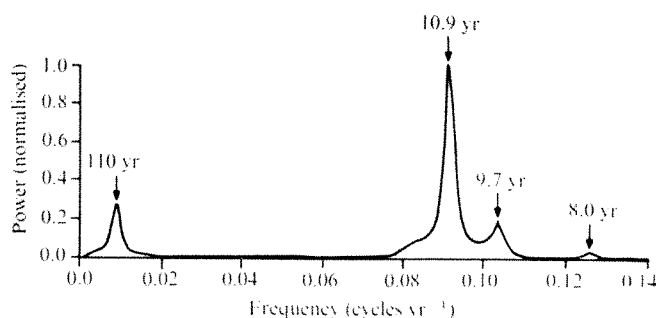


Fig. 2 Maximum entropy spectrum for the data interval 1844–1971.

MESA spectrum (Fig. 2) suggests that no significant power is present at periods greater than about 110 yr. The spectrum is dominated by three peaks with periods of about 110, 10.9 and 9.7 yr. Further, we note that the spectral components with periods 10.9 yr and 9.7 yr are separated in frequency by about $0.012 \text{ cycle yr}^{-1}$, and so the two sinusoids interfere with one another to produce beats with a period of roughly 167 yr. The similarity in behaviour of cycles 1, 2 and 3, and of cycles 17, 18 and 19 (Fig. 1) is apparently caused by this beat phenomenon.

That a long term periodicity can be observed in the data lends credence to the sunspot observations between 1750 and 1850. Therefore, we computed the MESA spectrum for a set of data which included the early mean sunspot observations. That analysis, which used 214 yr of data (1750–1963), yielded a spectrum with significant

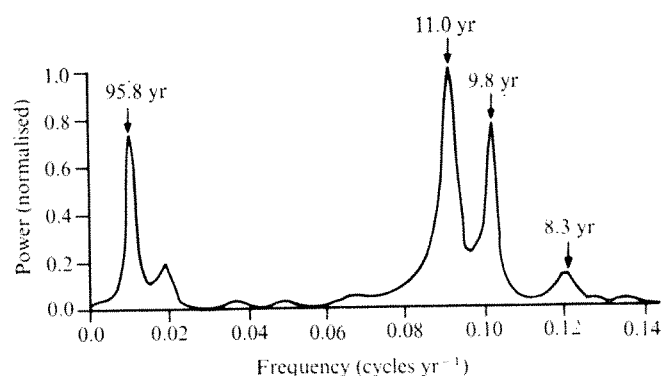


Fig. 3 Maximum entropy spectrum for the data interval 1750–1963.

signatures at periods of 95.8, 11.0 and 9.8 yr (Fig. 3). The frequency separation for the 11.0 and 9.8 yr spectral components yields an estimate of 181 yr for the long term periodicity. The similarity between the MESA spectra for the more recent sunspot observations (1844–1971) and the data incorporating the early observations (1750–1963) suggests that the sunspot data from 1850 to the present probably belong to the same population. This observation differs from the results of McNish and Lincoln⁶, who hypothesised that the very early data and the more modern data belong to two statistically different populations.

To refine our determination of the frequency separation between the 11.0 and 9.8-yr spectral components, we used all of the sunspot observations shown in Fig. 1 and correlated these data with a suite of sinusoids finely spaced in frequency about the peak frequencies in the 214-yr maximum entropy spectrum. The best correlations occurred at frequencies which correspond to periods of 11.2 and 9.9 yr. The actual frequency determinations for these correlation peaks are separated by $0.01116 \text{ cycle yr}^{-1}$, and as such, using this method the estimated period for the long

term undulation is 179 yr. This determination is in agreement with the average elapsed time between the peaks of cycles 1 and 17, 2 and 18, 3 and 19, and 4 and 20. Thus, although we estimate the length of the long period undulation in the sunspot cycle to be between 179 and 181 yr, we favour the 179-yr determination. This is somewhat longer than the 160–170 yr periodicity that Schöve⁷ observed in his data. On the other hand, our estimate is roughly 11 yr shorter than that of Cole⁸, who observed a 190-yr phase variation in Schöve's data. Regardless of these differences, the existence of a long period (160–190 yr) variation in sunspot activity seems certain.

If the major characteristics of the sunspot cycle repeat every 179 yr then, by definition, the data are periodic outside an interval of this length. It should, therefore, be possible to compute a periodogram using data covering 179 yr which is very similar to maximum entropy spectra computed previously. For example, the spectrum for the interval 1793–1971 (Fig. 4), exhibits the same spectral characteristics found in maximum entropy spectra computer previously.

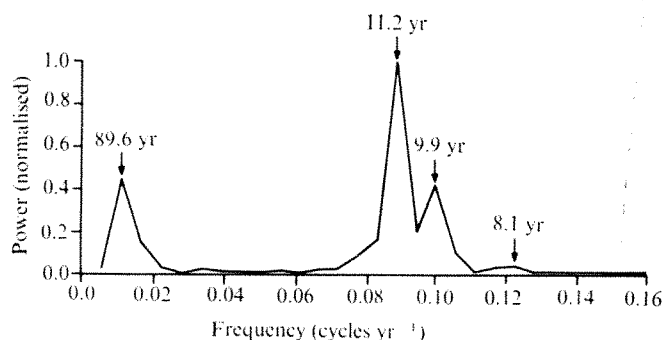


Fig. 4 Periodogram for the data interval 1793–1971.

We used the observation of the 179-yr periodicity to predict future sunspot activity. The ability to predict solar activity is important if efficient use is to be made of the high frequency spectrum for international communications. King⁹ has shown, however, that significant climatic features (such as droughts and unusually long growing seasons) may be related to the solar cycle; if such relationships can be established with certainty, long range weather forecasts can then be issued for agricultural planning purposes (see also ref. 10).

Sunspot activity could be predicted by taking the spectral components corresponding to the data for any 179-yr interval and then reconstructing the smoothed sunspot numbers in that interval. Then, by repeating the waveform outside the interval, smoothed sunspot numbers could be extrapolated forwards and backwards in time. This method was applied to the spectral components corresponding to the periodogram of Fig. 4 (1793–1971) together with a

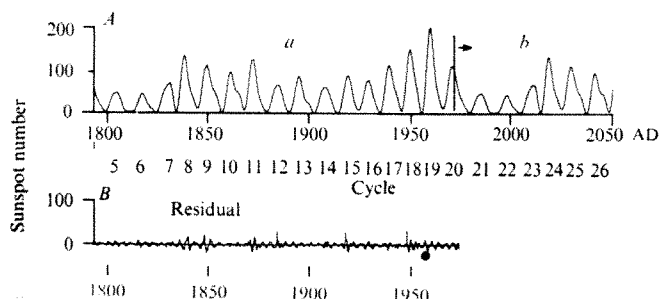


Fig. 5 Twelve-month mean sunspot numbers. A: a, reconstructed (1793–1971); b, predicted (1972–2050). B, Predicted minus reconstructed (1793–1971).

component of 47.4 smoothed sunspots (components at periods of less than 4.5 yr were eliminated). That yielded the results shown in Fig. 5A. In the interval 1793–1971, the reconstructed waveform has an r.m.s. residual (Fig. 5B) of five 12 month running mean sunspots.

Because these extrapolations can be projected backwards as well as forwards in time (we have assumed that the data are periodic, with a period of 179 yr), it follows that the predictions for the interval 1750–1792 correspond to the estimated sunspot numbers in the interval 1929–1971. As a measure of our prediction capability, therefore, we compared our predicted mean sunspot numbers for major features of the sunspot cycle from 1750 to 1792 inclusive with the corresponding observed values (Fig. 6). Although the data suggest that our predictions for the smoothed numbers at sunspot maxima may be in error by up to $\pm 25\%$, and that the time estimates for the occurrence of sunspot minima and maxima may differ from the true times by up to ± 2 yr, the general agreement between the trends of the observed and the predicted sunspot numbers is considered to be good.

Using the data in Fig. 5A, several predictions can be made:

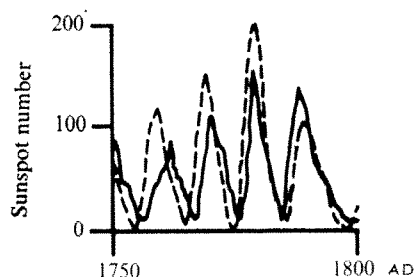


Fig. 6 Twelve-month running mean sunspot numbers (1750–1800). Solid curve, observed; dashed curve, predicted (1929–1971).

The current cycle, number 20, will exhibit a prolonged decay in sunspot activity, with a null-to-null period of nearly 13 yr. The activity of cycle 20 has already exhibited unusual decay behaviour by rising briefly, instead of falling continuously, in 1972. It seems plausible, therefore, that the next sunspot low will not be reached until after 1975, and possibly as late as the summer of 1977. At the minimum, mean sunspot numbers could be as low as 3.

Cycle 21 will have a rather broad peak, possibly reaching its maximum value late in 1982. Mean sunspot numbers for this peak may not, however, exceed 50. The sunspot minimum following cycle 21 should occur around 1988, and could exhibit smoothed sunspot numbers as low as 2.

Twelve month running mean sunspot numbers greater than 100 will not be observed again until approximately 2015. Thus, the next 40 yr may be characterised by relatively low sunspot activity as compared with the activity of the last 40 yr. This observation is supported by the results of Cole⁸.

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¹ Waldmeier, M., *The Sunspot Activity in the Years 1610–1960* (Technische Hochschule, Zurich, 1961).

² Waldmeier, M., *Monthly Sunspot Bulletin from the Swiss Federal Observatory* (Zurich, 1960–1972).

³ Jose, P. D., *Astr. J.*, **70**, 193–200 (1965).

⁴ Wood, K. D., *Nature*, **240**, 91–93 (1972).

⁵ Ulrich, T. J., *J. geophys. Res.*, **77**, 1396–1400 (1972).

- ⁶ McNish, A. G., and Lincoln, J. V., *Trans. Am. geophys. Un.*, **30**, 673 (1949).
⁷ Schove, D. J., *J. geophys. Res.*, **60**, 127-146 (1955).
⁸ Cole, T. W., *Sol. Phys.*, **30**, 103-110 (1973).
⁹ King, J. W., *Nature*, **245**, 443-446 (1973).
¹⁰ Gribbin, J., *Nature*, **246**, 453-454 (1973).

Asymptotic structure in torsional free oscillation data for the Earth

THE eigenfrequencies, ${}_n\sigma_l$, of the torsional modes, ${}_nT_l$, of a spherically symmetrical, nonrotating, elastic, isotropic Earth model, are defined, for fixed wave number $k=(l+\frac{1}{2})/a$, by the eigenvalue problem

$$\mu \left[\frac{d^2 U}{dr^2} + \frac{2dU}{rdr} \right] + \frac{d\mu}{dr} \left[\frac{dU}{dr} - \frac{U}{r} \right] + \left[{}_n\sigma_l^2 \rho - \frac{l(l+1)}{r^2} \mu \right] U = 0$$

(where $U=U(r)$; $\mu=\mu(r)$; $\rho=\rho(r)$; $a \leq r \leq b$)

and by the (zero stress) boundary conditions

$$\mu = [dU/dr - U/r] = 0, \quad r=a, \quad r=b;$$

where μ denotes rigidity, ρ density, a the radius of the core and b the radius of the Earth¹.

Using the Liouville transformation,

$$U = r^{-1}(\rho\mu)^{-1/4} Z, \quad r = \int_a^s \mu^{1/2} \rho^{-1/2} dt + a,$$

the asymptotic behaviour of ${}_n\sigma_l$ can be expressed in the form

$${}_n\sigma_l^2 = (n\pi)^2 \gamma^{-2} + A\gamma^{-2} + B\gamma^{-2} n^{-2} + O(n^{-3})$$

for fixed l and suitably large n , where $\gamma = \int_a^b \rho^{1/2} \mu^{-1/2} dr$,

and A and B are constants which depend only on the shear velocity and the density in the mantle, and on the radius of the core.

By its definition, γ corresponds to the radial travel time of an S wave between the surface of the Earth and the core-mantle boundary. On the basis of the asymptotic formula given, γ can be approximated by:

$${}_n\gamma_l^{(m)} = \pi(2nm + m^2)^{1/2} ({}_n\sigma_l^2 - {}_n\sigma_l^2)^{-1/2}$$

for sufficiently small values of m , if second order effects in the asymptotic formula are negligible. The values of ${}_n\gamma_l^{(m)}$ obtained from this expression may thus be expected to reach an asymptote, which is γ , for a sufficiently large overtone number $n=n_0$. Though n_0 is a function of l , it will be approximately constant over a small range of l values. This has been verified extensively using model data. For a given l , we estimate n_0 as the overtone number n at which, and beyond which, the values of ${}_n\gamma_{l-k_1}^{(m)}$, ${}_n\gamma_l^{(m)}$ and ${}_n\gamma_{l+k_2}^{(m)}$ match sufficiently closely. For example, Fig. 1 shows values of ${}_n\gamma_l^{(m)}$ calculated from the Earth model HBI of Haddon and Bullen for $m=1$ and $l=2, 18$. The slight oscillations of the two curves about the asymptote in Fig. 1 indicate that second order

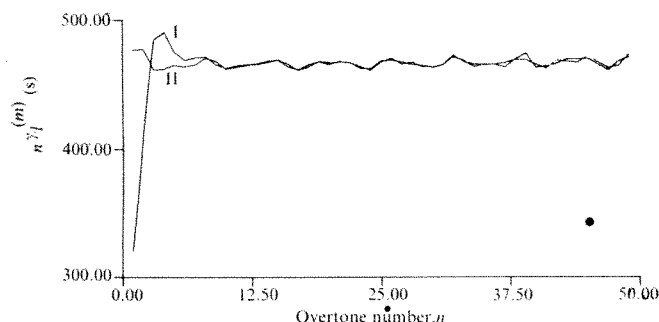


Fig. 1 Curves of ${}_n\gamma_l^{(m)}$ calculated from the Haddon and Bullen Earth model, HBI (ref. 2): I, $l=2$, $n=1$ to 49; II, $l=18$, $n=1$ to 49.

effects are not completely negligible in the asymptotic formula which applies to torsionals computed from the Earth model HBI (ref. 2). The structure in those second order effects is influenced by the position, size and sharpness of discontinuities in shear velocity, and by density in the mantle. The slight variation between the two curves for $n \geq 10$ is mainly because of numerical error in the computed periods.

The quantities γ and n_0 are obviously potentially important as constraints in the derivation of realistic Earth models by the inversion of torsional overtone data. Published observations of ${}_nT_l$ from free oscillation studies exist only for small overtone numbers², and calculations indicate that the asymptote has not been reached for these values of n . Some periods for ${}_nT_l$ up to $n=18$ have, however, been derived³ from data⁴ based on observations of the phase spectra of multiply-reflected S₀S waves. In particular, Brune and Gilbert³ list periods calculated for ${}_8T_{14}$, ${}_{12}T_{14}$, ${}_9T_{16}$, ${}_{14}T_{16}$, ${}_{10}T_{18}$, ${}_{16}T_{18}$, ${}_{11}T_{20}$ and ${}_{17}T_{20}$, with a standard error of approximately 1.2 s. Estimates of γ derived from these observations are listed in Table 1. The range of these four estimates is 1.22 s, and for the last three it is only 0.38 s. Despite the smallness of the sample, the consistency of these results strongly suggests that $n_0 < 10$ for $14 \leq l \leq 20$.

We have re-examined the data of Brune⁴ to see whether there is any additional information about the overtone structure. Included in example 4 of his Table 1 is a period of

Table 1 Values of ${}_n\gamma_l^{(m)}$ derived from the data of Brune and Gilbert

n	l	m	${}_nT_l$ periods (s)	${}_n\gamma_l^{(m)}$ periods (s)	${}_n\gamma_l^{(m)}$ (s)
8	14	4	109.64	75.81	469.30
9	16	5	97.18	65.10	470.14
10	18	6	87.29	75.03	470.44
11	20	6	79.27	53.54	470.52

147.659 s, which was not identified by Brune and Gilbert³, but which must correspond either to ${}_6T_{10}$ or ${}_6T_{11}$. Using Brune and Gilbert's³ periods for ${}_9T_{10}$ and ${}_{10}T_{11}$, this gives either ${}_6\gamma_{10}^{(3)} = 466.91$ s or ${}_6\gamma_{11}^{(4)} = 464.18$ s. Both are significantly smaller than the values of ${}_n\gamma_l^{(m)}$ in our Table 1, suggesting that $n_0 > 6$.

Brune and Gilbert acknowledge the possibility of bias towards continental values in the data, but point to the consistency with other normal mode data as an indication that there is no important regional bias. Although the value 470.36 s for the radial S time between the Earth's surface and the core-mantle boundary, obtained as the mean of the last three values in Table 1, does depend heavily on the 'structure' corrections applied by Brune⁴ to his data, the accuracy of the numerical calculation is unaffected. As far as the estimation of n_0 is concerned, we have found from model studies that the point at which the asymptote becomes discernible depends largely on the position, size and sharpness of discontinuities in the upper mantle, and that smoothing of the discontinuities tends to shift this point to smaller values of n . The periods of free oscillation modes are determined by the laterally averaged properties of the Earth, and it is certain that some smoothing of the velocity and density structure in the region of the discontinuities would result from this averaging process. Brune (personal communication) has pointed out that the Brune and Gilbert³ ${}_nT_l$ periods are strongly biased towards a model with no discontinuities, as a result of the fact that no reflections from any possible discontinuities were taken into account in the phase correlation. This no doubt accounts for the small scatter observed in the ${}_n\gamma_l^{(m)}$ values derived from these regional data. Our estimate of n_0 may therefore have to be revised when better data become available.

- Finally, we remark that the nature of the asymptotic structure in the ${}_nT_l$ periods will impose a quite delicate constraint on Earth models, especially in view of the effects of upper

mantle discontinuities noted above. An investigation of the significance of this constraint is now in progress.

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¹ Alterman, Z., Jarosch, H., and Pekeris, C. L., *Proc. R. Soc.*, **A252**, 80 (1959).

² Haddon, R. A. W., and Bullen, K. E., *Phys. Earth planet. Interiors*, **2**, 35 (1969).

³ Brune, J. N., and Gilbert, F., *Bull. seism. Soc. Am.*, **64**, 313 (1974).

⁴ Brune, J. N., *Bull. seism. Soc. Am.*, **54**, 2099 (1964).

H₂SO₄-HNO₃-H₂O ternary system in the stratosphere

RECENTLY, Friend *et al.*¹ reported a comprehensive laboratory study of a system of air containing trace quantities of H₂O, SO₂, NH₃ and O₃. By varying the proportions of these trace gases and the conditions of radiation of ultraviolet light and temperature, they obtained some detailed information for studying the mechanism of formation of stratospheric sulphate particles. They proposed a chemical model to interpret their laboratory observations and other observed features of stratospheric aerosol. The possible role of nitric acid, however, which is relatively abundant in the stratosphere², was not included in their investigation of stratosphere aerosol formation. Also, the possible solid phase of 75% H₂SO₄ by weight in water at -50° C, as discussed by Toon and Pollack³, was not taken into consideration in their proposed chemical model to interpret the formation mechanism of ammonium sulphate or ammonium persulphate particles in the stratosphere. Toon and Pollack examined some of the physical properties of nitric acid, sulphuric acid and ammonium sulphate in stratospheric thermodynamic conditions³. Using physical equilibrium phase diagram analysis techniques⁴, they compared the equilibrium vapour pressure over nitric acid solutions with observed water and nitric acid partial pressures in the stratosphere, and concluded that nitric acid cannot be present as an aerosol in the lower stratosphere. For sulphuric acid, they predicted that sulphuric acid aerosol particles in the stratosphere are 75% H₂SO₄ by weight in water, in agreement with observations by Rosen⁵. From the freezing curve of H₂SO₄ solutions⁶, Toon and Pollack pointed out that H₂SO₄ (75% in weight)-H₂O (25%) aerosol particles should exist in the lower stratosphere either as a solid or as a supercooled liquid. If most of the stratospheric sulphuric acid aerosol particles are in the solid phase, then the chemical models for the formation of ammonium sulphate using solution chemistry in sulphuric acid would not be applicable.

Here we present an order of magnitude estimate for the equilibrium vapour pressure over the ternary system H₂SO₄-HNO₃-H₂O to study the possibility of stratospheric aerosol formation involving HNO₃. Figure 1 shows the schematic equilibrium phase diagram for the H₂SO₄-HNO₃-H₂O ternary system at -50° C. The percentage labels refer to

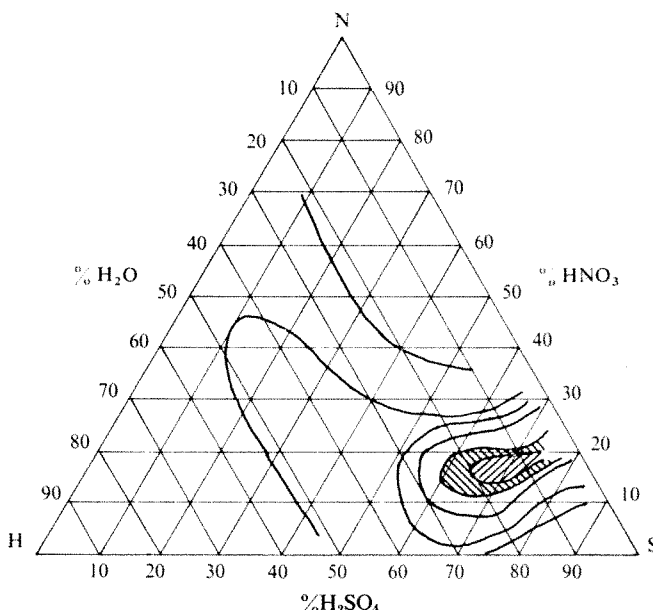


Fig. 1 Order of magnitude changes in vapour pressures for ternary system H₂SO₄-HNO₃-H₂O at -50° C. Vapour pressure curves are obtained by extrapolating from boiling point data. Each contour represents an order of magnitude change in vapour pressure. Note that in reading graph, weight percentages of the three components must add up to 100%. If graph is read incorrectly the sum will be 200%.

the % by weight. The total vapour pressure over the H₂SO₄-HNO₃-H₂O ternary system at -50° C is expressed as a function of the weight composition. The shaded area in the diagram indicates the lowest total vapour pressure region. To estimate the order of magnitude of these equilibrium total vapour pressure we have extrapolated the available data from the international critical tables⁷ to -50° C by using the Clausius-Clapeyron equation $dP/dT = L/Tv$, where P is the vapour pressure, T is the absolute temperature, v is the molar volume and L is the latent heat which is assumed to be constant over the temperature range. The vapour pressure obtained by this approximation will be larger than the values measured if the freezing point of these ternary systems is higher than -50° C, since the slope of the curve is proportional to L , and the curves will become steeper at the freezing point. From this crude approximation, we have found a region (Fig. 1, shaded area around 70-80% H₂SO₄, 10-20% HNO₃ and 10-20% H₂O) with the lowest vapour pressure. The vapour pressure in this region is about 10⁻⁴ to 10⁻⁵ times lower than the vapour pressure of 75% H₂SO₄ by weight in water. Toon and Pollack estimated the vapour pressure for 75% H₂SO₄ by considering the latent heat as a constant except at the melting point³ (-33° C). They found the vapour pressure for this composition at -50° C is about 10⁻⁴ mm Hg. For a supercooled liquid (without considering the freezing point) the vapour pressure for 75% H₂SO₄ at -50° C is about 10⁻³ mm Hg. Therefore, the vapour pressure of the shaded region is about 10⁻⁸ mm Hg or lower. We base this value on the vapour pressure of 75% H₂SO₄ rather than directly calculating it, because the vapour pressures calculated from ternary system data do not agree with binary system data⁷. This is also the reason why no numerical values for vapour pressure are presented in Fig 1. The curves are intended primarily to indicate the shape and depth of a region where aerosol growth is favoured. The accuracy of the results presented in Figs 1 and 2 depends upon two factors, the boiling point data for the ternary system⁷, and the accuracy of the extrapolation to -50° C. We feel that the results are accurate to within an order of magnitude.

For a more accurate estimate of the vapour pressure of this ternary system at -50° C, two factors should be taken

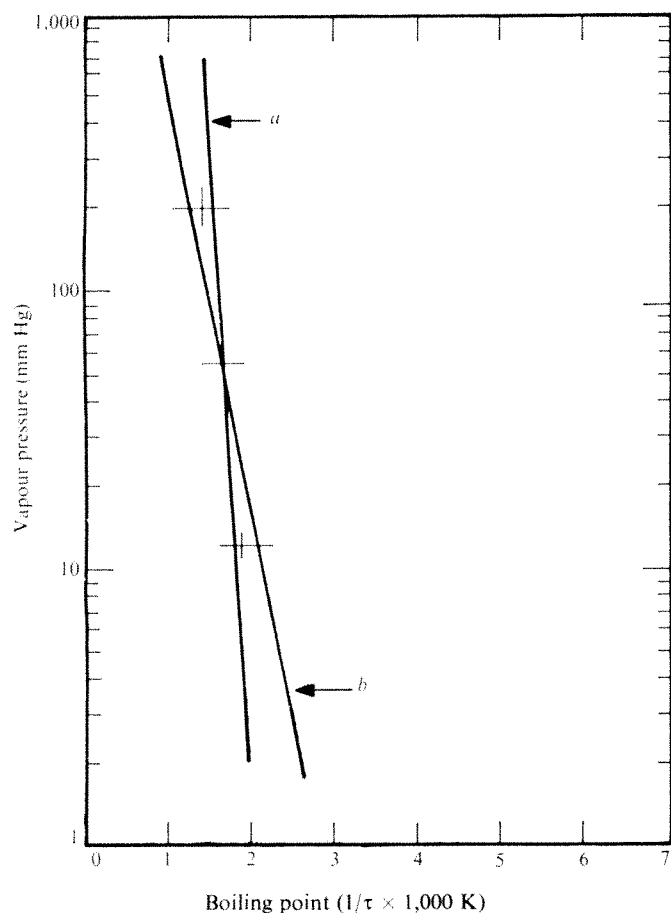


Fig. 2 Experimentally measured vapour pressure points of stratospheric aerosols determined from data obtained by Rosen¹. Also shown are vapour pressure curves for, *a*, the 75% H_2SO_4 -10% H_2O -15% HNO_3 ternary system, and *b*, the 75% H_2SO_4 -25% H_2O binary system.

into consideration. First, the latent heat L should be considered to be a function of temperature instead of a constant. In general, the latent heat decreases with increasing T , as it does for water. Then the vapour pressure at 50°C should be lower than what we have estimated. Second, the ternary system in the shaded region may exist as a solid. Since the vapour pressure of a solid is lower than that of a supercooled liquid at the same temperature, once again, our estimate for the vapour pressure could be too high. Therefore, as an order of magnitude estimate, it is probably safe to say that the vapour pressures for the ternary system H_2SO_4 - HNO_3 - H_2O with weight composition around 70-80% H_2SO_4 , 10-20% HNO_3 , 10-20% H_2O at -50°C are below the order of 10^{-8} mm Hg.

The measured partial pressure for HNO_3 in the stratosphere at -50°C (refs 2 and 3) is of the order of 10^{-7} to 10^{-6} mm Hg, for H_2O , the measured partial pressure is of the order 10^{-4} to 10^{-3} (ref. 3). Comparing the estimated total vapour pressure of the ternary system H_2SO_4 - HNO_3 - H_2O with the weight percentage mentioned above, there exists more than sufficient nitric acid and water vapour in the stratosphere to participate in ternary system aerosol formation at -50°C . Therefore, nitric acid should be found in stratospheric aerosols, provided H_2SO_4 is also present. Furthermore, we have compared the vapour pressure of the ternary system with data of stratospheric aerosols as observed by Rosen⁵ (Fig. 2). It is found that a ternary H_2SO_4 - HNO_3 - H_2O system with weight composition 75% H_2SO_4 , 10% HNO_3 and 15% H_2O , would conform to the experimentally determined vapour pressure curve as well as the one of 75% sulphuric acid solution fitted by Rosen⁵.

Because of the presence of HNO_3 , the freezing temperature of the sulphuric acid solution will be reduced. Based on the assumption of ideal solutions, we have estimated the decrease in the freezing temperature caused by adding 10% nitric acid to the sulphuric acid solution (with 75% H_2SO_4 and 25% H_2O). With the heat of fusion of 75% H_2SO_4 taken to be 4,360 cal mol⁻¹ (ref. 8), and the freezing temperature of the 67.5% H_2SO_4 , 22.5% H_2O and 10% HNO_3 ternary system is estimated to be -45°C . Since we have a real rather than an ideal solution, we expect the freezing temperature in the shaded region to be lower than -50°C .

We conclude that one cannot rule out the possibility that nitric acid may participate in aerosol formation in the stratosphere. More experimental measurements on the equilibrium vapour pressure for the ternary system H_2SO_4 - H_2O or other suitable ternary and multicomponent systems are strongly recommended. Furthermore, it is important that *in situ* measurements to determine whether stratospheric aerosol contain nitric acid are made.

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- ¹ Friend, J. P., Leifer, R., and Trichon, M., *J. atmos. Sci.*, **30**, 465 (1973).
- ² Lazrus, A. L., Gandrud, B., and Cadle, R. D., *J. appl. Meteorol.*, **11**, 389 (1972).
- ³ Toon, O. B., and Pollack, J. B., *J. geophys. Res.*, **78**, 7051 (1973).
- ⁴ Lewis, J. S., *Astrophys. J. Lett.*, **152**, L79 (1968); *Icarus*, **10**, 365 (1969).
- ⁵ Rosen, J. M., *J. appl. Meteorol.*, **10**, 1044 (1971).
- ⁶ Giauque, W. F., Hornung, E. W., Kunzler, J. E., and Rubin, T. R., *J. Am. chem. Soc.*, **82**, 62 (1960).
- ⁷ *International Critical Tables*, **4**, (McGraw-Hill, New York, 1928).
- ⁸ Rubin, T. R., and Giauque, W. F., *J. Am. chem. Soc.*, **74**, 800 (1952).
- ⁹ Kunzler, J. E., and Giauque, W. F., *J. Am. chem. Soc.*, **74**, 5271 (1952).

Potential method of geobarometry using quartz

As crystals grow from solution they may trap small amounts of the solvent and other solutes. It has long been known that minerals evolve gases on heating¹⁻⁴ and it has been assumed that, except for alteration during weathering, the gases are incorporated from the growth medium at the time of crystallisation. If the amount and composition of the gases trapped in minerals are to be used to make detailed geological interpretations it is essential that the location of the gas within the crystal structure is established. In minerals like carbonates and hydrates, 'gases' form part of the structure. Sites in which gas can occur include primary and secondary fluid inclusions, point defects, lattice dislocations, grain boundaries, and structure holes. Gas can also be adsorbed on crystal surfaces. Each site has its own characteristic temperature at which gas loss occurs, so that on heating a mineral the gases are released at different temperatures. This provides a method for distinguishing between gases from different sites.

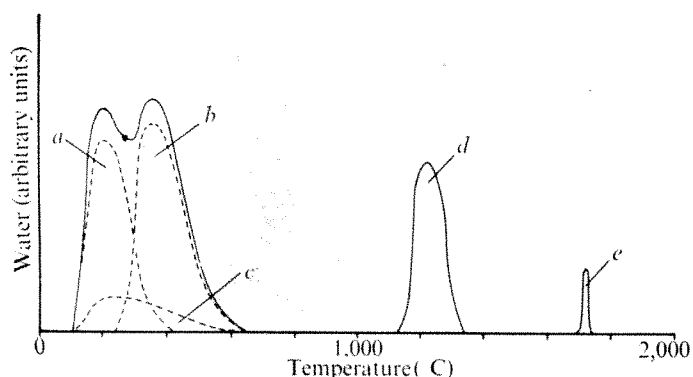


Fig. 1 Water release from quartz as a function of temperature. The solid line indicates the total response. This is made up of separate contributions coming from: *a*, secondary fluid inclusions; *b*, primary fluid inclusions; *c*, surface adsorption; *d*, grain boundaries and lattice defects; *e*, lattice water.

We have studied the location of water in quartz, using a mass spectrometer to monitor the release of water as a function of temperature. Quartz samples (0.5 g) were heated in a vacuum in outgassed mullite or alumina tubes connected to a heated stainless steel gas handling system which formed the inlet to a calibrated mass spectrometer⁵. A resistance-wound furnace was used to attain temperatures of 25–1,250° C, and a gas-torch furnace was used for temperatures up to 1,850° C. All the studied quartz samples showed three major episodes of water release (Fig. 1). In the temperature range 100–450° C water comes from fluid inclusion and from surface adsorption. (The detailed experimental support for this conclusion will be published elsewhere.) The second peak at 1,150–1,300° C is attributed to water released from grain boundaries and dislocations as they thermally anneal⁶. The remaining water is present in point defects and is not released until the quartz lattice is destroyed by melting. No release of water was associated with the α quartz– β quartz (573° C), quartz–tridymite (867° C) or tridymite–cristobalite (1,470° C) transitions. The quantities of water which remain in the liquid when the quartz melts are unknown, but they seem to be insignificant. Infrared studies of the glass produced show no adsorption characteristic of OH or H₂O. As the water vapour released by the

Table 1 Conditions of growth for synthetic quartz crystals

Pressure (N m ⁻² × 10 ⁻⁸)	Pressure (pound inch ⁻² × 10 ⁻³)	Top temperature (° C)	Bottom temperature (° C)	Growth rate (mm d ⁻¹)
1.38	20	349	399	0.67
1.59	23	349	399	1.30
2.14	31	350	396	2.21
2.41	35	352	396	2.26
2.76	40	374	396	2.61
2.90	42	371	410	2.29
3.10	45	360	416	2.64

sample is continuously pumped away through the mass spectrometer the partial pressure of water vapour over the melt remains low, and because of this the equilibrium amount of water in the melt should also be very small.

We have studied seven synthetic hydrothermal crystals of quartz, grown at pressures of 1.38×10^8 to 3.10×10^8 N m⁻² (45,000 pound inch⁻²), (Table 1), and natural hydrothermal quartz crystals from Brazil and Arkansas. In all cases the release of water as a function of temperature resembled the trend indicated by Fig. 1. As expected, the amount of water in fluid inclusions varied widely, even within a single crystal, but the water which was evolved at about 1,200° C showed a much smaller range. The amount of water released on melting crushed pieces (≈ 3 mm³) of single crystals of synthetic quartz varied from 1.2 ± 0.2 to 5.8 ± 0.2 cm³ of water vapour per gram

of quartz. Duplicate determinations confirmed the value of $0.2 \text{ cm}^3 \text{ g}^{-1}$. There is a clear trend of increasing water content with growth pressure (Fig. 2), indicating that the water incorporated into lattice sites is related to the partial pressure of water at the time of crystal growth. The distribution coefficients range from 0.8×10^{-5} to 2.0×10^{-5} ml (STP) per g atom. These are probably minimum values. We have not yet done any experiments to determine the variation of the distribution coefficients with growth temperature or with the composition of the growth medium. It has been reported that the presence of Li⁺ in the liquid decreases the total water content of the quartz⁷ but the particular location of the water was not investigated. Growth rate varies with growth pressure (Table 1), so there is a trend of increasing water content of the lattice with increasing growth rate. The data are insufficient to resolve completely the influences of pressure and growth rate on the water content. We feel that pressure is the more important because the 2.76×10^8 N m⁻² (40,000 pound inch⁻²) and 3.10×10^8 N m⁻² (45,000 pound inch⁻²) crystals were grown at the same rate and have very different water contents. Also, the 2.14×10^8 N m⁻² (31,000 pound inch⁻²), 2.41×10^8 N m⁻² (35,000 pound inch⁻²), and 2.90×10^8 N m⁻² (42,000 pound inch⁻²) crystals were grown at similar rates but have different water contents, which vary as a function of growth pressure.

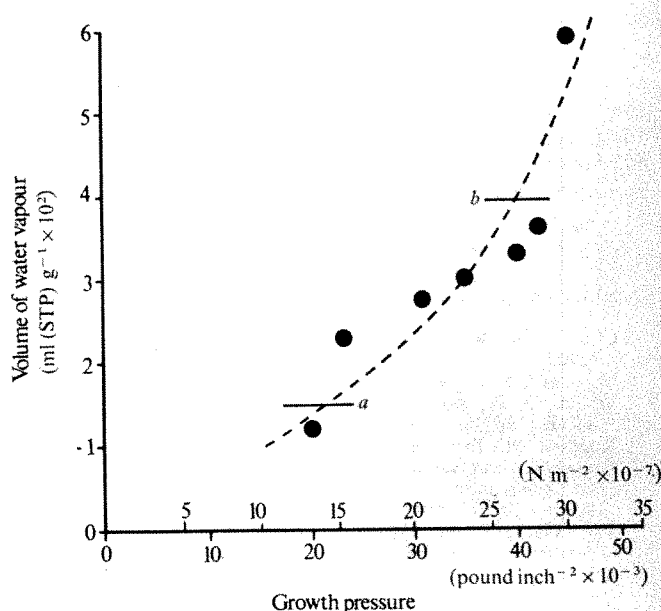


Fig. 2 Amount of water evolved from natural and synthetic quartz crystals between 1,650° C and the melting point, as a function of growth pressure. Values plotted at 1.38×10^8 N m⁻² (20,000 pound inch⁻²) and 3.10×10^8 N m⁻² (45,000 pound inch⁻²) are averages obtained from duplicate analyses. *a*, *b*, Water values obtained for the Arkansas and Brazilian quartz, respectively.

In most hydrothermal systems the partial pressure of water is approximately the same as to the total pressure, so a determination of the amount of water released at the melting point permits an estimate of the pressure at the time of crystal growth (assuming that the distribution coefficient is not appreciably altered by temperature, growth rate or composition of the growth medium). We have applied this potential method of geobarometry to the Brazilian and Arkansas quartz crystals, and it indicates pressures of 2.76×10^8 N m⁻² (40,000 pound inch⁻²) and 1.48×10^8 N m⁻² (21,500 pound inch⁻²) respectively. These values correspond to depths of 12,200 m (40,000 feet) and 6,550 m (21,500 feet), respectively, if the lithostatic pressure gradient has its usual value of 2.26×10^4 N m⁻² m⁻¹ (1.0 pound inch⁻² foot⁻¹). For a typical continental geothermal gradient of 30° C km⁻¹ these depths correspond to temperatures of 386° C for the Brazilian quartz, and 222° C

for the Arkansas quartz. If these temperatures and pressures are used to define a line of constant specific volume on a water phase diagram then the temperature at which the specific volume on a water phase diagram then the temperature at which the specific volume line intersects the two-phase boundary corresponds to the filling temperatures of the fluid inclusions. The calculated filling temperature for the Arkansas quartz is 138°C , which is in good agreement with the observed values of $100\text{--}150^{\circ}\text{C}$ (R. H. Konig, personal communication). For Brazilian quartz, filling temperatures ranging up to 190°C have been reported^{8,9}. The calculated value is somewhat high at 213°C . The calculated values were, however, obtained from the phase diagram for pure water and so they will be slightly higher than those calculated using data for saline solutions.

If the water content of the quartz lattice gives the partial pressure of water in the growth medium then this technique could probably be used with magmatic quartz to give the partial pressures of water in silicate melts. This method of determining partial pressures could also be extended to other minerals and to other gases if synthetic samples are available for calibrating the gas content as a function of the partial pressure in the growth medium.

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- ¹ Chamberlin, R. T., *Carnegie Institute of Washington, Publ.* 106 (1908).
- ² Shepherd, E. S., *Am. J. Sci.*, fifth ser., **35A**, 311 (1938).
- ³ White, D. E., and Waring, G. A., *Prof. Pap. US geol. Surv.*, **440-K** (1963).
- ⁴ Roedder, E., *Prof. Pap. US geol. Surv.*, **440-JJ** (1972).
- ⁵ Barker, C., and Sommer, M. A. in *Analytical Methods Developed for Application to Lunar Samples Analyses*, 56–70 (American Society for Testing Materials, Publication STP 539, 1973).
- ⁶ Van Vlack, L. H., *Material Science for Engineers* (Addison-Wesley, London, 1971).
- ⁷ Ballman, A. A., Laudise, R. A., and Rudd, D. W., *Appl. Phys. Lett.*, **8**, 53 (1966).
- ⁸ Ingerson, E., *Am. Miner.*, **32**, 375 (1947).
- ⁹ Skinner, B. J., *Econ. Geol.*, **48**, 541 (1953).

Fronts in the Irish Sea

A MARKED discontinuity in the sea surface temperature with horizontal gradients up to $1^{\circ}\text{C km}^{-1}$ is often observed in the western Irish Sea during the summer. This feature is the boundary between stratified and vertically mixed regimes¹. Here we describe observations by airborne radiation thermometer (ART) and conventional methods, and suggest a simple model accounting for the observed form and stable position of the front.

The region of low tidal energy to the west of the Isle of Man (IOM), (see Fig. 1) permits stabilisation of the water column by insolation during the early part of the summer. At the beginning of June stratification of this area is usually well established, and a rapid warming of the surface waters ensues. This leads to a sharp temperature discontinuity at

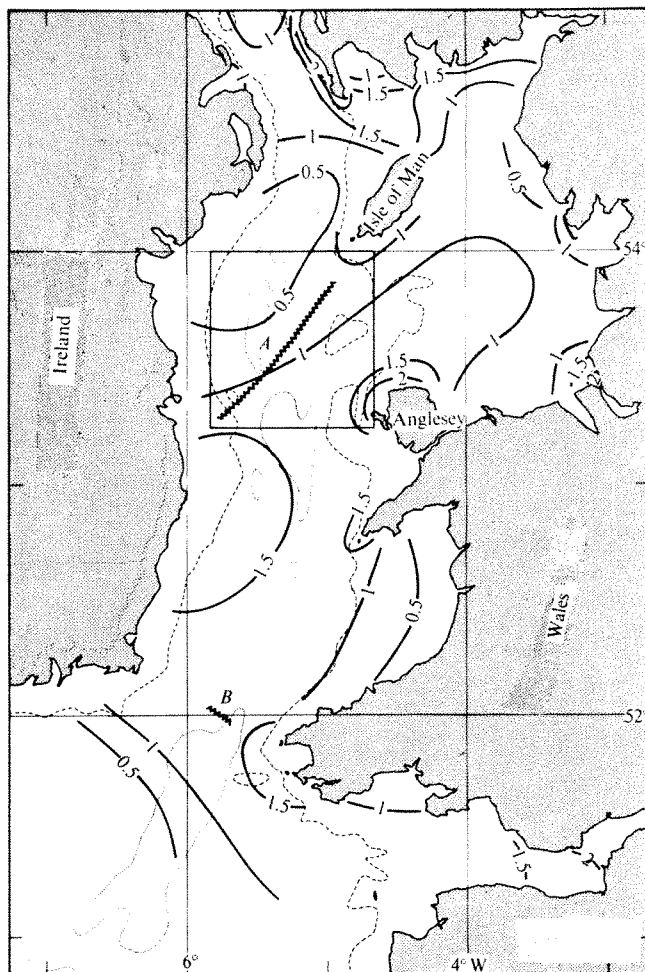


Fig. 1 Amplitude (m s^{-1}) of the discussed tidal streams (solid lines) in the Irish Sea at mean springs from Bowden¹. A, B, (heavy zigzag line), Observed positions of fronts. Depth contours are 100 m (dotted) and 50 m (dashed).

the surface, which may involve changes as large as 3°C in June and July. The temperature contrast is generally diminished in August as the temperature of the mixed regime approaches that of the surface layer in the stratified area. Strong horizontal temperature gradients remain, however, further down in the water column.

The position of the maximum surface temperature gradient, deduced from surface temperature surveys, is shown in Fig. 2. Surveys a–e are based on thermograph data supplemented by TSD stations. Coverage is limited as a result of the ship's slow speed (about 10 knots) and significant evolution of the temperature field resulting from surface heating and wind mixing may well have occurred during the survey. These difficulties preclude investigation of the detail form of the front from ship's observations alone, and we therefore proceeded to obtain alternative data using an ART. Surveys f and g (Fig. 2) represent results of ART surveys. In these two cases the position of the front has been corrected for tidal movements on the basis of the best available tidal stream data for the area. Errors which occurred in the earlier surveys because of tidal displacements, should not exceed 10 km.

The position of the front does not vary greatly (Fig. 2). With the exception of survey c all the observed positions lie within 10 km of a mean position. The front observed on this occasion was not well defined, and may have represented a transient condition during the establishing of stratification.

In aerial survey work, the radiation temperature of the sea surface is measured using a portable radiation ther-

nometer fitted to an aircraft. The instrument is calibrated in flight with a constant temperature black body, and simultaneous measurements of the true sea surface temperature at a depth of 3 m are made from a ship at selected points on the flight plan. With these controls the errors in the final estimate of sea surface temperature may be kept as low as $\pm 0.1^\circ \text{C}$. The aircraft operates at an altitude of approximately 150 m, from which the radiation sensor effectively samples an area of 50 m^2 of the surface of the sea.

Navigation is accomplished using the Decca Chain 3B/MP, with the operational legs flown approximately north-south along red lanes at intervals of $\sim 2 \text{ km}$ (3 Decca lanes). A tidal correction is applied to the data, which are then transformed on to a square grid with a mesh size of 2.54 km . This is accomplished for each grid point by meaning all the temperature values falling in a square of length 3.81 km which is centred on that point. The resultant smoothing helps to reduce the noise inherent in the raw data. Objective contouring is then accomplished by linear interpolation between adjacent grid points.

Figure 3a is the result of an ART flight on June 5, 1973. It shows a well developed frontal situation extending for almost 50 km through the survey area. The flight was made under nearly ideal conditions (clear sky, wind less than 10 knots) following a long period of almost calm weather. In the three days before the flight, the wind at Ronaldsway (IOM) did not exceed 15 knots and no winds greater than 20 knots were reported during the preceding two weeks. It therefore seems reasonable to assume that the observed position of the front represents an equilibrium undisturbed by wind mixing and transient motions produced by wind stress.

The relative stability of the front is also indicated by observations made by the aircrew during the flight. The pilot reported that the line of the front was clearly visible because of an accumulation of surface material in the vicinity of the maximum surface temperature gradient. The

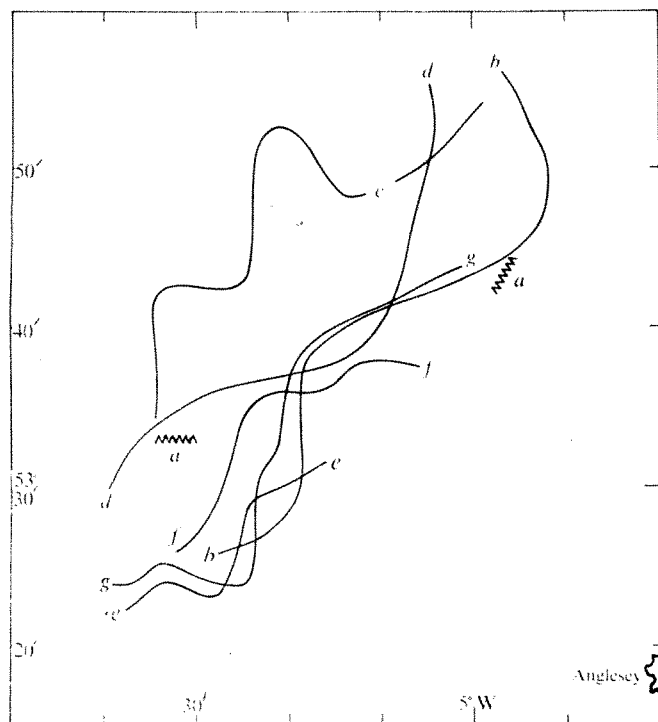


Fig. 2 Position of the maximum temperature gradient in the boundary front A as deduced from sea surface temperature surveys. Date of measurements: a, August 18-21, 1969; b, June 15-19, 1970; c, June 7-11, 1971; d, July 12-15, 1971; e, June 26-30, 1972; f, August 24, 1972; g, June 5, 1973. a, b, c, d, and e, thermograph and TSD data; f, and g, ART data.

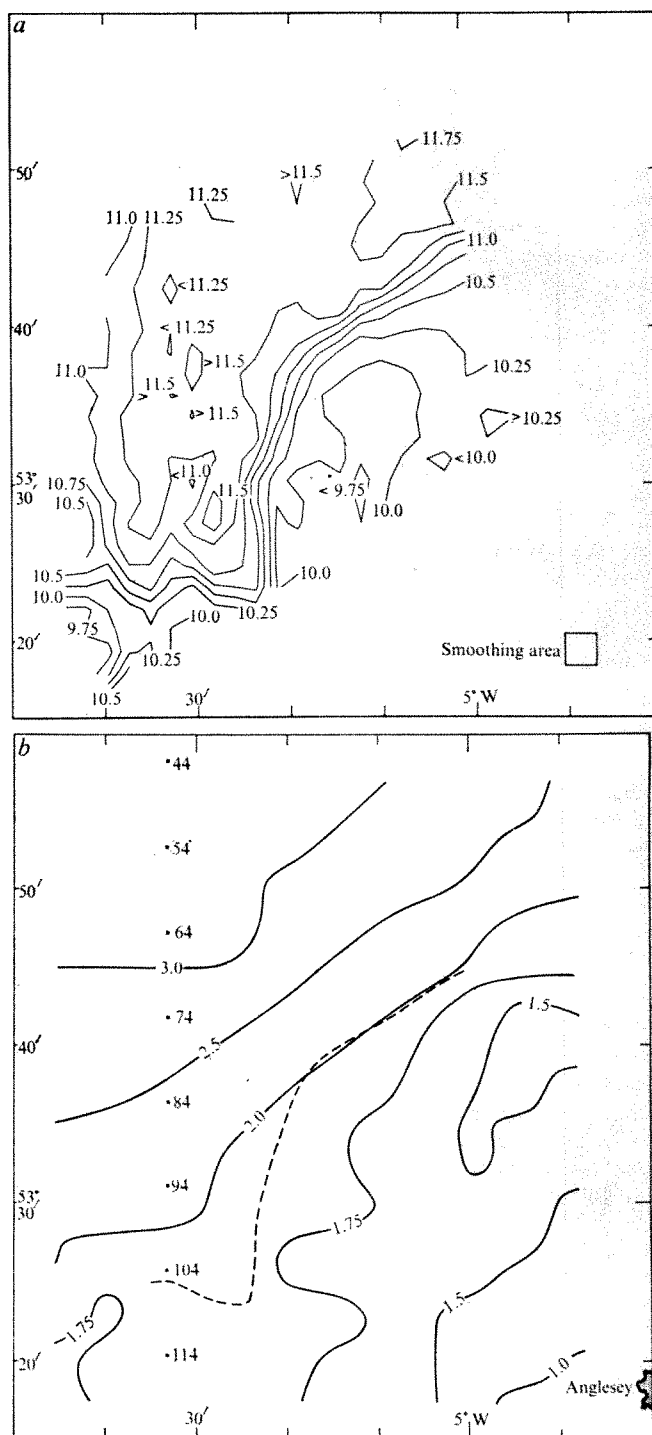


Fig. 3 a, Sea surface temperature distribution for June 5, 1973. Contours are at intervals of 0.25°C and have been drawn by an objective contouring programme. The data have been corrected for the observed difference between the radiation and thermograph temperatures of the sea surface. b, Contours of $\log_{10} h/u^2$ with h in metres and u in m s^{-1} . The dashed line shows the position of maximum gradient in Fig. 3a. The station positions shown (black squares) refer to the section shown in Fig. 4, which was worked in August 1970.

aircrew also noted changes in the colour of the sea and in the state of the sea as the front was crossed. The change in colour (greener in the mixed water, bluer in the stratified region) is probably a manifestation of the changes in the standing crop of phytoplankton in the frontal region. Estimates made in July 1971 indicate that the chlorophyll concentration decreases from $\sim 1.5 \mu\text{g l}^{-1}$ in the mixed water to $\sim 0.5 \mu\text{g l}^{-1}$ in the surface water of the stratified

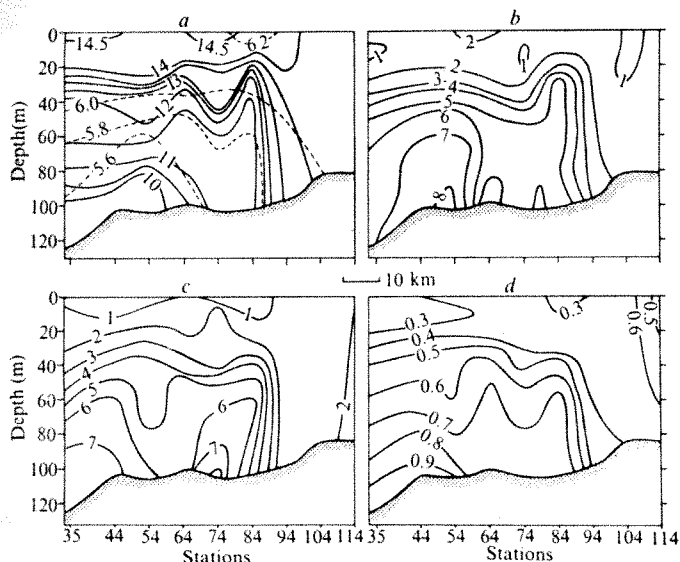


Fig. 4 North-south section across front A, showing the distribution of: a, temperature ($^{\circ}\text{C}$), (solid line), and oxygen (ml l^{-1} at NTP), (dashed line); b, silicates ($\mu\text{g atom silica l}^{-1}$); c, nitrate ($\mu\text{g atom nitrate nitrogen}$); d, phosphates ($\mu\text{g atom phosphate phosphorus}$). The positions of the stations are shown in Fig. 3b. Data taken August 17–20, 1971.

region. These changes in the standing crop of phytoplankton may be associated with limitations which are imposed by the availability of nutrients in the two regimes.

The distribution of nutrients in a section approximately perpendicular to the front is shown in Fig. 4 together with the corresponding temperature and dissolved-oxygen data. These results were obtained in mid-August 1971 but are representative of the distribution of nutrients throughout the summer months. The contrast in surface temperature is slight, but the front is clearly apparent in the intensified horizontal gradients below the surface mixed layer between stations 84 and 94. The distributions of all three nutrients, nitrate, phosphate and silicate, and the dissolved oxygen, are strongly correlated with the temperature structure. In the cases of nitrate and phosphate, the concentration in the mixed water (stations 104, 114) is generally higher than in the surface layer of the stratified regime, but large concentrations of all of the nutrients remain in the bottom water of the stratified regime, which is also characterised by depletion of the dissolved oxygen content. The higher values of nitrate and phosphate at the surface in the vertically mixed regime probably result from replenishment of nutrients from the bottom, which is not possible in the stratified area because vertical mixing is strongly inhibited.

The relative consistency of the observed position of the front suggests that the transition between stratified and unstratified regimes is essentially controlled by the level of tidal mixing. To examine this hypothesis we have considered the energetics of tidal mixing in a simple case.

Suppose that an amount, q , of heat is introduced into the surface of a water column of depth h , and an initial density ρ . This heat input produces a density change $\Delta\rho$ in a thin surface layer Δh . To mix the column vertically to a uniform density ρ' in a depth h' the potential energy must be increased by an amount $V_2 - V_1$ where

$$V_1 = \frac{1}{2} \rho g h^2 - \Delta \rho \Delta h g h + P h = \text{potential energy before mixing}$$

$V_2 = \frac{1}{2} \rho' g h'^2 + P h' = \text{potential energy after mixing; where } P = \text{atmospheric pressure.}$

Assuming that the density is a linear function of temperature, and neglecting salinity as a source of buoyancy input, then it is easily shown that $h = h'$ and

$$V_2 - V_1 = \alpha q g h / 2c$$

where α is the linear expansion coefficient and c is the

specific heat. For a rate of heat input Q , the demand for potential energy to maintain mixing would be

$$dV/dt = \alpha Q g h / 2c$$

Energy is lost from the tidal motion at a mean rate of

$$dE/dt = \tau_b u_b = k \rho |u_b|^3 = (4k/3\pi) \rho u_o^3$$

where u_b and τ_b are the velocity and shear stress respectively, near the bottom, and u_o is the amplitude of u_b . k is a constant in the assumed quadratic friction law. A fraction ϵ of this kinetic energy is considered to be available for increasing the potential energy of the water column so that at transition there is a balance defined by

$$h/u_o^3 = 8c\rho k\epsilon/3\pi\alpha g Q$$

If the area and time of interest are limited, Q can be regarded as constant. Then, providing ϵ and k are also constants, the locus of the front should be defined by a critical value of h/u_o^3 . Assuming that u_o is proportional to the observed surface tidal velocity amplitude u_s , h/u_s^3 can be used as the parameter which controls the formation of a front. Results for h/u_s^3 , based on the computations of a numerical model², are shown in Fig. 3b, along with the line of the front observed in June 1973. The front is approximately parallel to the contours of h/u_s^3 at a value between 65 and 100.

Assuming that ϵ and k are constant, this approach can be used to predict the occurrence of stratification and fronts for other parts of the continental shelf where the appropriate value of Q is known. As a more exhaustive test of our hypothesis we have a programme in hand to compare all the available data on stratification on the shelf, with the distribution of h/u_s^3 . We have already examined a front in the south-western approaches, the position of which (front B, Fig. 1) was predicted in this way. Observations in August 1972 revealed a transition occurring for $h/u_s^3 = 55$. Considering the uncertainties in u_s this value is not significantly different from that given for the A front. Provisional data for a less well defined transition in the Channel suggest a similar critical value of h/u_s^3 .

At present, relatively little is known of the residual velocity field in the vicinity of the front, largely because of the difficulties of making measurements of a velocity field which is being displaced tidally through an excursion of about 10 km. Observations with parachute drogues, which were tracked by radio in both the A and B fronts, show residual velocity vectors with speeds up to 10 cm s^{-1} approximately parallel to the front. Whether or not these velocities are generally in geostrophic balance with the density field remains uncertain on the basis of present knowledge. Such a balance is, however, suggested by data obtained in observations of the B front in August 1972, and if confirmed, would indicate longitudinal velocities of up to 50 cm s^{-1} in parts of the front.

We are grateful to Graham Savidge of the Marine Science Laboratories for the nutrient data in Fig. 4 and to the Meteorological Research Flight for providing the ART facilities.

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¹ Simpson, J. H., *Deep-Sea Res.*, **18**, 309 (1971).

² Hunter, J. R., thesis University College of North Wales, Bangor (1972).

³ Bowden, K. F., *The Physical Oceanography of the Irish Sea*, Fish. Invest., Series II, **XVIII**, No. 8 (1955).

Irregularities in dendrochronological calibration

Most of the 'wiggles' in the Suess curve¹, which is used for calibrating radiocarbon dates (denoted b.p.; sidereal dates denoted BP) in terms of dendrochronology, are likely to be artefacts². Curves with almost indiscernible fluctuations have been produced. There are, however, physical reasons for expecting some changes in the ^{14}C content of the atmosphere to have occurred and it is important to locate them by a method that does not depend either on intuition or on minimising deviations over the whole range of the curve⁴⁻⁶.

It should be possible to locate wiggles, if they exist, by using the estimated radiocarbon ages of samples themselves. If the samples have a uniform age distribution in sidereal time, then the apparent distribution in radiocarbon years would be uneven around the date at which the wiggle occurs; in other words, the apparent distribution of the samples in radiocarbon years would act as an indicator of the first differential of the slope of the calibration curve. Simulation trials showed that the uneven distribution can indeed be picked up, particularly if the sample ages are grouped at intervals which are small in comparison with the expected duration of the wiggle, but that the method is much more likely to be successful with a short lived sinusoidal irregularity than with a gradual but permanent change in slope. Stuiver (see ref. 7) has apparently tried a similar idea but his results are not available to us.

If the method is to be successful, there have to be enough sample ages available to ensure that, first, their distribution is uniform (although not necessarily constant) and, second that small regions of local high frequency are swamped. To meet these requirements we have considered all of the radiocarbon

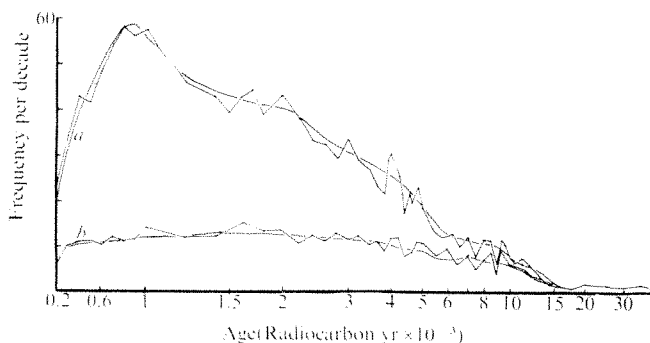


Fig. 1 Frequency distribution of published radiocarbon dates. *a*, All dates; *b*, geological dates. In each case a smoothed curve has been constructed (see text).

dates recorded in volumes 1 to 15(2) of the journal *Radiocarbon*. All of the dates used had the half life of 5,570 yr; deep sea samples were omitted but pelagic shell samples were not, because there is some doubt about whether any correction is necessary for shallow waters^{8,9}. No attempt was made to separate samples from the southern hemisphere. 'Archaeological' and 'geological' dates were collected separately. We recorded approximately 13,800 archaeological, and 12,500 geological, dates going back 50,000 radiocarbon years.

The archaeological dates are not so suitable for testing, because almost all occur in the first 4,000 yr b.p. They will be discussed elsewhere, and here we report findings from the geological dates, and from the combined series, going back to 40,000 yr b.p.

A smoothed frequency curve was constructed by applying first-order smoothing algorithms (IBM SSP routines SE13 and SE15) successively to the grouped totals for intervals of 200 yr. From this a Kolmogorov-Smirnov statistic was calculated and was used to provide confidence limits¹⁰ for a curve corresponding to at least 150 dates, or extending over at least 150 yr. The sections were made to overlap, so that each decade interval had more than one chance of being in the centre of the sectional

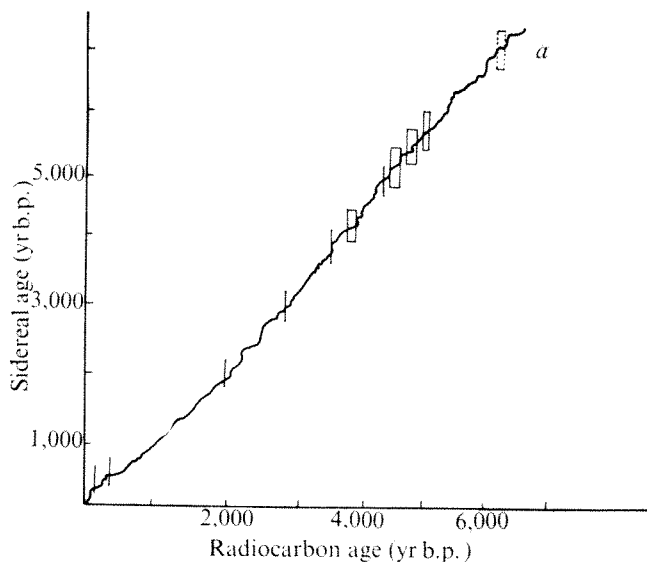


Fig. 2 Correlation of wiggles predicted by frequency analysis with MASCA calibration curve¹² *a*, geological series.

curve. The actual frequencies of the dates, grouped in decades, were then tested against this smoothed curve, using 1%, 5% and 10% probability criteria¹⁰.

It was possible to identify several probable wiggles. Beyond 14,000 yr b.p. the frequency of the dates is too low (Fig. 1) to pick up with certainty anything other than a possible irregularity at about 29,000 yr b.p. This correlates moderately well with the shift in the $^{18}\text{O}/^{16}\text{O}$ ratio reported from the Camp Century ice sheet between 30,000 and 35,000 yr BP¹¹. Excluding irregularities which extend over just a single decade, only five wiggles are significant at the 1% level: they occur in two major groups at about 3,800, 4,400 and 4,700 yr and 8,800 and 8,900 yr b.p. The archaeological data, at a low significance level, indicate two wiggles, at 200 and 400 yr b.p., which may be assigned to the original Suess and de Vries deviations. There is quite a good correlation between the irregularities predicted by this study and the small scale variations (which are nevertheless important for archaeological dating) in the recently published MASCA dendrochronological calibration curve (Fig. 2). The chief difference is the almost complete absence of wiggles in the third millennium BC.

There is also a remarkably good correlation between the irregularities found in this study and Bray's¹³ estimates of the dates of maximum glacial retreat (we assume that his dates are uncorrected radiocarbon years). It is therefore likely that the most intense and long lasting wiggles predicted by us—at about 8,600–9,000 yr b.p.—correspond to the large climatic change at the beginning of the present interglacial. This could be used to try to obtain an absolute dating for this irregularity. If the slope of the dendrochronological calibration curves from 1,000 to 5,000 yr BP were to continue back indefinitely, the period 8,600 to 9,000 yr b.p. (radiocarbon) would correct to 10,500–11,000 yr BP. There is some difficulty in obtaining estimates of climatic events which do not depend on uncorrected radiocarbon dating. The oxygen isotope profiles published by Johnsen *et al.*¹¹ show that the Allerød change was completed by about 11,000 yr BP in the Greenland ice core, which fits rather well with our extrapolated correction. On the other hand, Stuiver's curve relating the age determined by the ^{14}C method to varve counts⁷, shows a large deviation in both Swedish and Minnesota varves at about 8,000–8,500 yr BP. If these are sidereal dates, identical with the irregularity under discussion, the dendrochronological correction would be zero at about this time. On this graph, however, the Swedish varves show absolutely no shift in $\Delta^{14}\text{C}$ over the period from 8,500 to 12,500 yr BP (ref. 14), which does not seem to correlate with the $^{18}\text{O}/^{16}\text{O}$ record at

Table 1 Significant irregularities (radiocarbon yr) in frequencies of geological ^{14}C dates

Date b.p.	Significance level	Date of temperature maximum (from Bray ¹³)
890	$P < 0.1$	900
1,620	0.05	1,600
3,180	0.1	—
3,350 – 3,390	0.1	3,300
*		3,800
4,200 – 4,210	0.01	—
4,460 – 4,470	0.05	4,400
—		4,500
4,660 – 4,690	0.05	—
—		5,800
—		5,900
6,000, 6,060		—
6,550 – 6,640	0.05	6,500
†		7,100
8,600 – 8,800	0.01	8,500
8,910 – 9,050	0.01	8,800
—		9,600
—		9,700
10,140 – 10,190	0.05	—
10,990	0.05	11,110
11,980 – 12,020	0.05	11,800
—		12,250
12,590 – 12,640	0.1	—
—		13,400
—		14,100
14,640 – 14,790	0.1	14,700 – 15,500
—		16,100, 16,800
—		18,000, 18,300
—		19,100, 20,300
27,430 – 27,490	0.1	—
27,630 – 27,660	0.1	—

Estimates of significance are conservative (see ref. 10).

* Irregularity at 3,770 – 3,810 yr b.p. in combined data ($P < 0.01$).

† Irregularity at 6,910 – 7,020 b.p. in archaeological data ($P < 0.05$).

all. Moreover, this interpretation of the Swedish data has been disputed¹⁵. At present, we prefer to correlate our chart of irregularities with the oxygen isotope profile, partly because of the coincidence at 30,000 yr BP and (with the Southern Hemisphere profile) at 4,000 yr BP, and partly because a massive ^{14}C correction factor would explain the present dearth of dates for the Mesolithic in the period 8,000 to 10,000 yr BP (J. B. Campbell, personal communication). This identification is very tentative.

Nonparametric search techniques require a point estimate for each event that forms part of the frequency, whereas radiocarbon determinations, by their nature, include a statistical uncertainty. We are aware that in converting from one to the other we have lost the information contained in the estimate of standard deviation accompanying each published date, but it was inevitable that many different kinds of sample, with different inherent errors, should be brought together, if enough dates were to be assembled to cover adequately a long time-span. No single laboratory could have provided enough dates for this purpose. We feel that our approach was justified in that, up to about 7,000 yr b.p. (after which archaeological dates become very infrequent), there is substantial agreement between the dates of wiggles predicted by the geological and archaeological dates taken separately, particularly in the period of intense disturbance from 4,000 to 5,000 yr b.p. It is likely that some smaller wiggles may have become blurred, but in any case we do not think that the method described here would pick up the recently reported decadal rhythms. We wished in the first instance to see whether independent evidence could be provided for any of the fluctuations of about a century's duration, which characterise the Suess curve. For a similar reason we included dates from both the Old World and the New World, and from both hemispheres. There has been an increasing tendency for archaeologists to point out that the dendrochronological evidence for ^{14}C calibration is at present derived from a relatively small area of the United States, and to draw attention to apparent discrepancies in certain millennia and in certain areas

of the Old World (for example, the Mediterranean basin). It therefore seemed important to establish 'global' wiggles, although it would undoubtedly be interesting in later studies to test separately dates from all quadrants of the earth.

We are satisfied that there are about four serious wiggles in the dendrochronological calibration curve, concentrated in the second and third millennia BC. It should not be difficult to determine the shapes of the irregularities by standard statistical techniques, now that the regions in which to search have been identified.

We would like to thank Dr H. E. Suess for discussion on this topic.

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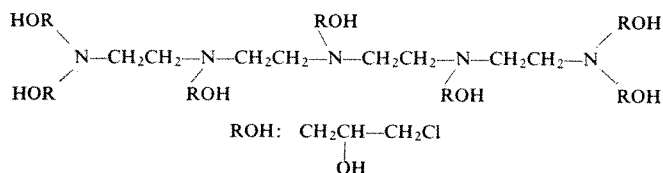
- ¹ Suess, H. E., in *Radiocarbon Variation and Absolute Chronology* (edit. by Olssen, I. U.), 303, (Wiley, New York, 1970).
- ² Ottaway, B., and Ottaway, J. H., *Nature*, **239**, 512 (1972).
- ³ Switsur, V. R., *Antiquity*, **47**, 131 (1973).
- ⁴ Ralph, E. K., and Michael, H. N., *Proceedings of the eighth International Conference on Radiocarbon Dating*, 28 (1972).
- ⁵ Damon, P. E., Long, A., and Wallick, E. I., *Proceedings of the eighth International Conference Radiocarbon Dating*, 45 (1972).
- ⁶ Clark, R. M., and Renfrew, C., *Nature*, **243**, 266 (1973).
- ⁷ Stuiver, M., *Nature*, **228**, 454 (1970).
- ⁸ Olssen, I. U., *Earth-Sci. Rev.*, **4**, 203 (1968).
- ⁹ Buddemeier, R. W., and Hufen, T. H., *Radiocarbon*, **15**, 70 (1973).
- ¹⁰ Hoel, P. G., *Introduction to Mathematical Statistics*, third ed. 345 (Wiley, New York, 1962).
- ¹¹ Johnsen, S. J., Dansgaard, W., Clausen, H. B., and Langway, C. C., *Nature*, **235**, 429 (1972).
- ¹² Ralph, E. K., Michael, H. N., and Han, M. C., *MASCA Newsletter*, **9**, 1 (1973).
- ¹³ Bray, J. R., *Nature*, **237**, 277 (1972).
- ¹⁴ Tauber, H., in *Radiocarbon Variations and Absolute Chronology* (edit. by Olssen, I. U.), (Wiley, New York, 1970).
- ¹⁵ Morner, N. A., in *Radiocarbon Variations and Absolute Chronology* (edit. by Olssen, I. U.), (Wiley, New York, 1970).

Polymer structures and turbulent shear stability of drag reducing solutions

THE phenomenon of frictional drag reduction in the turbulent flow of Newtonian fluids, by the addition of high molecular weight polymers, has been known for some time. Because of the great potential it offers in various technological applications this phenomenon has generated considerable engineering interest. Unfortunately, when a limited volume of polymer solution is continuously exposed to mechanical shearing action, either by repeated use¹ or by passing through long pipelines², drag reduction rapidly declines, indicating a rapid breakdown of the polymer molecules³. This polymer degradation greatly limits the application of drag reducing polymers. When considering the use of high molecular weight polymers in operating systems their shear stability must be considered as equally important as their effectiveness in drag reduction. Polymer degradation effect is likely to be caused by the scission of molecular entanglements or the breaking of individual molecules induced by the shear stresses associated with very high local shear rates⁴. But previous studies were made mainly with linear polymers such as polyethylene oxide (PEO) and polyacrylamide (PAM), because a linear structure was believed to be most effective⁵. We have studied the effect of polymer structure on turbulent drag reduction by synthesising drag-reducing agents of different structures⁶. Here we present the results of an investigation of the degradation behaviour of a highly branched

PAM, and compare the results with those observed with ordinary linear polymers.

The branched PAM sample was synthesised by grafting PAM chains on to a small, nucleus molecule. The nucleus was prepared by reacting tetraethylene pentamine with epichlorohydrin. A typical structure of this backbone molecule may be represented as follows:



There was a total of seven potential chain-grafting sites in this molecule. Through the redox reaction of the molecule with ceric ion, the graft polymerisation of acrylamide was initiated on the ROH groups⁷. The polymerisation started instantaneously and proceeded at a much higher rate than with the ceric ion alone. This suggested that the probability of ungrafted acrylamide polymerisation occurring is negligibly small. The polymerisation was carried out in aqueous solution using the redox system under a nitrogen atmosphere for 5 h at 32° C, resulting in a gel-like, transparent material which was completely water-soluble. The intrinsic viscosity of this PAM compound was 6.13 dl g⁻¹, leading to an estimated molecular weight of 1.7×10^6 (using the relationship in ref. 8). But as this relationship was developed for linear PAM, it may not be relevant to the present highly branched structure. The details on the polymerisation will be published elsewhere.

The drag-reducing capability of the branched PAM sample was characterised in a capillary flow system of diameter 0.1575 cm. A detailed description of the apparatus was presented elsewhere⁹. Using the characterisation technique developed

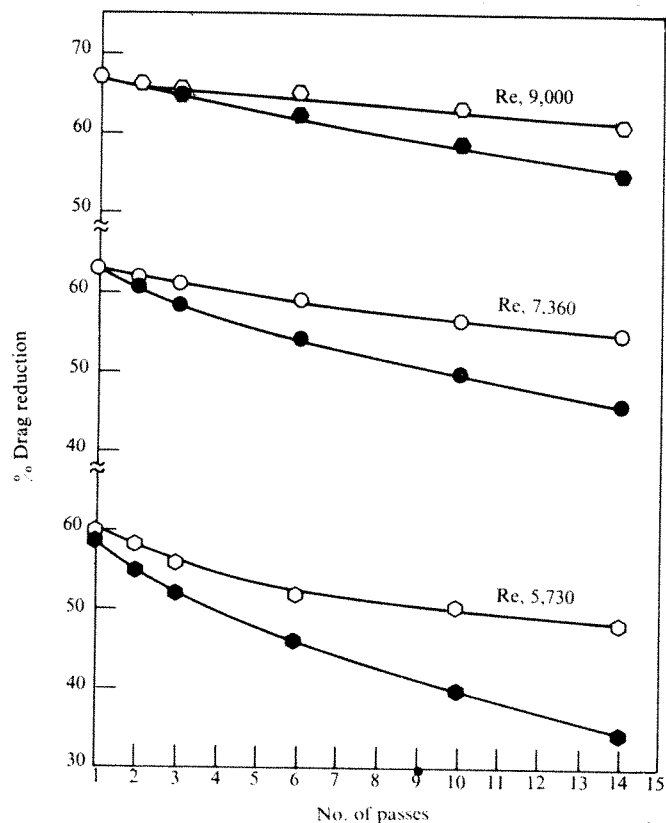


Fig. 1 Degradation behaviour of the 100 p.p.m. solutions of PEO (closed symbols) and branched PAM (open symbols).

earlier¹⁰, the effectiveness of the branched PAM was evaluated at a fixed Reynolds number of 9,000. On a unit concentration basis at infinite dilution this compound had an effectiveness measure (Eff) = 4.14 in units of percentage drag reduction per parts per million (%/p.p.m.). The degradation behaviour of commercial linear PEO and PAM compounds with similar effectiveness was compared with that of the branched PAM. Polyox-WSRN-3000 (Union Carbide Corp.; Eff = 4.15 %/p.p.m.) and Magnifloc 905N (American Cyanamid Co.; Eff = 3.78 %/p.p.m.) were used. The solutions were repeatedly passed through the capillary and the change in percentage drag reduction recorded for each pass. These measurements provided

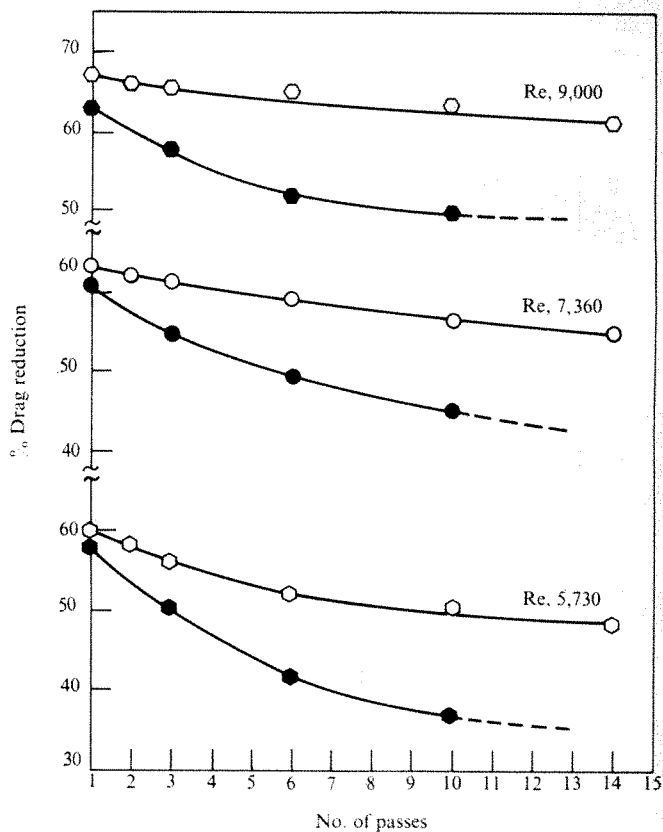


Fig. 2 Degradation behaviours of the 100 p.p.m. solutions of linear PAM (closed symbols) and branched PAM (open symbols).

a relative measure of the shear stability of these compounds of different molecular structure.

The degradation behaviour of 100 p.p.m. solutions of the branched PAM, the linear PAM and PEO are shown in Figs 1 and 2. The data show comparisons on an equal concentration basis with approximately equal initial percentage drag reduction. At three different Reynolds numbers, the solutions of linear PAM and PEO showed a very rapid decline in percentage drag reduction with increasing number of passes through the capillary. The rate of the decrease in percentage drag reduction is much slower for the branched PAM. Such a contrast in degradation behaviour clearly demonstrates the superior shear stability gained by molecular branching. Physically, this may be explained by the molecular scission concept. Recent experimental results of a degradation study of polyacrylamide in a high shear flow¹¹ showed general agreement with the theoretical prediction of Bueche's midpoint break theory¹². According to this theory, polymer chain breaking does not take place at random along the chain but occurs predominantly in the central portion of the chain where the extending forces are maximum. In the case of a linear polymer, this kind of chain breakage will immediately reduce the polymer molecular weight to approximately one-half. The drag reducing ability of

the compound is therefore greatly reduced because of the strong dependence of drag reduction on polymer molecular weight⁶. In the case of the branched PAM sample, however, the reduction in molecular weight is apparently less drastic probably because the shear forces acting on the polymer are distributed among the individual chains. Any reduction in molecular weight, when it occurs, will probably be of the order of a chain length rather than one-half of the molecule.

It can be seen that the improvement of the shear stability by branching is more pronounced at lower Reynolds numbers. If turbulent drag reduction is considered as a manifestation of the intensive interactions between polymer molecules and the dissipative turbulent eddies, this Reynolds number effect may be explained as follows. In the dissipation range, the Komolgorov eddy scale, η , is related to the kinematic viscosity ν and the dissipation ϵ by $\eta = (\nu^3/\epsilon)^{1/4}$. It can easily be shown that¹³

$$\eta/d \sim (\nu^3 d^3 / u^3)^{1/4} / d = (\nu / u d)^{3/4} = Re^{-3/4}$$

or

$$\eta \sim d / Re^{3/4}$$

where d is the capillary diameter and Re the Reynolds number. Since d is fixed in the present case, the turbulent dissipative eddies have relatively smaller scales at higher Reynolds numbers than those in flows of lower Reynolds numbers. The degraded polymer molecules, presumably having smaller coil sizes, may still be able to interact effectively with the dissipative eddies in flows of higher Reynolds numbers, but have become less effective in lower Reynolds number flows.

The present results also suggest that the linear PAM solution shows as poor shear stability as the PEO solution. This is in contrast to the general belief that the PAM solution is more shear stable than the PEO solution in turbulent flows. From the chemical point of view, a better shear stability for PAM can be possible only if some turbulence-induced oxidation is involved in the degradation process, as proposed by White¹⁴, but this is not obvious according to the present data. We showed that the presence of counter-ions surrounding the domain of an ionic polymer could greatly improve the polymer shear stability in turbulent flow¹⁵. Most of the previous PAM degradation studies used commercially manufactured compounds. These materials, after long handling and storage periods, could very possibly have become partially hydrolysed to form copolymers of PAM and polyacrylic acid. This is especially true if the materials were exposed to moisture for a long time. This variation, as we have shown¹⁵, could very well introduce higher resistance to mechanical shearing to these so-called PAM compounds.

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- ¹ Ellis, A. T., Ting, R. Y., and Nadolink, R. H., *J. Hydronautics*, **6**, 66 (1972).
- ² Paterson, R. W., and Abernathy, F. H., *J. Fluid Mech.*, **43**, 689 (1970).
- ³ Gadd, G. E., in *Encycl. Polym. Sci. Tech.*, **15**, 224 (Interscience, New York, 1971).
- ⁴ Barnard, B. J. S., and Sellin, R. H. J., *Nature phys. Sci.*, **236**, 12 (1972).
- ⁵ Hoyt, J. W., and Fabula, A. G., *Fifth Nav. Hydrodynamics Symp., ONR*, 947 (1964).
- ⁶ Kim, O. K., Little, R. C., and Ting, R. Y., *AIChE Symp. Ser.*, **69**, 39 (1973).
- ⁷ Mino, G., Kaiserman, S., and Rasmussen, E., *J. Polym. Sci.*, **38**, 393 (1959).
- ⁸ Sholton, W., *Makromolec. Chem.*, **14**, 169 (1954).
- ⁹ Little, R. C., and Wiegard, M., *J. appl. Polym. Sci.*, **14**, 409 (1970).

- ¹⁰ Ting, R. Y., and Little, R. C., *J. appl. Polym. Sci.*, **17**, 3345 (1973).
- ¹¹ Abdel-alim, A. H., and Hamielec, A. E., *J. appl. Polym. Sci.* (in the press).
- ¹² Bueche, F., *J. appl. Polym. Sci.*, **4**, 101 (1960).
- ¹³ Tennekes, H., and Lumley, J. L., *A First Course in Turbulence* (MIT Press, Boston, 1972).
- ¹⁴ White, D. A., *Chem. Engng Sci.*, **25**, 1255 (1970).
- ¹⁵ Ting, R. Y., and Little, R. C., *Nature phys. Sci.*, **241**, 42 (1973).

Hardening of immersed metals by ultrasound

DURING electrodeposition in an ultrasonically agitated solution, the hardness of copper anodes increases, and sometimes they perforate in a nonuniform manner¹. Deposits plated from electrolytes which have been subjected to ultrasound have a higher hardness than those produced from still solutions^{2,3}. Here we report on the change in hardness of a wide range of metals.

We used strips of metal about 10 cm long, 1 cm wide and 0.1 cm thick. The majority of these were annealed for 2 h at a temperature of about 60% of the melting point (in degrees absolute) and the hardness (HV) was measured with a microhardness tester using a load of 25, 50 or 100 g. The same load was used in all the measurements of any one metal.

The metal strips were subjected to ultrasound by immersing them in tap water in an ultrasonic tank. The specimens were treated individually to prevent shielding and were clamped centrally and perpendicularly about 1 cm above a 12.5 cm diameter transducer probe. The ultrasonic field, of frequency 13 kHz and intensity 930 W m⁻², was applied for 5 min. The specimens were then removed and the hardness of an area in the centre of the strip was measured immediately. This procedure was repeated keeping the metals in the ultrasonic field for increasing intervals of up to a maximum of 1,500 min. Tin and zinc, however, perforated before the end of this time. The hardness was measured again one month after the completion of the experiment to check whether any change had occurred during storage at room temperature.

The changes in the hardness of the various metals during the time of ultrasonic bombardment are shown in Fig. 1 and the relevant data are given in Table 1.

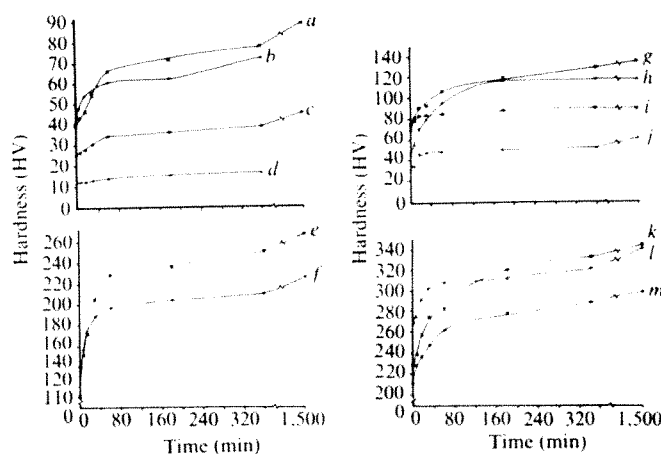


Fig. 1 The effect of ultrasonic agitation on the hardness of immersed metals. Metals with the same crystallographic structure are grouped together wherever possible: a, Mg; b, Zn; c, Cd; d, Sn; e, mild steel; f, Ni; g, brass; h, Cu; i, Nb; j, Al; k, Zr; l, Ti; m, Ta.

Table 1 Changes in hardness of immersed metals produced by ultrasound and during subsequent storage

Metal	Crystal structure	Hardness HV			Hardness changes (%)		Storage temperature (K) Melting point (K)
		Initial	After 1,500 min agitation	After 1 month storage	After 1,500 min agitation	After 1 month storage	
Aluminium	f.c.c.	32†	61	43	+ 91	-29	0.31
Copper	f.c.c.	47†	117	93	+149	-21	0.22
Nickel	f.c.c.	110†	225	190	+105	-16	0.17
Brass 70/30	f.c.c.	75†	134	113	+ 79	-16	0.23
Stainless Steel 18/8	f.c.c.	249‡	450	294	+ 81	-35	0.17
Niobium	b.c.c.	70†	89	75	+ 27	-16	0.11
Tantalum	b.c.c.	210‡	299	276	+ 42	- 8	0.09
Mild steel	b.c.c.	142†	251	169	+ 77	-33	0.16
Cadmium	c.p.h.	25.5*	45	39	+ 76	-13	0.49
Magnesium	c.p.h.	40*	92	69	+130	-25	0.32
Zinc	c.p.h.	41†	72§		+ 76		
Zirconium	c.p.h.	227‡	345	281	+ 52	-16	0.14
Titanium	c.p.h.	270‡	342	274	+ 27	-20	0.15
Tin	Tetragonal	11.8*	16.0§		+ 36		

f.c.c., face-centred cubic; b.c.c., body-centred cubic; c.p.h., close-packed hexagonal. Microhardness load: *25 g; †50 g; ‡100 g. §After 360 min.

The increase in hardness produced by ultrasound was not uniform but varied considerably over the surface; this was particularly noticeable on some metals which perforated during bombardment. Variations were also observed when the microhardness was measured at different areas on the surface, and because of this the readings were always taken on the same central area of the specimen. These variations may result from the presence of a standing wave which would form nodes and antinodes in the water above the probe.

The hardness of all the metals except tin, which showed only a slight change, increased rapidly at first and then tended to reach a steady value; a further increase in hardness was usually observed over the period of bombardment 360–1,500 min (Fig. 1).

There are certain similarities in the patterns of the percentage increase in hardness of metals which have the same crystallographic structure (Fig. 1). Face-centred cubic metals increased by about 80–150%, whereas body-centred cubic metals showed a much lower change of 27–77% and the change in close-packed hexagonal metals ranged from 27–130%. Tantalum, which is much purer than niobium showed less resistance to dislocation movement and underwent a higher increase in the percentage hardness. The lattice of brass was more strained than that of copper, which resulted in a much lower increase.

The increase in hardness of a metal exposed to ultrasound may result from the cavitation that occurs at the metal surface. The pressure in cavitation bubbles before they collapse has been calculated to be several thousand atmospheres⁴ and, as the cavities implode, shock waves deform and harden the surface. Thus the lattice of the material at the surface of the metal may be distorted, and internal strains may be present in the surface layers.

A very significant decrease in the hardness during storage was observed with many of the metals, and in some cases, such as Ti, the value dropped to almost the initial reading. This softening process is too high to be a result of annealing at room temperature but could be caused by recovery at room temperature. There is a definite trend in metals with the same crystal structure (Table 1) between the percentage decrease and the ratio of storage temperature to melting point. Greater percentage decreases occur with the higher ratios which suggests that it is the recovery of point defects which gives the observed softening.

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- 1 Walker, C. T., and Walker, R., *Nature*, **244**, 141 (1973).
- 2 Walker, C. T., and Walker, R., *Electrodeposition and Surface Treatment*, **1**, 457 (1972/73).
- 3 Walker, R., and Clements, J. F., *Metal Finish. J.*, **16**, 100 (1970).
- 4 Rayleigh, J. W. S., *Phil. Mag.*, **34**, 94 (1917).

Anion coordination geometry as a determining factor in crystallographic shear

ATTENTION has been drawn to some of the consequences of anion polarisation in one crystallographic shear (CS) system, the reduced titanium oxides¹; particularly to the effect on crystal energy and cation coordination geometry. The existence of polarisation in typical CS structures is indicated by the values of certain common properties—the dielectric constant and refractive index are both high—by the more direct, experimental determination of net ion charges in some double CS (column, or so-called 'block') structures², and by the characteristic persistence of the cation coordination geometry³, which is invariably octahedral.

Polarisation produces some directed covalency. The implications of this for anion coordination geometry are immediate and simple, but far reaching. For a second row element of the periodic classification, such as oxygen, the maximum coordination number for covalent bonding is $CN(O) = 4$. Thus, in ordered, reduced-rutile structures there can be only one set of CS planes: this raises the coordination number of anions at the CS planes from $CN(O) = 3$ in the rutile parent, to the maximum $CN(O) = 4$. On the other hand, when an ReO_3 -type structure is reduced by CS, $CN(O)$ is increased from 2 to 3 for one set of CS planes (slab structures). A second, intersecting set of CS

planes is possible before the maximum $CN(O) = 4$ is attained, thus producing column structures. True block structures—with three intersecting sets of CS planes—could not exist, because $CN(O) = 5$ would be produced (as it would in column structures derived from a rutile type parent).

Because $CN(O) \leq 4$, the minimum stoichiometry for any CS structure MO_x will be $x_{min} = 1.0, 1.5, 2.0$ for cation coordination numbers $CN(M) = 4, 6, 8$, respectively. (This considers only the $CN(M)$ values appropriate to the common regular cation coordination polyhedra: tetrahedron, octahedron and cube.) The stoichiometry of the parent structure must be $x > x_{min}$. Therefore, for octahedrally coordinated cations, the most reduced CS derivative will have $x = x_{min} = 1.5$ (compare with corundum type M_2O_3). On the other hand, CS in reduced fluorite type oxides is immediately ruled out, even though their bonding is of a covalent character⁴; because already, in the parent structure, $CN(O) = 4$, and $x = x_{min} = 2.0$.

These deductions are all in complete accord with a vast amount of experimental evidence. Furthermore, the 'distortions' from conventionally idealised structures (with regular $[MO_6]$ octahedra) to the real structures, which occur in the parents and in CS, column and bronze type derivatives, clearly show the strong tendency towards anion coordination symmetries appropriate to sp, sp^2 and sp^3 hybridisation, and characteristic of $CN(O) = 2, 3$ and 4 .

For tetrahedrally coordinated cations the situation is unclear: no transition metal oxide is known to retain exclusively $CN(M) = 4$ on reduction. For example, although CrO_3 contains only $[CrO_4]$ tetrahedral groups, the (high pressure) structure of Cr_6O_{15} contains $[CrO_6]$ octahedra in addition to $[CrO_4]$ tetrahedra, whereas the (ambient pressure) structures of the reduced β and γ oxides are unknown⁵.

In other metal oxide systems either the cations are not of variable valency (which is essential for CS in a binary system) or the situation is complicated by the effects of stereochemically active lone pairs of electrons (Ge, Sn, Pb, Sb, Bi)⁶. Perhaps they have been insufficiently studied. That is certainly true of systems with other anions, such as fluoride. There, however, polarisation effects will be much smaller than with the oxide ion, and there is certainly no evidence so far of CS in metal fluorides, although it does occur in oxide fluorides.

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¹ Bursill, L. A., Hyde, B. G., and O'Keeffe, M., *Solid State Chemistry* (edit. by Roth, R. S., and Schneider, S. J.), 197–204 (US Government Printing Office, Washington, DC, 1972).

² Anstis, G. R., Lynch, D. F., Moodie, A. F., and O'Keeffe, M. A., *Acta crystallogr.*, **A29**, 138–147 (1973).

³ Wadsley, A. D., *Helv. chim. Acta*, Werner centenary volume, 207–222 (1967).

⁴ Caro, P., and Derouet, J., *C.r. hebd. Seanc. Acad. Sci., Paris*, **271**, 1370–1373 (1970).

⁵ Wilhelmi, K. A., thesis, Univ. Stockholm (1966).

⁶ Andersson, S., and Åström, A., *Solid State Chemistry* (edit. by Roth, R. S., and Schneider, S. J.), 3–14 (US Government Printing Office, Washington, DC, 1972).

Molecular mechanism for missense suppression in *E. coli*

THE missense mutation *trpA36* results in a Gly (GGA) → Arg (AGA) amino acid substitution at position 211 of the tryptophan synthetase A protein¹. Suppressors of *trpA36* have been derived by mutation in *glyT*, the gene specifying tRNA^{Gly 2}_{GGA/GG}, and in *glyU*, which specifies tRNA^{Gly 1}_{GGG} (refs 2–4). Transfer RNA from strains carrying either of the *glyTsuA36* or *glyUsuA36* alleles inserts glycine into polypeptides, *in vitro*

and *in vivo*, in response to the arginine codon, AGA^{3,5,6}. We report here a comparison of the nucleotide sequences of tRNA^{Gly 2}_{GGA/GG} and tRNA^{Gly 2}_{suA36(HA)}; the *glyTsuA36(HA)* mutation results in a C → U base change at the 3'-end of the anticodon of tRNA^{Gly 2}_{GGA/GG}, which causes a subsequent, enzyme-catalysed modification of the A directly adjacent to the 3'-end of the anticodon.

³²P-labelled tRNA^{Gly 2}_{GGA/GG}, obtained from *glyT*⁺ *E. coli* strains, was purified by the methods of Gillam *et al.*^{7,8}. ³²P-labelled tRNA^{Gly 2}_{suA36(HA)} was obtained by infection of strain BF266 with the phage, λ h80d*glyTsuA36*, (ref. 9) and was purified by BD-cellulose⁷ and RPC-5 reverse phase chromatography¹⁰. The tRNAs were sequenced by the methods of Sanger and co-workers, as described by Barrell¹¹. A complete account of the purifications and sequence procedures will be presented elsewhere.

Ribonuclease T₁ fingerprints of the two tRNA species are quite similar, differing only in the position, and sequence, of oligonucleotide T-13 (Fig. 1), which forms the anticodon loop of the tRNA molecule (Fig. 2). Oligo T-13, from tRNA^{Gly 2}_{GGA/GG}, has the sequence CCUCCAAG; oligo T-13', from tRNA^{Gly 2}_{suA36(HA)}, has the sequence CCUUCU^UAAG. A portion of both species has an unidentified modified U in the wobble position of the anticodon (Fig. 2). The *glyTsuA36(HA)* mutation, therefore, replaces the C in the third position (3'-

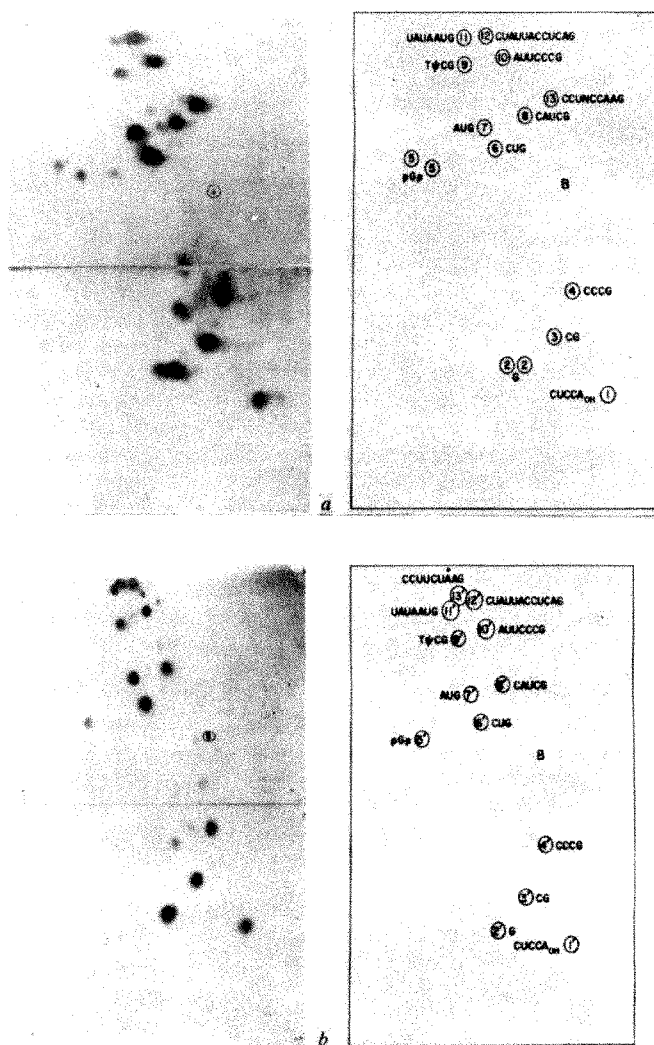


Fig. 1 A comparison of ribonuclease T₁ fingerprints of: (a) tRNA^{Gly 2}_{GGA/GG}; (b) tRNA^{Gly 2}_{suA36(HA)}. First dimension electrophoresis on cellulose acetate at pH 3.5 was from right to left. Second dimension electrophoresis on DEAE paper in 7% formic acid was from top to bottom. The symbol B denotes the position of the blue dye marker (xylene cyanole FF).

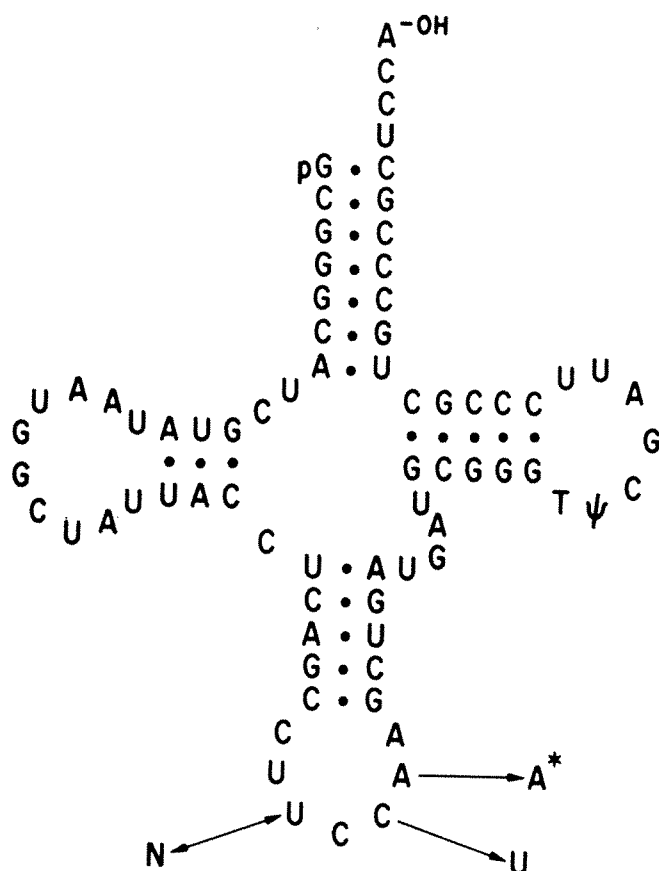


Fig. 2 tRNA^{Gly 2}_{GGA/G} drawn in the cloverleaf configuration. The arrows indicate the changes resulting from the *glyTsuA36* (HA) mutation. N, an unidentified derivative of U, is present in a portion of both species. A*, as yet unidentified, is apparently a derivative of N⁶-carbamoylthreonyl-A.

end) of the anticodon of tRNA^{Gly 2}_{GGA/G} with a U, changing the anticodon sequence from UCC to UCU. The observed anticodon sequences are, as might be expected, complementary to the codons recognised by the tRNA species *in vitro* and *in vivo*.

Other missense suppressors can arise by alteration of the anticodons of, and thus the codons recognised by, particular tRNA species. For example, tRNA^{Gly 3}_{suA78}, which inserts glycine into polypeptides in response to the cysteine codons, UGU and UGC, was derived from tRNA^{Gly 3}_{GGU/C} by a C→A base change at the 3'-end of the anticodon¹². tRNA^{Gly 3}_{ins}, which recognises the glycine codons GGA and GGG, was derived from tRNA^{Gly 3}_{GGU/C} by a G→U base change in the wobble position of the anticodon¹³. tRNA^{Gly 1}_{suT}, which recognises the glutamic acid codon, GAG, was derived by a C→U base change in the middle position of the anticodon of tRNA^{Gly 1}_{GGU} (ref. 14). In all cases, the anticodons of the suppressor tRNAs are complementary to the missense codons suppressed by these tRNAs.

As shown in Figs 2 and 3, the A adjacent to the anticodon of tRNA^{Gly 2}_{suA36(HA)} is modified, while the corresponding A in tRNA^{Gly 2}_{GGA/G} is not. The modified nucleotide, as yet unidentified, is converted to Ap by treatment with alkali; it exhibits an electrophoretic mobility at pH 3.5 (relative to Up) of 0.98, and chromatographic mobilities in systems A and B (ref. 11) (relative to Up) of 0.66 and 0.5 respectively. Since derivatives of N-[9-(β-D-ribofuranosyl)-purin-6-ylcarbamoyl]threonine (t⁶A) are found adjacent to the anticodons of tRNA species recognising codons beginning with A (ref. 15), we suggest that the modified A adjacent to the anticodon of tRNA^{Gly 2}_{suA36(HA)} might be such a derivative. The enzymes responsible for this modification must recognise, at least in part, the anticodon sequences of prospective tRNA substrates. An analogous

situation exists with tRNA^{Gly 3}_{suA78}, which has 2-thiomethyl-6-isopentenyl-adenine (ms²i⁶A) adjacent to the anticodon; the corresponding A in tRNA^{Gly 3}_{GGU/C} is not modified (Fig. 3)¹². Some suggestions have been made concerning the physiological significance of such modifications; for example, tRNA^{Tyr}_{su111} lacking the isopentenyl modification adjacent to the anticodon shows a greatly reduced ability to recognise the codon, UAG, *in vitro*, but appears to function normally in other respects¹⁶.

The following observation suggests that the greatly reduced rate of aminoacylation of tRNA^{Gly 2}_{suA36(HA)} (10⁻⁴ times that of tRNA^{Gly 2}_{GGA/G} under identical conditions)^{2,3,17} is a result of the base change in the anticodon, rather than the modification of the adjacent A. A tRNA capable of inserting glycine into polypeptides, *in vitro*, in response to the arginine codon, AGA, has been derived from tRNA^{Gly 2}_{GGA/G} by nitrous acid deamination, presumably of the C at the 3'-end of the anticodon. This altered tRNA^{Gly 2}, chromatographically different from tRNA^{Gly 2}_{suA36(HA)} and probably lacking modification of the A adjacent to the anticodon, exhibits a greatly reduced rate of aminoacylation¹⁷. The role of the anticodon sequence in the aminoacylation reaction has been suggested for other tRNA species. If the anticodon of tRNA^{Tyr}_{UGG} is changed from CCA to CUA, the altered tRNA can be enzymatically aminoacylated with glutamine¹⁸. If a C in the anticodon of tRNA^{Met} or tRNA^{Val} is chemically modified to U, the tRNA can no longer be enzymatically aminoacylated^{19,20}. Preliminary attempts to isolate unmodified tRNA^{Gly 2}_{suA36(HA)}, in order to test this hypothesis directly, have been unsuccessful; in fact, modification of the A adjacent to the anticodon has been found in the uncleaved precursor of tRNA^{Gly 2}_{suA36(HA)} (S. Chang, and J. C., unpublished results).

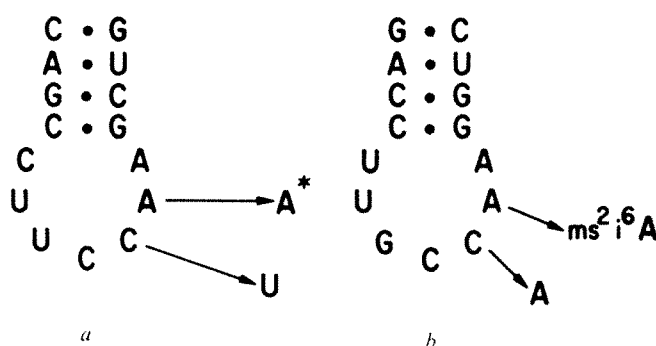


Fig. 3 A comparison of the anticodon changes and resulting modification changes of: (a) tRNA^{Gly 2}_{GGA/G} → tRNA^{Gly 2}_{suA36(HA)}, and (b) tRNA^{Gly 3}_{GGU/C} → tRNA^{Gly 3}_{suA78}. In each case, substitution of the base at the 3'-end of the anticodon results in a modification of the adjacent A.

GlyTsuA36 mutations cause pleiotropic effects, of varying severity, on the cell; these effects are due, at least in part, to the loss of GGA translating ability, since they are reversed by the presence of *glyT*⁺ (normal tRNA^{Gly 2}_{GGA/G}) or *glyVins* (tRNA^{Gly 3}_{GGU/C} → tRNA^{Gly 3}_{GGA/G}) (refs 2, 4 and 13). For example, the *glyTsuA36*(HA) allele is semilethal when haploid⁴. The *glyTsuA36*(159) allele, which is probably the result of two or more closely linked mutational events, renders the cell tryptone-sensitive; when shifted from minimal to complex medium, cells carrying the *glyTsuA36*(159) allele cease normal cell division, form long aseptate filaments, and die^{2,21}. Nucleotide sequence studies of tRNA^{Gly 2}_{suA36(159)} may suggest a molecular basis for the less severe pleiotropic effects of *glyTsuA36*(159); *glyTsuA36*(159) may specify a tRNA^{Gly 2} with the *glyTsuA36*(HA) anticodon alteration plus a second nucleotide substitution which allows the tRNA to recognise with low efficiency

the glycine codon, GGA, in addition to the arginine codons AGA and AGG (ref. 4).

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- ¹ Yanofsky, C., Ito, J., and Horn, V., *Cold Spring Harbor Symp. quant. Biol.*, **31**, 151-162 (1966).
- ² Hill, C. W., Squires, C., and Carbon, J., *J. molec. Biol.*, **52**, 557-568 (1970).
- ³ Carbon, J., Squires, C., and Hill, C. W., *Cold Spring Harbor Symp. quant. Biol.*, **34**, 505-512 (1969).
- ⁴ Carbon, J., Squires, C., and Hill, C. W., *J. molec. Biol.*, **52**, 571-584 (1970).
- ⁵ Brody, S., and Yanofsky, C., *Proc. natn. Acad. Sci., U.S.A.*, **50**, 9-16 (1963).
- ⁶ Carbon, J., Berg, P., and Yanofsky, C., *Cold Spring Harbor Symp. quant. Biol.*, **31**, 487-497 (1966); *Proc. natn. Acad. Sci., U.S.A.*, **56**, 764-771 (1966).
- ⁷ Gillam, I., Millward, S., Blew, D., von Tigerstrom, M., Wimmer, E., and Tener, G. M., *Biochemistry*, **6**, 3043-3469 (1967).
- ⁸ Gillam, I., Blew, D., Warrington, R. C., von Tigerstrom, M., and Tener, G. M., *Biochemistry*, **7**, 3459-3468 (1968).
- ⁹ Squires, C., Konrad, B., Kirschbaum, J., and Carbon, J., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 438-441 (1973).
- ¹⁰ Kelmers, A. D., and Heatherly, D. E., *Analyt. Biochem.*, **44**, 486-495 (1971).
- ¹¹ Barrell, B. G., in *Procedures in nucleic acid research* (edit. by Cantoni, G. L., and Davis, D. R.) (Harper and Row, New York, 1970).
- ¹² Carbon, J., and Fleck, E. W., *J. molec. Biol.*, **85**, 371-391 (1974).
- ¹³ Squires, C., and Carbon, J., *Nature new Biol.*, **233**, 274-277 (1971).
- ¹⁴ Hill, C. W., Combrato, G., and Dolph, W., *J. Bact.*, **117**, 351-359 (1974).
- ¹⁵ Ishikura, H., Yamada, Y., Murao, K., Saneyoshi, M., and Nishimura, S., *Biochem. biophys. Res. Commun.*, **37**, 990-995 (1969).
- ¹⁶ Geftter, M. L., and Russell, R. L., *J. molec. Biol.*, **39**, 145-157 (1969).
- ¹⁷ Carbon, J., and Curry, J. B., *J. molec. Biol.*, **38**, 201-216 (1968).
- ¹⁸ Yaniv, M., Folk, W., and Soll, L., quoted by Berg, P., in *The Harvey Lectures*, series 67, 247 (Academic Press, New York, 1973); *J. molec. Biol.* (in the press).
- ¹⁹ Schulman, L. H., and Goddard, J. P., *J. biol. Chem.*, **248**, 1341-1345 (1973).
- ²⁰ Chambers, R. W., Aoyagi, S., Furikawa, Y., Zuwadska, H., and Bhanot, O. S., *J. biol. Chem.*, **248**, 5549-5551 (1973).
- ²¹ Hill, C. W., Foulds, J., Soll, L., and Berg, P., *J. molec. Biol.*, **39**, 563-581 (1969).

X-ray diffraction studies of DNA at reduced water contents

REDUCED water contents probably stimulate most closely the local environment about DNA when it is complexed with protein. At low water contents, less than about six water molecules per nucleotide, the equilibrium conformation for pure DNA is the A form^{1,2}. The A form does not alter with base composition³ whereas the B states of DNA are strongly correlated with chemical composition⁴. The A conformation (A state) is the only one which can be called a distinct form. There are many B type conformations all having ten base pairs per turn. 'C form' is a misnomer. We use the term 'C state' or type for structures having a pitch between 29 and 33 Å and a diffraction intensity distribution of weak, strong, then weak on the first three layer lines.

Since the discovery of the A form¹, all experiments on A-DNA where lattice formation could be detected, have shown the presence of a crystalline state. We have specifically looked for noncrystalline A-DNA but have not found it. In fibres, one might argue that orientation would favour and highlight crystalline regions, but this would not be

the case for unoriented gels. We have dried wet gels to about 70% DNA concentration and subsequent X-ray scattering experiments have shown only B or crystalline A patterns.

As it has been reported that the circular dichroism and Raman spectra of DNA in 80% ethanol is the same as A-DNA in films and fibres^{5,6}, we decided to study the conformation of DNA in this solvent.

Indeed, we found that DNA did adopt the A conformation in 80% ethanol and that this A state was always crystalline and quite similar to that previously observed in gels and fibres (see Fig. 1). We also found that, after the addition of ethanol to a DNA gel, complete formation of the A state could require up to several hours and that, upon the B to A transition, spontaneous preferential orientation sometimes occurred.

In the nucleus, conditions for DNA crystallisation are obviously very unfavourable because of the protein-DNA and water-DNA linkages which are stronger than DNA-DNA interactions. Consequently, it is of biological interest to examine the dehydration of DNA under conditions where A transitions are inhibited.

At high water contents in solution and in fibres DNA assumes conformations of the B type^{2,7} and when a B-DNA gel or fibre is slowly dried at lower relative humidity (r.h.), a transition to the A form is nearly always observed. But, if a hydrated fibre is maintained under tension while it is dried rapidly, the 30% contraction in length required for a B to A transition is inhibited and other conformations are adopted. When a wet gel of *Clostridium perfringens* DNA (69% A-T) is maintained under tension while drying at 20°-25° C, subsequent X-ray fibre patterns show diffraction of the P type at NaCl contents less than 2% and of the

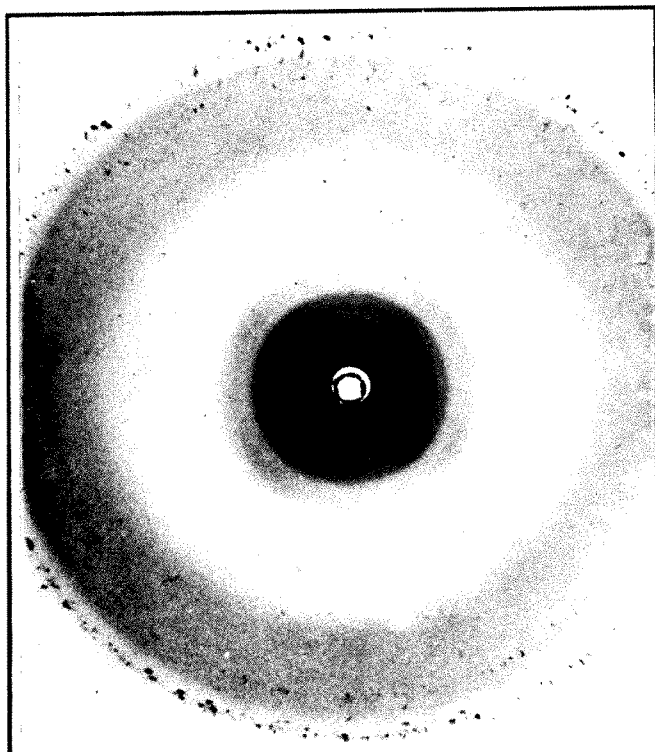


Fig. 1 A pattern of calf thymus DNA in 80% ethanol taken with a torroidal camera in air in a glass capillary tube. The concentration of DNA was about 5%, but it was present as a precipitate. The diffraction is that of the crystalline A state and preferential orientation was observed perpendicular to the length of the capillary. The ring at 3.14 Å is from silicon powder on the capillary. Similar A patterns have been observed with *Cl. perfringens* DNA in 80% ethanol.

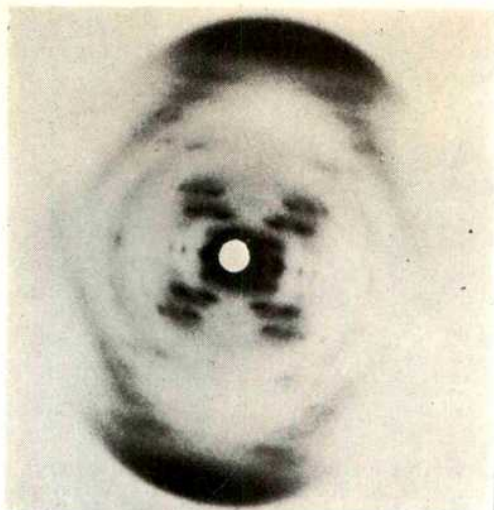


Fig. 2 A crystalline B pattern from *Cl. perfringens* DNA at 44% r.h. showing a small amount of A form (notice the slight splitting of the second layer line). The fibre contained 5% excess salt was dried on a fixed platinum support at 37° C.

T type at higher salt concentrations. (The nature of these patterns has been described previously⁸; in short both the T and P structures are more highly wound and have smaller radii than the A, B or C structures.) The P and T states are stable for at least several days at 66% r.h. even if the fibre is freely suspended. But if the r.h. is raised to 98% and then returned slowly to 66% r.h., an A diagram appears. (Similar hysteresis effects have also been observed by Dr. A. M. Levelut at the Laboratoire de Physique des Solides, Orsay.)

If a gel of *Cl. perfringens* DNA on a fixed support is quickly dehydrated by drying at 37° C at r.h. < 66%, a B state is often obtained. These X-ray patterns are more crystalline than any other sodium B patterns yet observed. Some patterns (as in Fig. 2), however, show the presence of small amounts of A-DNA implying the existence of a metastable mixture. This low humidity B state is stable for at least 2 d at 44% r.h. The lithium salt of his DNA gives a crystalline B pattern at lower r.h. (ref. 8) but no C pattern has been observed with the lithium salt. Experiments with the magnesium salt of *Cl. perfringens* DNA (Mg-DNA) give a pattern at lower humidities which resembles those of C states in the intensity distribution on the lower layer lines but in which the ninth layer line, which has been related to a slight base tilting, is much weaker than in the C patterns. Thus *Cl. perfringens* DNA can adopt at least five different forms at the same water content.

It was accidentally observed that the sodium salt of calf thymus DNA (Na-DNA) gave a C pattern (S.B. and P. Tougard, unpublished) which was almost identical to the published lithium salt C diagrams (see Fig. 3). Subsequent experiments with a variety of pulling conditions and salt contents failed to give C patterns. It was then found, however, that calf thymus DNA dried at 37° C (between 30 and 66% r.h.) usually gives a C pattern. This state is metastable at 66% r.h. and 'decays' into the A form after about a day. An immediate C to A transition occurs at lower r.h. if a C-DNA fibre is cut loose at one end from its support. This rapid transformation is accompanied by a 30% contraction in fibre length. Calf thymus DNA can also give very crystalline B patterns at 30-66% r.h. when dried at 37° C. For calf thymus DNA, it seems that high salt conditions favour the metastable B state, whereas at salt contents less than about 3%, C structures seem to predominate.

Stable states are produced by several agents which are

known to block the B to A transitions. The result is that at lower humidity new conformations are adopted, all of which are more highly wound than the A state. One agent known to block B to A transition is the lithium ion. The A form has never been observed with calf thymus Li-DNA (nor with *Micrococcus luteus* or *Cl. perfringens* DNA in this laboratory). Instead a C state is found at lower r.h. (ref. 10). Calf thymus Mg-DNA also does not exist in the A form but instead adopts a C configuration at lower r.h. (ref. 11); and in mixtures of sodium and magnesium salts, the relative amount of A-DNA we observed was far less than the $\text{Na}^+/\text{Mg}^{2+}$ ratio. When glucose residues are present, as in phage T2 DNA, or with nonintercalating acridine orange or proflavin, instead of the A form a structure having eight or nine base pairs per turn exists at r.h. less than 80% (refs 12, 13). In fact, the fibre patterns shown by T2 DNA at low r.h. (ref. 12) are very similar to those of our P form. In chromatin the A form has never been detected and experiments in this laboratory show that small amounts of histone protein also inhibit the B to A transition in fibres. Thus, even if the requirement for crystallinity were not sufficient to prevent an A transition *in vivo*, these blocking agents, some of which are quite common in the cell, would strongly inhibit it.

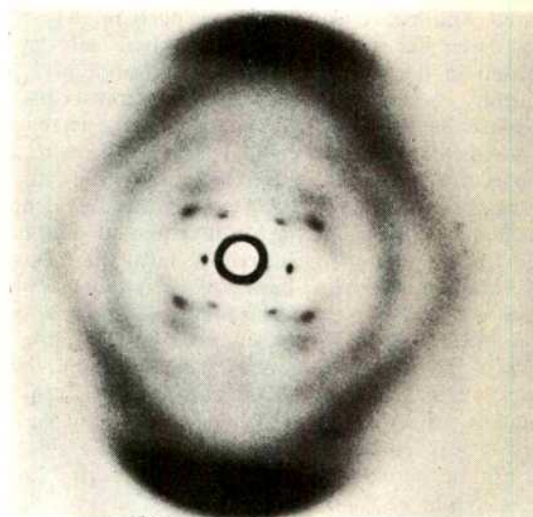


Fig. 3 A sodium C pattern from calf thymus DNA at 44% r.h. having a layer line separation of 31 Å. The fibre was dried on a fixed support at 37° C and over 44% r.h.

On the other hand, A-DNA, the only state which is invariant with base composition, might provide the best substrate for both RNA and DNA polymerases. Yet our results strongly imply that the A form of DNA can exist *in vivo* only when hybridised with RNA, for here DNA is forced to adopt the A configuration¹⁴. It may be that RNA or ribonucleotides play the role of a catalyst in transcription and replication by driving DNA to the A-state.

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¹ Riley, D. P., and Oster, G., *Biochim. Biophys. Acta*, 7, 525 (1951).

- ² Franklin, R. E., and Gosling, R. G., *Nature*, **171**, 740 (1953).
- ³ Hamilton, L. D., Barclay, R. K., Wilkins, M. H. F., Brown, G. L., Wilson, H. R., Marvin, D. A., Ephrussi-Taylor, H., and Simmons, N. S., *Biophys. Biochem. Cytol.*, **5**, 397 (1954).
- ⁴ Bram, S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2127 (1973).
- ⁵ Ivanov, V. I., Mitchenkova, L. E., Schgolkin, A. K., and Poletayev, A. I., *Biopolymers*, **12**, 89 (1973).
- ⁶ Peticolas, W. (in the press).
- ⁷ Bram, S., *J. molec. Biol.*, **58**, 277 (1971).
- ⁸ Bram, S., and Tougaard, P., *Nature new Biol.*, **239**, 128 (1972).
- ⁹ Azoulay, R., and Bram, S., *C.r. hebd. Séanc. Acad. Sci. Paris*, **276**, 2977 (1973).
- ¹⁰ Marvin, D. A., Spencer, M., Wilkins, M. H. F., and Hamilton, L. D., *J. molec. Biol.*, **3** (1961).
- ¹¹ Skuratovsky, I. I., and Mokulsky, M. A., *Dokl. Akad. Sci. U.S.S.R.*, **200**, 637 (1971).
- ¹² Mokulsky, M. A., Kapitonova, K. A., and Mokulskaya, T. D., *Fourth int. Biophys. Congr. Abstr.*, **VII**, 195 (1972).
- ¹³ Neville, D. M., and Davies, D. R., *J. molec. Biol.*, **17**, 57 (1966).
- ¹⁴ Milman, G., Langridge, R., and Chamberlin, M. J., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 1804 (1967).

RNA synthesis specific for an integrated adenovirus genome during the cell cycle

WHEN adenovirus transforms a cultured mammalian cell, substantial portions of the viral genome can be integrated into the host cell chromosomes and at least a fraction of this integrated genome is transcribed¹. Approximately 50% of the early sequences observed in the lytic infections are synthesised in the transformed cell². In randomly growing transformed rat embryo cells, the virus transcripts in the nucleus are in the large molecular weight, heterogeneous RNA covalently linked to cellular RNA³. These transcripts are apparently processed before they appear on cytoplasmic polyribosomes³. To date, there has been little evidence concerning the synthesis and post-transcriptional modifications of viral transcripts during the cell cycle in the transformed cells. The first question to be asked is, is there continuous synthesis of viral transcripts from at least some portion of the integrated genome, or is synthesis limited to one phase of the cell cycle? We have investigated this question in synchronised rat embryo cells transformed with adenovirus type 2 (Ad2-T)⁴. The synthesis of viral RNA transcripts was correlated with the life cycle of the cell which can be defined as consisting of a period of DNA synthesis (S phase) followed by a period of growth before mitosis (G₂), mitosis (M), and another growth period following division prior to DNA synthesis (G₁). Our data indicate that virus-specific RNA was transcribed throughout the cell cycle along with cellular RNA synthesis. Viral RNA transcription was restricted during mitosis coincident with the restriction of cellular RNA synthesis.

All experiments were performed with a cloned population⁵ which demonstrated levels of T antigen and synthesis of virus-specific RNA at least equivalent to those of the uncloned population. Cells were maintained in spinner cultures in Eagle's MEM (without calcium)⁶ enriched with 5% dialysed calf serum and 1% foetal bovine serum.

Cultures were synchronised in S phase by exposure to thymidine. Incorporation of ¹⁴C-thymidine into acid-precipitable material began immediately after release from a double thymidine blockade (Fig. 1). Incorporation reached a maximum at about 3 h and then declined during the 4–6 h after synchronisation. Autoradiography revealed that 85% of the cells were synthesising DNA during the period 1–5 h following resuspension as opposed to 22% at 7–11 h. Approximately 90% of the culture passed through mitosis during a 4 h interval beginning at about 6 h after synchronisation. If the cells were released in the presence of colcemid, between 60 to 70% of cells accumulated in metaphase by about 10 h and an additional 20 to 30% seemed to be arrested in prophase.

Synthesis of cellular and viral RNA was compared in a

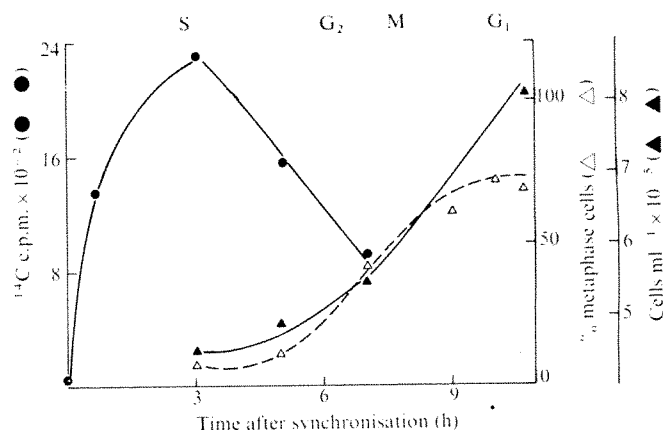


Fig. 1 Synchronisation of Ad2-T cells following thymidine blockade. A suspension culture of Ad2-T was synchronised with 2.5 mM thymidine by incubating the cells for 15 h in the presence of thymidine, resuspending in fresh medium for 9 h followed by an additional 14 h exposure to thymidine. At the indicated times after blockade 2 ml aliquots (approximately 10⁶ cells) were transferred to tubes containing 0.5 μ Ci ¹⁴C-thymidine (specific activity 40–60 mCi mmol⁻¹, New England Nuclear, Boston, Massachusetts). After incubation for 20 min at 37° C, the acid-insoluble radioactivity was determined as previously described⁷. Data were normalised to c.p.m. per 10⁶ cells. At the time of resuspension an equal amount of the culture was treated with colcemid (0.025 μ g ml⁻¹) and the accumulation in metaphase scored. If apparent prophase cells were counted along with metaphase cells, the final proportion of cells not in interphase was generally 85–90%. Cell concentration was determined with a haemocytometer and the mitotic index was determined with phase microscopy. ●, ¹⁴C-thymidine; ▲, cells ml⁻¹; Δ, % metaphase.

population of cells progressing from S phase, through G₂ and into metaphase (Fig. 2). Aliquots of cells were exposed to ³H-uridine for 1 h intervals during this period. RNA was prepared from whole cells⁸ and analysed for total radioactive incorporation and for incorporation into virus-specific material using the low temperature DNA-RNA hybridisation procedure in the presence of 7.5 M urea⁹. Figure 2a illustrates that viral RNA synthesis corresponds to total cellular RNA synthesis. Incorporation of uridine into both viral and cellular RNA increased following release from thymidine, reached a maximum in S and steadily declined as the population proceeded through G₂ into metaphase-arrest.

The extent of restriction of viral RNA synthesis during mitosis was determined using metaphase-arrested cells collected from albumin density gradients as described in the legend of Table 1. Interphase cells, subjected to the same manipulative procedures, were added to the metaphase cells to form populations of defined content. These mixtures were incubated at 37° C with ³H-uridine for 1 h and the total cellular RNA was prepared as described (Fig. 2). Incorporation into virus-specific RNA decreased linearly with increasing proportion of metaphase cells and approached zero. This decrease paralleled that of total cellular RNA synthesis suggesting that synthesis of both species is restricted to the same extent during metaphase.

Since the G₂ period is short and is not clearly defined following thymidine synchronisation (Fig. 1), it could be argued that viral RNA was synthesised primarily during S and that restriction during G₂ was masked by the entry of cells into mitosis. The nearly complete restriction of viral RNA synthesis during metaphase made a more precise examination of the G₂ phase possible. A culture was synchronised with thymidine, and colcemid was added at the time of release from the second blockade (Table 1). The culture was divided into three aliquots which were continuously exposed to ³H-uridine for 6, 4, and 2 h before

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Fluorescence Techniques in Cell Biology

Proceedings of the Conference on "Quantitative Fluorescence Techniques as Applied to Cell Biology" held at Battelle Seattle Research Center, Seattle, Washington March 27–31, 1972

Editors: Dr. **Andreas A. Thaler** and Dr. **Manfred Sernetz**, Battelle-Institut e.V.,

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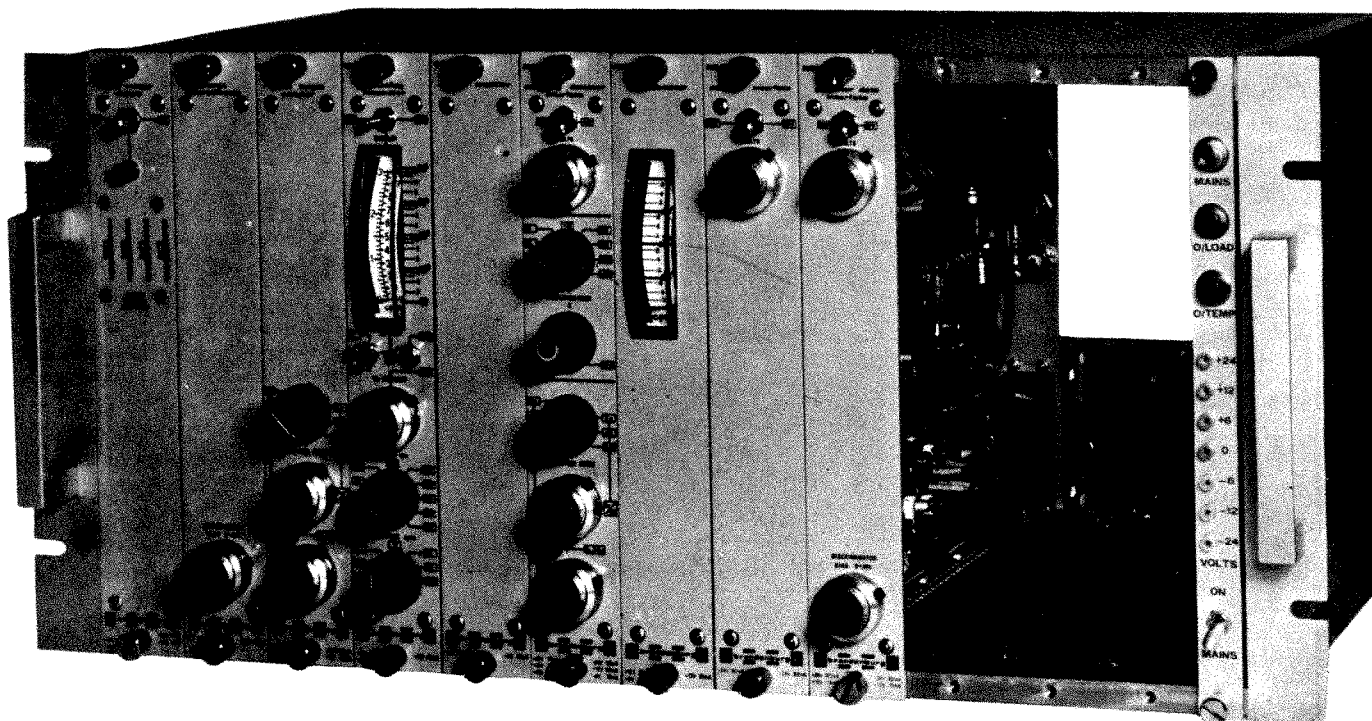
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collection of metaphase cells. The advantage of this approach is that by isolating metaphase cells, the incorporation during a given labelling period represents synthesis for a maximum of that time before mitosis. For example, cells incorporating radioactivity in the 2 h interval immediately preceding the collection of metaphase-arrested cells should represent synthesis by a fairly homogeneous population of cells in G_2 . As expected metaphase cells collected from the population exposed to radioactivity for 6 and 4 h contained significant amounts of labelled virus-specific RNA. A lower level of incorporation was found in the third population labelled for 2 h. This latter result clearly indicates synthesis of viral RNA during G_2 .

Between 6 and 10 h after thymidine synchronisation, the population of cells passed from G_2 through mitosis into G_1 (Fig. 1). No evidence of DNA synthesis was observed in this population until 9 h later. Aliquots of cells were exposed to 3H -uridine for 1 h intervals beginning 8 h after synchronisation in S phase and RNA was prepared as previously described (Fig. 2b). There was minimal incorporation of uridine into both cellular and viral RNA coincident with the time of mitosis. Incorporation then rose to fairly constant levels as the population progressed into G_1 . Viral RNA synthesis seems to follow the resumption of cellular synthesis following mitosis. There is a steady decay in synchrony as the population progresses from S phase to G_1 . The following techniques to initiate synchrony in G_1 were surveyed but proved unsuccessful: selective detachment of mitotic cells¹⁰, release from density growth inhibition¹¹, serum deprivation¹², and isoleucine deletion¹³.

We conclude that at least some virus-specific RNA was synthesised at all times during the cell cycle except mitosis.

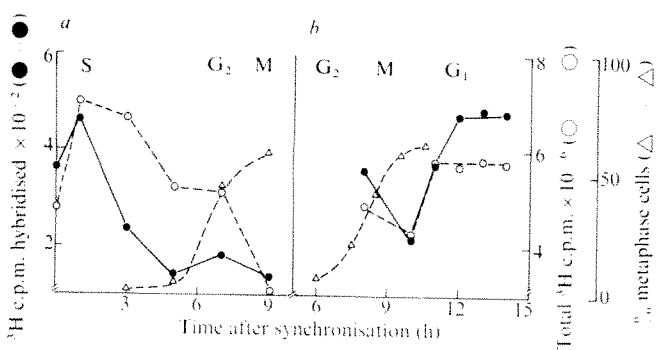
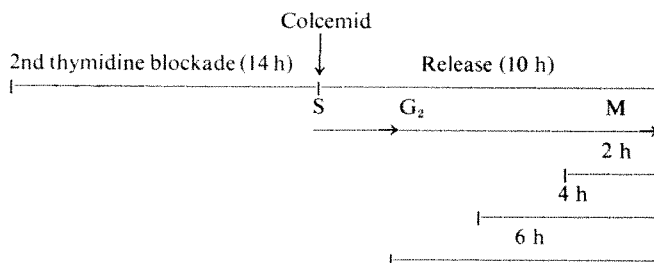


Fig. 2 Cellular and viral RNA synthesis following thymidine blockade. Ad2-T cells were used directly as S phase cells following thymidine synchronisation. At the indicated times 4×10^7 cells were suspended in 4.0 ml of complete medium at 37° C containing 200 μ Ci of 3H -uridine (specific activity 720 Ci mmol⁻¹, New England Nuclear, Boston, Massachusetts). After incubation for 1 h, cells were washed, resuspended in STE (0.05 M Tris-HCl, pH 7.4; 0.001 M EDTA; 0.1 M NaCl) containing 1% sodium dodecyl sulphate (SDS) and 1% mercaptoethanol, and extracted three times at room temperature with phenol saturated with 20% STE. Nucleic acid was precipitated for 16 h at -20° C with 0.15 M NaCl and 2.5 volumes ethanol. The precipitate was suspended in 1.0 ml HSB (0.01 M Tris-HCl pH 7.4, 0.5 M NaCl and 0.05 M MgCl₂, digested with 100 μ g DNase I at room temperature for 30 min, and the RNA reprecipitated. RNA was dissolved in $2 \times$ SSC and the Cl_2CCOOH insoluble radioactivity and virus-specific radioactivity determined. DNA-RNA hybridisation was carried out in a solution of $2 \times$ SSC containing 7.5 M urea, 10^{-2} M Tes (N-tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid) buffer, pH 7.0, and 0.05% SDS. Equal aliquots of individual samples were incubated with nitrocellulose filters containing 8 μ g of alkali denatured adenovirus 2 DNA and blank filters at 37° C for 4 d. Pancreatic RNase resistant radioactivity was determined. Virus-specific RNA was corrected for recovery from phenol extraction and for nonspecific binding using the amount of radioactivity bound to filters not containing adenovirus DNA. This represented 20-25% of the specific binding. *a*, Colcemid (0.025 μ g ml⁻¹) was added at the beginning of S phase; *b*, colcemid was not added allowing the cells to pass through mitosis into G_1 . ●, Virus-specific radioactivity; ○, total radioactivity; Δ, % metaphase cells.

Table 1 Extent of viral RNA synthesis in G_2

Interval before metaphase (h)	Cell cycle stage	Total c.p.m. $\times 10^7$	Hybridisable c.p.m.
6	S + G_2	3.87	545
4	late S + G_2	1.62	228
2	G_2	0.77	178



The diagram below the table outlines the experiment as described in the text. RNA was extracted and assayed as described in Fig. 2 from metaphase arrested cells. At the end of each incubation period 4×10^7 cells with approximately 60-70% in metaphase arrest were mixed with bovine albumin resulting in a final concentration of 30%. Gradients were formed by successive addition of 1 ml each of the cell mixture, 23%, 20%, and 17% albumin and 0.5 ml of 10% albumin at the top and were centrifuged in a Spinco SW65 rotor at 17,500 r.p.m. for 45 min at 5° C. $2 \times 10^7 - 3 \times 10^7$ cells with a metaphase index of 95-97% were recovered from the 17-10% interphase of two gradients.

During mitosis, the synthesis of viral RNA seemed to be restricted to the same extent as cellular RNA. These data permit the generalisation that the transformed state can be correlated with continuous functioning throughout interphase of at least some portions of the integrated genome. This result is in sharp contrast to that observed with H and L chain biosynthesis¹⁴, induction of tyrosine amino transferase¹⁵, and synthesis of histone mRNA¹⁶, in cultured mammalian cells, none of which is continuous throughout interphase. The relative composition of the RNA transcripts synthesised in each phase must be explored. This analysis seems particularly important, since individual clones of Ad2-T have incorporated not only different portions of the adenovirus genome but also different proportional amounts of the same fragment (J. Sambrook, personal communication). Comparison of the RNA synthesised during the same phase of the cell cycle in different clones may provide information about the site of integration of the viral genome, especially if sufficient quantities of virus-specific RNA can be obtained for limited structural analysis. Of equal importance is the post-transcriptional processing in each phase. We are at present examining whether there is continuous export or selective exit of particular viral transcripts at unique times in the cell cycle. Further investigation with clones containing defined adenovirus segments, when synchronised, should provide useful information concerning both the maintenance of the transformed state and the expression of the host cell genome.

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¹ Petterson, U., and Sambrook, J., *J. molec. Biol.*, **73**, 125-130 (1973).

- ² Green, M., Parsons, J. T., Pina, M., Fujinaga, K., Caffier, H., and Landgraf-Leurs, L., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 803-818 (1970).
- ³ Wall, R., Weber, J., Gage, Z., and Darnell, J. E., *J. Virol.*, **11**, 953-960 (1973).
- ⁴ Freeman, A. E., Black, P. H., Vanderpool, E. A., Henry, P. H., Austin, J. B., and Huebner, R. J., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1205-1212 (1967).
- ⁵ Puck, T. T., Marcus, P. I., and Cieciura, S. J., *J. exp. Med.*, **103**, 273-283 (1956).
- ⁶ Eagle, H., *Science*, **130**, 432-437 (1959).
- ⁷ Hodge, L. D., and Scharff, M. D., *Virology*, **37**, 554-564 (1969).
- ⁸ Lerner, R. A., and Hodge, L. D., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 544-551 (1969).
- ⁹ Tsuei, D., Fujinaga, K., and Green, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 427-430 (1972).
- ¹⁰ Terasima, T., and Tolmach, L. J., *Expl Cell Res.*, **30**, 344-362 (1963).
- ¹¹ Stoker, M., O'Neill, C., Berryman, S., and Waxman, V., *Int. J. Cancer*, **3**, 683-693 (1968).
- ¹² Strohl, W. A., *Virology*, **39**, 653-665 (1969).
- ¹³ Tobey, R. A., Ley, K. D., *J. Cell Biol.*, **46**, 151-157 (1970).
- ¹⁴ Lerner, R. A., and Hodge, L. D., *J. Cell Physiol.*, **77**, 265-276 (1971).
- ¹⁵ Martin, D. W., jun., Tomkins, G. M., and Granner, D., *Proc. natn. Acad. Sci. U.S.A.*, **62**, 248-255 (1969).
- ¹⁶ Boron, T. W., Scharff, M. D., and Robbins, E., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1977-1983 (1967).

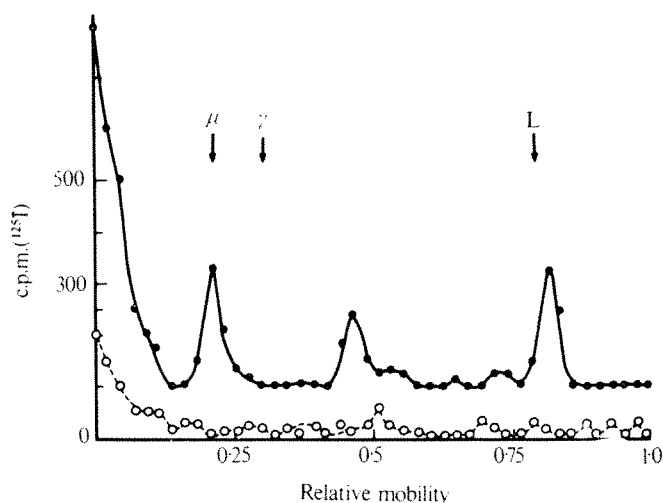


Fig. 1 Analysis by polyacrylamide gel electrophoresis in SDS-containing buffer of reduced ^{125}I -labelled surface immunoglobulins of chicken bursa lymphocytes. Sample is adult bursa, experiment I, Table 1. ●, Specifically precipitated immunoglobulin (RAFγG + GARG); ○, control precipitate (NRS + GARG). μ, γ and L indicate the positions at which human μ chain, γ chain and light chains migrated in this 9% gel.

Isolation of surface immunoglobulins from lymphocytes from chicken thymus and bursa

ALTHOUGH the chicken is more closely related to dinosaurs and crocodiles than to the mouse¹, the clear-cut demarcation between thymus-derived (T) and bursa-derived (B) lymphocytes in this bird^{2,3} offers an excellent system for study of the two lymphocyte types. B cells have been demonstrated to possess surface immunoglobulin³⁻⁵, while T cells have not, although it has been established that light chains at least are expressed on specifically activated chicken T cells⁶. So far, however, intact polypeptide chains have not been demonstrated on T and B cells. We now describe the isolation and partial characterisation of surface immunoglobulins of chicken B and T lymphocytes. We have evidence that both lymphocyte types have surface immunoglobulins consisting of light chains, μ chains and a heavy chain of molecular weight 40,000.

Lymphocyte suspensions were prepared from thymuses, bursas and spleens of normal chickens (outbred white leghorn-australorp hybrids; newly hatched or 3-4 months old) and thymuses and spleens of hormonally bursectomised⁷ chickens (Table 1). The cell populations were greater than 95% viable and consisted predominantly of lymphocytes (bursa and thymus, approximately 95%; spleen approximately 80%). These suspensions were surface-radioiodinated as previously described (refs 8 and 9 and Table 1 and Figure legends); ^{125}I -labelled surface proteins were extracted by various methods as described in the legend to Table 1, and immunoglobulin was isolated by specific immunological precipitation using antiserum to chicken 7S immunoglobulin (IgY) which possessed activity directed against light chains and the γ (Y) heavy chain.

As Table 1 shows, ^{125}I counts were specifically precipitated from labelled surface protein preparations obtained from thymic, bursa and splenic lymphocytes. In one case where thymus cells of neonatal chickens (day 1) were extracted with 1% Nonidet P-40, 6 M urea (apparent pH 7.2) no significant ^{125}I counts were precipitated by antiserum to chicken immunoglobulin. Because the other thymus lymphocyte preparations contained specifically precipitable immunoglobulin, we believe that the negative result represents a technical problem which arises from the release of proteolytic enzymes during the lysis of whole cells with the detergent urea mixture (D. Hausteine and J. J. M., unpublished observations). Between 1.5 and 3.5% of ^{125}I -iodide counts in high molecular weight cell surface protein

of bursal lymphocytes were precipitated as immunoglobulin. Corresponding results for thymus lymphocyte populations varied from 1.0 to 2.4% under these conditions.

Although these results show that surface immunoglobulin can be isolated from chicken lymphocytes, they tell nothing about the class of such immunoglobulins because the antiserum used possessed activity for light chains. To investigate this problem we dissolved the precipitates in 3% sodium dodecyl sulphate (pH 6.8) containing 10% glycerol, 6M urea and 2% mercaptoethanol to cleave interchain disulphide bonds, and resolved heavy and light polypeptide chains by electrophoresis in 9% (w/v) polyacrylamide gels under conditions described by Laemmli¹⁰. Figures 1 and 2 illustrate the polypeptide chain distribution patterns for adult (3 months) bursal lymphocytes (Fig. 1) and neonatal thymus lymphocytes (Fig. 2). These patterns are representative of all experiments. In both cases illustrated, components with mobilities characteristic of μ chains and light chains were resolved. Each pattern also contained a component with a relative mobility of approximately 0.5 which corresponded to a molecular weight of about 40,000 in terms of the standard proteins used. High molecular weight aggregate which did not enter the gel was also present in all chicken samples analysed. No definite components resembling the chicken γ (Y) chain (molecular weight 65,000; ref. 11) were found in any preparations.

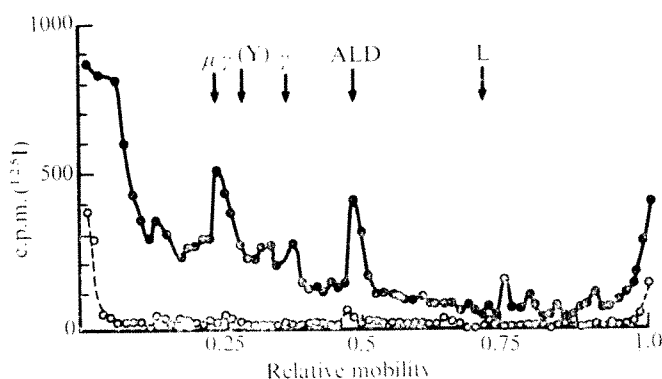


Fig. 2 Analysis by polyacrylamide gel electrophoresis in SDS-containing buffer of reduced ^{125}I -labelled surface immunoglobulins on neonatal thymus lymphocytes (experiment IV, Table 1, turnover sample). ●, Specifically precipitated sample (FγG + RAFγG); ○, control, precipitate (MγG + RAMγG). μ, μ chain (chicken); γ (Y) chicken γ (Y) heavy chain; γ, human γ chain; ALD, aldolase; L, light chain (chicken). 9% polyacrylamide.

Table 1 Isolation of ^{125}I -labelled surface immunoglobulin from chicken lymphocytes

Experiment	Cell source	Extraction procedure	Specific system	(¹²⁵ I-iodide in precipitate)	Control system	Difference	% Initial counts (high mol. wt protein in sample) precipitated as Ig
I	Bursa (adult)	Turnover	12,600 ± 700 (a)	3,600 ± 100 (b)	9,600	1.5	
II	Bursa (neonatal)	Turnover	15,000 ± 940 (c)	2,400 ± 90 (d)	12,600	3.5	
III	Bursa (neonatal)	Turnover	9,600 ± 600 (c)	1,200 ± 50 (d)	8,400	3.5	
		Acid-urea	4,800 ± 350 (c)	1,200 ± 80 (d)	3,600	3.2	
IV	Thymus (neonatal)	Turnover	8,300 ± 700 (c)	2,800 ± 30 (d)	5,500	2.4	
		Nonidet P-40 extract of pellet	11,700 ± 1,300 (c)	4,100 ± 300 (d)	7,600	0.6	
V	Thymus (bursectomised)	Nonidet P-40 urea	1,800 ± 200 (c)	1,500 ± 80 (d)	N.S.	—	
VI	Thymus (bursectomised)	Acid-urea	3,900 ± 300 (c)	900 ± 70 (d)	3,000	1.0	
	Spleen (bursectomised)	Acid-urea	2,500 ± 130 (c)	300 ± 20 (d)	2,200	0.7	
	Thymus (normal)	Acid-urea	5,900 ± 120 (c)	2,000 ± 40 (d)	3,900	1.3	
	Spleen (normal)	Acid-urea	1,100 ± 40 (c)	400 ± 30 (d)	700	0.5	
	Bursa (normal)	Acid-urea	12,600 ± 400 (c)	4,900 ± 130 (d)	7,700	2.5	

Chicken lymphocytes were radioiodinated as follows. To 10^7 lymphocytes in 50 μl phosphate buffered saline (0.02 M phosphate; 0.15 M NaCl, pH 7.2) containing 20 μg lactoperoxidase (either prepared by the method of Morrison and Hultquist²³ or purchased from J. L. B. Laboratories, Granada Hills, California) were added 4 μl (approximately 300 μCi) carrier-free ^{125}I -iodide (Radiochemical Centre, Amersham). The reaction was initiated by addition of 10 μl of 0.03% H_2O_2 followed by mixing. The cells were incubated at 30°C for 5 min, after which the reaction was stopped by addition of large volumes of chilled phosphate buffered saline (PBS). Cells were washed twice at 4°C by centrifugation before lysis or metabolic release. Uptake of ^{125}I -iodide ranged from 10–20% of the initial amount. Between 5 and 10% of this cell-associated value was high molecular weight as judged by dialysis or trichloroacetic acid precipitation. Metabolic release (turnover) of surface immunoglobulins of labelled cells was achieved as before²⁴. For extraction with Nonidet P-40 cells were incubated in 0.5% Nonidet P-40 in PBS at a concentration of 10^7 cells ml for 15 min at room temperature. Nuclei and insoluble particulate matter were removed by centrifugation. Acid-urea extraction was performed as described elsewhere⁹. In extraction with Nonidet P-40 urea, the cells were dissolved in 1% Nonidet P-40–6M urea; otherwise the conditions were as described above. All samples were dialysed against 0.15 M NaCl buffered to pH 8.0 with Tris-HCl before immunological analysis. Coprecipitated samples were washed three or four times. Data given here refer to ^{125}I counts (means \pm s.e. of at least four replicates) associated with specific or control precipitates. Precipitation systems were (a) rabbit antiserum to chicken IgG (Y) immunoglobulin (contains specificity for light chains and γ (Y) chains) plus goat antiserum to rabbit IgG (Commonwealth Serum Laboratories, Melbourne); (b) normal rabbit serum plus goat antiserum to rabbit IgG; (c) carrier chicken IgG (Y) plus rabbit antiserum to chicken IgG (Y), and (d) mouse IgG plus rabbit antiserum to mouse IgG. All systems were calibrated to precipitate more than 90% of their homologous antigen. Data given represent counts from $2\text{--}5 \times 10^6$ lymphocytes.

A significant finding was the release of IgM by turnover from thymuses of newly hatched chickens. In these chickens the only existing immunoglobulin is maternal IgG (Y) released from the yolk, and there is no detectable IgM in the serum¹². A second significant finding was that chicken bursa lymphocytes express surface immunoglobulin polypeptide light chains, μ chains and a component of molecular weight 40,000. It is not surprising to find light chains and μ chains because several workers using methods similar to ours isolated IgM immunoglobulin from human¹³ and murine^{14,15} B lymphocytes. The 40,000 molecular weight component was unexpected, but is consistent with the report that chicken B lymphocytes synthesise and apparently express on their surfaces a component of similar size which is antigenically related to μ chain¹⁶ and termed H_μ chain. A heavy chain of this size is interesting from an evolutionary viewpoint because the major immunoglobulin class in certain reptiles¹⁷ and birds¹⁸ contains a heavy chain of mass approximately 40,000 daltons. This heavy chain has been termed the nu chain¹⁹. Although such a component is not readily detectable in chicken serum, it might constitute an important surface-associated receptor molecule in this species. We do not believe that this surface-associated heavy chain is a degradation product of μ chain because of the biosynthetic results of Choi and Good¹⁶ and because the molecule has been obtained under conditions where we have not observed proteolysis in studies of mammalian

lymphocyte surface immunoglobulin²⁰. Ladoulis *et al.*²⁵ reported immunoglobulins of molecular weight 200,000 and 130,000 on the plasma membranes of rat thymus and spleen lymphocytes. The chicken surface immunoglobulins tended to aggregate and we could not estimate intact molecular weights. If they consist of L_2H_2 units analogous to surface immunoglobulins of man^{13,15,20} and mouse^{14,15,20}, they should have molecular weights of about 185,000 and 125,000.

Our finding of immunoglobulins consisting of μ and light chains on chicken T cells is consistent with similar studies of mammalian T cells^{15,20,21}. Biosynthesis has demonstrated immunoglobulin in thymocyte populations of normal chickens²² and Theis and Thorbecke⁶ inhibited specific delayed type hypersensitivity reactions in bursectomised chickens by antisera to immunoglobulin, concluding that chicken T lymphocytes have at least light chain antigenic determinants on their surfaces. We have now established that such cells also express μ chains and a chain similar to the H_μ heavy chain described by Choi and Good¹⁶. Because our method gives a bulk average, it is not possible to decide whether μ chain and H_μ chain occur on the same T or B lymphocytes.

The existence of IgM on the surface of mammalian T cells has been confirmed independently in several laboratories^{21,25,26}. The presence of IgM on the thymocytes from newly hatched chickens which lack detectable circulating IgM, makes it improbable that

this immunoglobulin has been adsorbed from the serum.

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- ¹ Walker, A. D., *Nature*, **237**, 257 (1972).
- ² Warner, N. L., Szenberg, A., and Burnet, F. M., *Austr. J. exp. biol. med. Sci.*, **40**, 373 (1962).
- ³ Kincade, P. W., and Cooper, M. D., *J. Immun.*, **106**, 371 (1971).
- ⁴ Rabellino, E., and Grey, H. M., *J. Immun.*, **106**, 1418 (1971).
- ⁵ Albin, B., and Wick, G., *Int. Arch. Allergy*, **44**, 804 (1973).
- ⁶ Theis, G. A., and Thorbecke, G. J., *J. Immun.*, **110**, 91 (1973).
- ⁷ Meyer, R. K., Rao, M. A., and Aspinall, R. L., *Endocrinology*, **64**, 890 (1959).
- ⁸ Marchalonis, J. J., Cone, R. E., and Santer, V., *Biochem. J.*, **124**, 921 (1971).
- ⁹ Cone, R. E., and Marchalonis, J. J., *Biochem. J.* (in the press).
- ¹⁰ Laemmli, U. K., *Nature*, **227**, 680 (1970).
- ¹¹ Leslie, G. A., and Clem, L. W., *J. exp. Med.*, **130**, 1337 (1969).
- ¹² Martin, L. N., and Leslie, G. A., *Proc. Soc. exp. biol. Med.*, **143**, 241 (1973).
- ¹³ Marchalonis, J. J., Atwell, J. L., and Haustein, D., *Biochim. biophys. Acta* (in the press).
- ¹⁴ Vitetta, E. S., Baur, S., and Uhr, J. W., *J. exp. Med.*, **134**, 242 (1971).
- ¹⁵ Marchalonis, J. J., Cone, R. E., and Atwell, J. L., *J. exp. Med.*, **135**, 956 (1972).
- ¹⁶ Choi, Y. S., and Good, R. A., *J. exp. Med.*, **135**, 1133 (1972).
- ¹⁷ Chartrand, S. L., Litman, G. W., Lapointe, N., Good, R. A., and Frommel, D., *J. Immun.*, **107**, 1 (1971).
- ¹⁸ Grey, H. M., *J. Immun.*, **98**, 811 (1967).
- ¹⁹ Marchalonis, J. J., and Cone, R. E., *Austr. J. exp. Biol. med. Sci.*, **51**, 461 (1973).
- ²⁰ Marchalonis, J. J., and Cone, R. E., *Transplant. Rev.*, **14**, 3 (1973).
- ²¹ Moroz, C., and Hahn, Y., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3716 (1973).
- ²² Bienenstock, J., Gauldie, J., and Perey, D. Y. E., *J. Immun.*, **111**, 1112 (1973).
- ²³ Morrison, M., and Hultquist, D. E., *J. biol. Chem.*, **238**, 2847 (1963).
- ²⁴ Cone, R. E., Marchalonis, J. J., and Rolley, R. T., *J. exp. Med.*, **134**, 1373 (1971).
- ²⁵ Ladoulis, C. T., Gill, III, T. J., Chen, S.-C., and Misra, D. N., *Prog. Allergy* (in the press).
- ²⁶ Boylston, A. W., and Mowbray, J. F., cited in Greaves, M. F., Owen, J. J. T., and Raff, M. C., *T and B lymphocytes*, 116 (Excerpta Medica Foundation, Amsterdam, 1973).

Effect of T cell depletion on the potentiated reagin response

In the rat, infection with helminth parasites can have the remarkable effect of causing nonspecific potentiation of IgE (reaginic) antibody responses against antigens unrelated to those of the parasite. This phenomenon, which has been called the potentiated reagin response¹, has been found to encompass IgE responses to antigens as diverse as egg albumin, keyhole limpet haemocyanin and house dust² and has been produced with representatives of both nematode and trematode parasites³.

The potentiated reagin response is but one manifestation of the potent IgE stimulating effect⁴ of helminths. Infections with these parasites also induce high levels of reaginic antibody specific for parasite derived antigens, and at least in man, cause greatly elevated levels of total serum IgE. Parasite-specific reagins which were first described in in-

fections of the rat with *Nippostrongylus brasiliensis* and the rat and monkey with *Schistosoma mansoni*⁵ have been demonstrated in various host-parasite systems in many species including man^{5,6}.

The potentiated reagin response provided a basis for experimental investigation of the mechanism of IgE stimulation in helminth infections. It is known that parasitic infection can only amplify an already existing IgE response: in the rat this must be induced before infection by inoculation of the antigen together with a conventional adjuvant^{1,7}. IgE responses against different antigens may be simultaneously potentiated² while IgG responses remain largely unaffected^{7,8}. The indications from these results are that live worms produce a factor which has the capacity to stimulate those immunocytes previously programmed for IgE production.

To find out whether this factor acts directly on IgE-producing B cells or whether its effect is produced through T cells, we examined the effect of T cell depletion and show here that this prevents potentiation of reagin responses in rats infected with the nematode *N. brasiliensis*.

Outbred female Hooded Lister rats (Animal Suppliers (London) Ltd), were used. Thymus deprived (B) rats were prepared by thymectomy at 4 weeks of age, followed by 850 rad whole body irradiation 2-4 weeks later. During

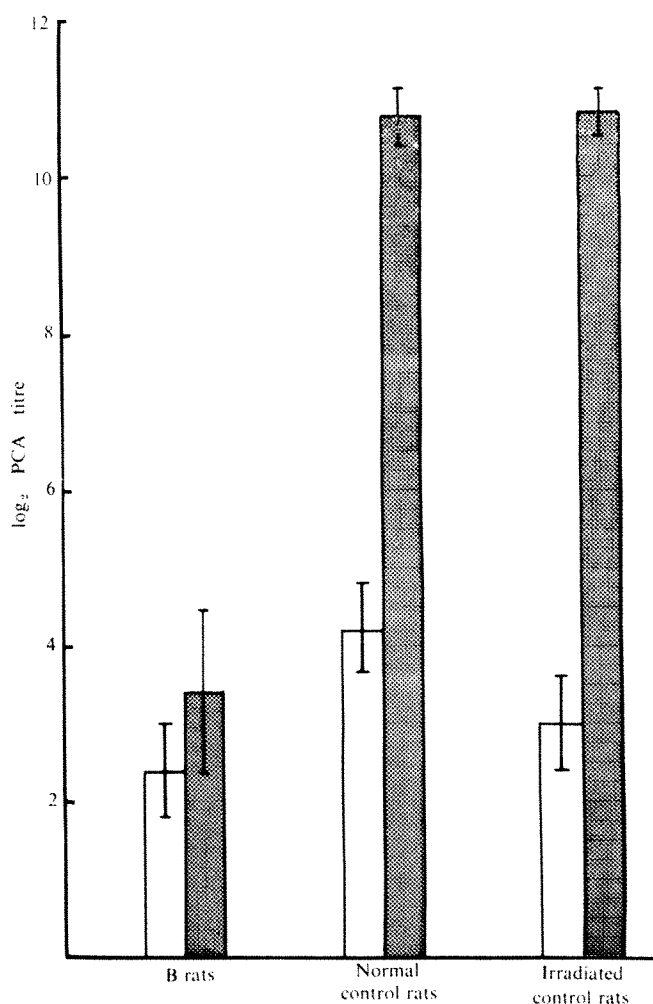


Fig. 1 Mean EA reaginic antibody levels 4 d before (□) and 14 d after (▨) *N. brasiliensis* infection. Eighteen B rats, 10 normal control rats and 8 irradiated control rats were immunised with 1 mg EA (58 d after thymectomy; 38 d after irradiation). Of these 9 B rats, 10 normal rats and 6 irradiated rats produced an EA IgE response and were infected with *N. brasiliensis* 28 d after immunisation. The means and standard errors shown above were calculated using logarithmically transformed antibody titres.

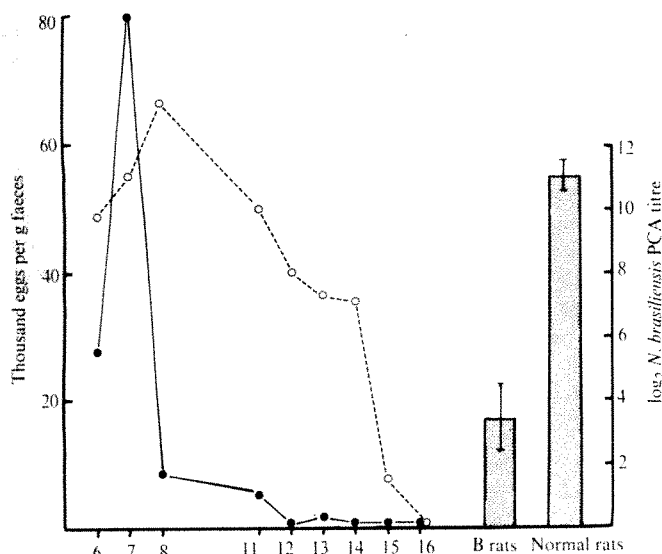


Fig. 2 Prolonged egg output in B rats (○) infected with *N. brasiliensis* compare with infected normal rats (●). Groups of 10 B rats and 5 normal rats were killed 19 d after infection when mean worm burdens in the small intestine were found to be 618 ± 255 (s.e.) for the B rats and 3 ± 2 (s.e.) for the normal controls. Of these animals 5 B rats and 5 normal rats had detectable circulating *N. brasiliensis* reagins and the mean levels (\pm s.e.) are shown above.

irradiation the distal parts of both hind limbs were shielded to allow auto-repopulation by bone marrow cells. This technique⁹ has been shown to produce animals whose T deficiency resembles that of thymectomised animals prepared by irradiation and reconstitution with bone marrow cells. The animals were immunised 1–2 months later by intraperitoneal inoculation of 1 mg of either egg albumin (EA) (Sigma grade 5) or keyhole limpet haemocyanin (KLH) (Cal Biochem A grade) together with 10^{10} *Bordetella pertussis* organisms (Wellcome Biological Reagents). At appropriate times after immunisation, the animals were bled from the tail vein and IgE antibody levels were determined by titration of individual sera by the passive cutaneous anaphylaxis (PCA) technique¹⁰. The IgE-producing B rats together with IgE-producing normal rats were then infected by subcutaneous inoculation of 4,000 *N. brasiliensis* larvae and were bled for antibody estimations 12–14 d later when the potentiated reagin response normally reaches its peak.

Table 1 shows the results of one such experiment involving groups of B and normal rats immunised with either EA or KLH. It can be seen from the individual titres that whereas reagin responses were markedly potentiated in eight out of ten normal animals a significantly raised response occurred in only one of ten B rats. This latter animal is in fact the only B rat in which potentiation has occurred in four experiments.

Figure 1 shows the results of another similar experiment which included an additional control group of rats which had been irradiated without previous thymectomy. Again it is clear that the parasitic infection did not have any significant effect on the levels of EA reagin antibody in B rats whereas the response of the normal animals was substantially raised. The results also show that the process of irradiation does not in itself interfere with the ability to form potentiated reagin responses. Two other experiments were performed which gave consistent results.

In these experiments we also studied some parameters of the immune response to the parasite itself since T-cell deficiency has been described as depressing immune responses in parasitic infections^{11,12}. In normal adult rats,

Table 1 Effect of *N. brasiliensis** infection on egg albumin (EA) and keyhole limpet haemocyanin (KLH) IgE responses in normal and thymus deprived (B) rats.

	PCA titres 4 d before and 12 d after infection†			
	B rats		Normal rats	
	Before infection	After infection	Before infection	After infection
EA reagin responses	4	0	16	32,768
	4	16	16	1,024
	8	2	64	8,192
	16	16	8	64
	8	512	32	2,048
KLH reagin responses	16	64	8	64
	8	1	16	1,024
	8	2	8	2,048
	8	8	128	2,048
	8	16	4	1,024

The Hooded Lister strain of rats which we use normally produce a good IgE response on immunisation with doses of EA as low as 1 μ g (ref. 18). Preliminary experiments with B rats showed that these did not have an IgE response following doses of 10 μ g EA but that 33–50% did so following doses of 1 mg EA. The ability to overcome effects of T cell deficiency by increasing the antigen dose has been reported in other systems^{19,20}. In this experiment, 27 B rats were divided into groups of 15 and 13 and the animals of each group were immunised with 1 mg EA or KLH respectively (44 d after thymectomy, 30 d after irradiation). Groups of normal rats were similarly immunised. Five animals in each of the B groups developed a reagin antibody response and they, together with a similar number of normal control animals, were infected with *N. brasiliensis* 26 d after immunisation.

* The method of culture of *N. brasiliensis* and preparation of the infective larval dose have been previously described^{21,22}.

† To estimate reagin antibodies by the PCA technique 0.1 ml quantities of saline dilutions of the test sera were injected intradermally in Hooded Lister recipient rats, each injection being duplicated on a different animal. 48–72 h later the animals were injected intravenously with 2.5 mg of either EA or KLH together with 0.5 ml of 1% Evans blue which acts as an indicator of vasodilation. The skin reactions were examined after 20 min and the titres recorded above are the greatest dilutions of serum which gave reactions larger than 5 mm in diameter.

primary infection with *N. brasiliensis* is followed 10–12 d later by an immunological expulsion of worms from the small intestine. This so called 'self-cure' reaction proceeds exponentially over several days and is preceded by a fall in egg production by the parasite. Reagin antibodies specific for *N. brasiliensis* appear during or shortly after worm expulsion and rise to reach high circulating levels. These manifestations of parasitic immunity were found to be markedly depressed in the B rats in our experiments. Egg production continued for a longer period than in the control animals, the expulsion rate of worms was decreased and the parasite specific IgE response was depressed or undetectable (Fig. 2).

Although it seems clear that T cells are involved in the potentiation of existing IgE responses by *N. brasiliensis* infection, their mode of action is still conjectural. Other workers using hapten-carrier immunisations have shown that carrier-specific T cells are heavily involved both as helpers^{13–15} and suppressors^{16,17} of B cells in anti-hapten IgE responses of rats and mice. In these situations, the T cell effect is antigen specific. The potentiated reagin response on the other hand is a nonspecific event and any theories about its mechanism must accommodate this element of nonspecificity as well as the requirement for T cells and the fact that it is only, or largely, IgE responses which are affected. T cells are known to produce a variety of soluble factors which can affect both cell-mediated and humoral immunity and it would seem most likely that it is through such factors that they exert their role in the potentiated reagin response.

Assuming that T cells exert a helper effect, potentiation could be brought about in one of two ways. In the first,

the parasite-derived potentiating factor activates a population of T cells to produce a substance which is capable of selectively stimulating IgE B cells regardless of their antigenic specificity. In the second, the parasitic factor activates T cells so that those which have preordained IgE helper functions (induced by previous immunisation) are driven to perform them. In the latter mechanism, the T cell effects on the B cell is antigen specific. It is equally possible that the potentiation of reagins is the result of release of B cells from the inhibiting effect of suppressor T cells but, in essence, the mechanisms outlined above would remain the same.

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- ¹ Orr, T. S. C., and Blair, A. M. J. N., *Life Sci.*, **8**, part II 1073-1077 (1969).
- ² Jarrett, E. E. E., and Stewart, D. C., *Immunology*, **23**, 749-755 (1972).
- ³ Jarrett, E. E. E., *Immunology*, **22**, 1099-1101 (1972).
- ⁴ Ogilvie, B. M., *Nature*, **204**, 91-92 (1964).
- ⁵ Sadun, E. H., *Immunity to Animal Parasites* (edit. by Soulsby, E. J. L.), 97-129 (Academic Press, 1972).
- ⁶ Bennich, H., and Johansson, S. G. O., *Adv. Immun.*, **13**, 1-55 (Academic Press, 1971).
- ⁷ Jarrett, E. E. E., Henderson, D., Riley, P., and White, R. G., *Int. Archs Allergy appl. Immun.*, **42**, 775-781 (1972).
- ⁸ Bloch, K. J., Ohman, J. L., Waltin, J., and Cygan, R. W., *J. Immun.*, **110**, 197-204 (1973).
- ⁹ Parrott, D. M. V., and Ferguson, A., *Immunology*, **26**, 571-588 (1974).
- ¹⁰ Ovary, Z., *Immunological Methods* (edit. by Ackroyd, J. F.), 259-283 (Blackwell, Oxford, 1964).
- ¹¹ Ogilvie, B. M., and Jones, V. E. *Parasitology*, **57**, 335-349 (1966).
- ¹² Walls, R. S., Carter, R. L., Leuchars, E., and Davies, A. J. S., *Clin. exp. Immun.*, **13**, 231-242 (1973).
- ¹³ Tada, T., and Okumura, K., *J. Immun.*, **107**, 1137-1145 (1971).
- ¹⁴ Hamaoka, T., Katz, D. H., and Benacerraf, B., *J. exp. Med.*, **138**, 538-556 (1973).
- ¹⁵ Okudaira, H., and Ishizaka, K., *J. Immun.*, **111**, 1420-1428 (1973).
- ¹⁶ Okumura, K., and Tada, T., *J. Immun.*, **107**, 1682-1689 (1971).
- ¹⁷ Tada, T., Okumura, K., and Taniguchi, M., *J. Immun.*, **111**, 952-961 (1973).
- ¹⁸ Jarrett, E. E. E., and Stewart, D. C., *Immunology* (in the press).
- ¹⁹ Aird, J., *Immunology*, **20**, 617-624 (1971).
- ²⁰ *Transpl. Rev.* (edit. by Möller, G.), **1** (1969).
- ²¹ Jennings, F. W., Mulligan, W., and Urquhart, G. M., *Exp. Parasit.*, **13**, 367-373 (1963).
- ²² Jarrett, E. E. E., Jarrett, W. F. H., and Urquhart, G. M., *Parasitology*, **58**, 625-639 (1968).

homogeneous populations of glucocorticoid target cells has advanced our understanding of the molecular mechanisms involved^{2,3}. Using a specific antiserum we have now shown that cortisol induces glycerol phosphate dehydrogenase (GPDH, EC 1118) in a rat brain tumour cell line by increasing the number of GPDH molecules rather than by increasing the activity of each molecule. Also, the induction does not involve isozymes since GPDH from induced and uninduced cells is identical with respect to immunological titration, electrophoretic mobility and thermal sensitivity.

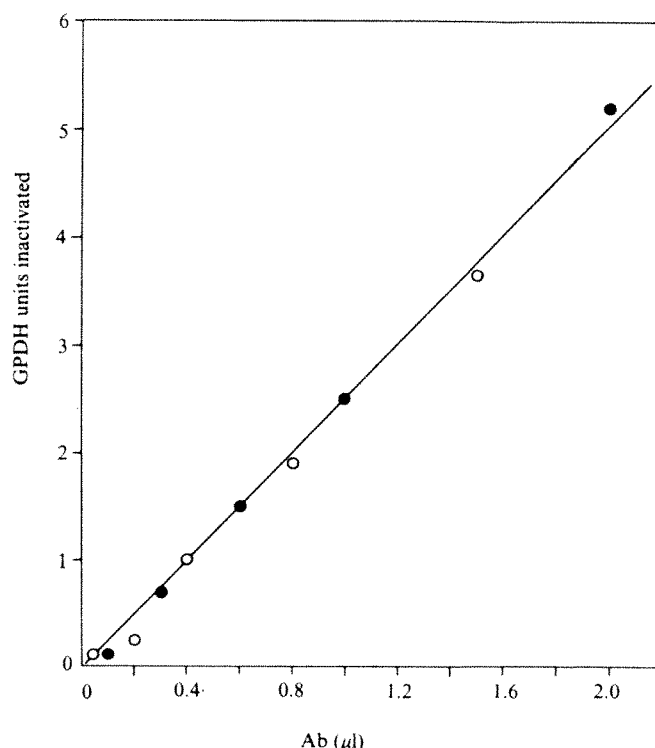


Fig. 1 Immunological titration of GPDH from control and cortisol-induced cells. Cells were grown in Ham's F-10 medium supplemented with 10% foetal calf serum, in Falcon T-250 plastic flasks as described previously^{6,8}. Cortisol (when present) was added to a final concentration of $0.5 \mu\text{g ml}^{-1}$ for 3 d before collection. The cells were first washed twice with 3 ml volumes of 10 mM Na phosphate buffer, pH 7.5, containing 5 mM mercaptoethanol and 1 mM EDTA and then were collected in 1 ml of the same buffer using a rubber policeman to scrape the cells from the flask. After disruption by sonication cellular debris was removed by centrifugation for 1 h at 30,000 r.p.m. in a 30 rotor using a Beckman L2-65-B ultracentrifuge. The supernatants from induced and uninduced cells were assayed for GPDH activity as described previously¹⁵ and the concentration of enzyme was equalised by concentrating the supernatant from uninduced cells using an Amicon ultrafiltration apparatus (PM30 membrane). Rabbit antiserum against purified rat brain GPDH was obtained as described previously¹⁵. The serum was initially diluted in 0.01 M phosphate buffer, pH 7.5 containing 1 mg of bovine serum albumin ml^{-1} , 1 mM EDTA and 5 mM mercaptoethanol and from this stock solution a series of dilutions of antiserum was made. A sample of 0.2 ml of each of the dilutions was added to tubes in triplicate. The high speed supernatant from induced and uninduced cells was used as the source of GPDH and a constant amount (0.1 ml, 200 U ml^{-1}) was added to each tube. After incubation at 37°C for 30 min an aliquot was removed and remaining GPDH activity was determined. Incubation overnight at 4°C did not result in further inactivation of GPDH and since the antibody-GPDH complex was enzymatically inactive it was not necessary to centrifuge the samples. The amount of enzyme remaining after incubation with antiserum was subtracted from that present in the absence of antiserum and the results were plotted as the number of units of enzyme removed against the microlitres of antiserum present. O, GPDH from uninduced cells; ●, GPDH from cortisol induced cells.

Cortisol induction of glycerol phosphate dehydrogenase in a rat brain tumour cell line

HORMONAL regulation of enzyme levels in mammalian cells has been known for more than a decade¹ and the use of

The increase in the level of brain GPDH during the development of the rat is regulated specifically by cortisol¹. This hormonal dependence has also been demonstrated in primary cultures of brain cells⁵ and in RGC6 (C6) cells⁶, a line of rat glial tumour cells⁷. The addition of cortisol or other glucocorticoids^{8,9} to confluent C6 cells increases GPDH activity three-fold within 24 h and up to ten-fold over 5 d. This induction requires RNA synthesis, protein synthesis and the continual presence of cortisol^{6,9}.

Simple explanations for this increased activity—a direct effect of cortisol in the assay, increased overall protein synthesis and the presence of diffusible inhibitors or activators—have been ruled out⁶. We have investigated which of the alternative mechanisms is responsible for cortisol-dependent increase in GPDH in C6 cells. An increase in enzyme specific activity can occur if the catalytic activity of each molecule increases^{10,11} or if the number of molecules increases^{2,3,12,13}. These mechanisms are not mutually exclusive nor are they comprehensively explanatory since each could be achieved by various changes in the regulatory machinery of the cell.

Using rabbit antiserum directed against rat brain GPDH,

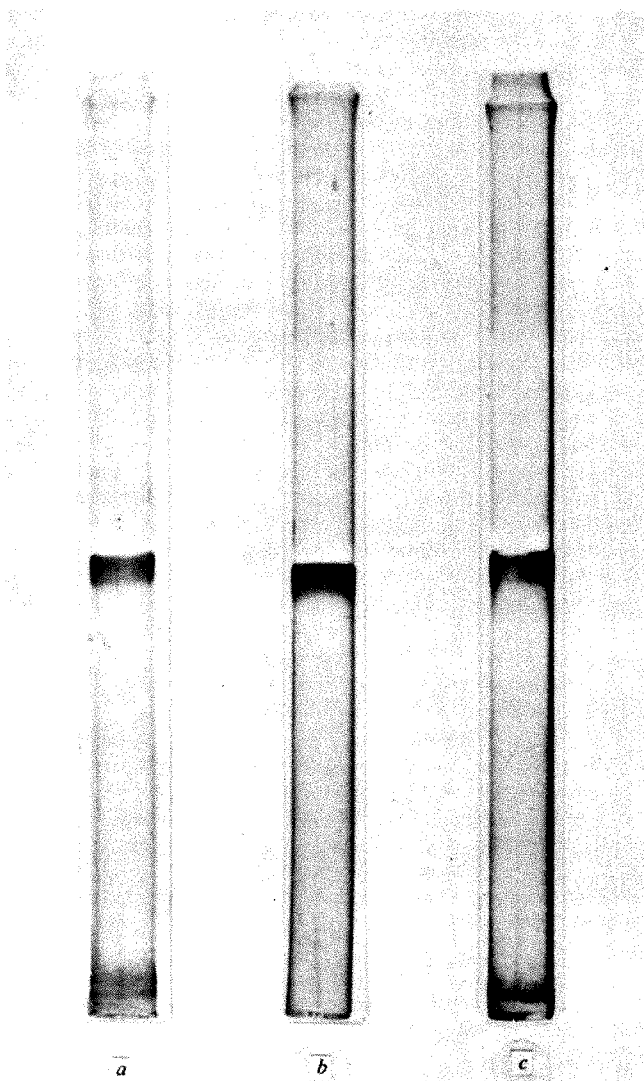


Fig. 2 Electrophoresis of GPDH from C6 cells (clone 2B-13). The cells were grown and collected as described in Fig. 1. Electrophoresis was by the method of Davis¹⁸ and the gels were stained for GPDH activity as described previously¹⁵. Each gel contained a total of 20 U of GPDH, where 1 U of activity is equal to the amount of enzyme which oxidises 1 nmol of NADH min⁻¹ under the conditions of the assay. Supernatants were from induced cells (a), uninduced and induced cells (b) and uninduced cells (c).

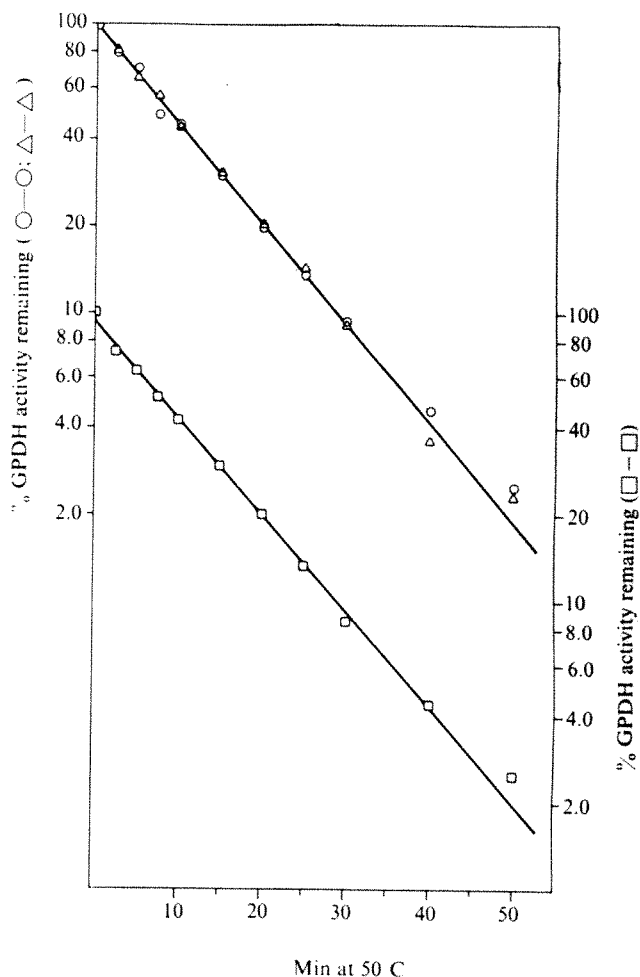


Fig. 3 Heat inactivation of GPDH from induced and uninduced cells. Cells were grown and collected as described in Fig. 1. Before concentration by ultrafiltration, the specific activities of GPDH in the high speed supernatants were 45 U mg⁻¹ of protein and 188 U mg⁻¹ of protein, for uninduced and induced cells respectively. The concentrations of GPDH at the zero time points (100% activity) were 207 U mg⁻¹ for the induced (O), 206 U mg⁻¹ for the uninduced (Δ) and 214 U ml⁻¹ for the mixture (□) containing equal amounts of induced and uninduced enzyme activity. For clarity the data from the mixture are plotted using the ordinate on the right. Three sets of tubes (in duplicate) containing 200 μl of each of the enzyme solutions were incubated in a circulating water bath at 50.0 ± 0.05° C for the indicated times. The tubes were sealed with parafilm to prevent evaporation. Immediately after removal from the bath, the samples were quickly cooled by swirling in an ice-salt slurry (-15° C) for 5 s and were then maintained at 0° C until completion of the experiment. Aliquots from each tube were assayed as described¹⁵.

we have distinguished between the above mechanisms (Fig. 1). If the induction represents an increased number of molecules then equal amounts of antiserum will be needed to inactivate the same number of GPDH units from either induced or uninduced cells. If the induction is due to an increased activity per molecule then the amount of antiserum required to inactivate the same number of enzyme units from each source will be different. Figure 1 shows that equal amounts of enzyme are inactivated by equal amounts of antiserum. Therefore, the cortisol-dependent increase in GPDH activity in C6 cells represents an increase in the number of enzyme molecules.

The existence of more than one enzyme able to catalyse the same reaction has been known for some time. GPDH isozymes have been isolated from tissues of various species and at least two forms of GPDH have been demonstrated in mouse brain¹⁴, while two distinct GPDH have been purified from rat brain¹⁵. In addition, the basal level of

GPDH in C6 clones¹⁶ and in hybrids¹⁷ is apparently regulated independently of the induced level of GPDH. Such data are consistent with the possibility that there is more than one structural gene for GPDH.

Since two electrophoretically distinct forms of GPDH have been purified from rat brain¹⁵, we examined the mobility of GPDH by polyacrylamide gel electrophoresis as a test of the identity of GPDH in hormone-treated and untreated cells. Only one form of GPDH was found in induced (Fig. 2a) and uninduced (Fig. 2c) C6 cells. In addition, since only one band of enzyme activity was found when a mixture was run (Fig. 2b), we conclude that the GPDH in induced cells is electrophoretically identical to that in uninduced cells. Identity by polyacrylamide gel electrophoresis is not definitive proof that the basal enzyme is identical to the induced form, but shows that one of them does not represent an oligomeric form of the other and that there is no net charge difference between them.

Further confirmation of the identity of the induced and basal forms of GPDH was obtained from heat denaturation experiments. The ability of GPDH from control and treated cells to survive heating at 50° C (Fig. 3) showed that there was only one heat labile component in induced and uninduced cells. The single straight line obtained when supernatants from induced and uninduced cells are mixed and heated indicates that the GPDH from both sources is identical. These results, together with the electrophoretic and immunological data, argue against the possibility that the induction of GPDH activity in C6 cells involves the formation of an isozyme. The sensitivity of these procedures in detecting isozymes has been discussed¹⁸ and more recently similar techniques were used to demonstrate the absence of isozymes in tyrosine aminotransferase induction in hepatoma cells²⁰.

We are now investigating the mechanism(s) by which the number of GPDH molecules increases. Obviously an increase in the rate of synthesis^{12,13} of the enzyme or a decrease in its rate of degradation²¹ or a combination of these could lead to an accumulation of GPDH molecules. The determination of the specific rates of synthesis and degradation will allow us to distinguish between these possibilities.

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- ¹ Knox, W. D., *Br. J. exp. Path.*, **32**, 462 (1961).
- ² Tomkins, G. M., Thompson, E. B., Hayashi, S., Gelehrter, T., Granner, D. K., and Peterkofsky, B., *Cold Spring Harbor Symp. quant. Biol.*, **31**, 349 (1966).
- ³ Reel, J. R., Lee, K. L., Kenney, F. T., *J. biol. Chem.*, **245**, 5800 (1970).
- ⁴ de Vellis, J., and English, D., *J. Neurochem.*, **15**, 1061 (1968).
- ⁵ Breen, G., de Vellis, J., and Cole, R., *Trans. third Meeting Soc. Neurosci.*, San Diego (1973).
- ⁶ de Vellis, J., English, D., Cole, R., and Molson, J., in *Influence of hormones on the nervous system* (edit. by Ford, D.), 25 (Karger, Basel, 1971).
- ⁷ Benda, P., Lightbody, J., Sato, G., Levine, L., and Sweet, W., *Science*, **151**, 370 (1968).
- ⁸ de Vellis, J., and Brooker, G., in *Tissue culture of the nervous system* (edit. by Sato, G.) (Plenum Press, New York, 1973).
- ⁹ de Vellis, J., in *Development and Aging in the Nervous System* (edit. by Rockstein, M.) (Academic Press, New York, 1973).
- ¹⁰ Griffin, M. J., and Cox, R. P., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 946 (1966).

- ¹¹ Ghosh, N. I., Rubenstein, A., Baltimore, R., and Cox, R. P., *Biochim. biophys. Acta*, **286**, 175 (1972).
- ¹² Kenney, F. T., *J. biol. Chem.*, **237**, 3495 (1962).
- ¹³ Granner, D. K., Hayashi, S. I., Thompson, E. B., and Tomkins, G. M., *J. molec. Biol.*, **35**, 291 (1968).
- ¹⁴ Kozak, L. P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3170 (1972).
- ¹⁵ McGinnis, J. F., and de Vellis, J., *Biochim. biophys. Acta* (in the press).
- ¹⁶ de Vellis, J., and English, D., *Prog. Brain Res.*, **40**, 321 (1973).
- ¹⁷ Davidson, R. L., and Benda, P., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1870 (1970).
- ¹⁸ Davis, B. J., *Ann. N.Y. Acad. Sci.*, **121**, 404 (1964).
- ¹⁹ Paigen, K. P., in *Enzyme synthesis and degradation in mammalian systems* (edit. by Rechcigl, M.), 1 (Karger, Basel, 1971).
- ²⁰ Gelehrter, T. D., Emanuel, J. R., and Spencer, C. J., *J. biol. Chem.*, **247**, 6197 (1972).
- ²¹ Schimke, R. T., Sweeney, E. W., and Berlin, C. W., *J. biol. Chem.*, **240**, 322 (1965).

***In vitro* restoration of deficient β -galactosidase activity in liver of patients with Hurler and Hunter disease**

HUNTER'S and Hurler's syndromes are recessively inherited errors of metabolism, in which accumulation of mucopolysaccharides in tissues is accompanied by skeletal abnormalities and mental retardation. Patients suffering from these diseases show a strikingly reduced activity of β -galactosidase in the liver although this enzyme is not directly involved in the degradation of the glycosaminoglycans that accumulate in the tissues¹⁻³. This enzyme reduction which is accompanied by an abnormal isoenzyme pattern⁴ can be understood as the result of complex formation between stored mucopolysaccharides and the β -galactosidase enzyme. Indeed artificial mixtures of chondroitin sulphate and liver β -galactosidase show an isoenzyme profile quite analogous to that seen in Hurler's and Hunter's diseases⁵. The isoenzyme abnormality and the reduction in enzyme activity of such an artificial mixture can be neutralised by cetylpyridinium chloride (CPC), a quaternary base which is known to form complexes with mucopolysaccharides⁶. Therefore it seemed possible to restore the β -galactosidase activity of the Hurler and Hunter patients.

When increasing amounts of CPC were added to a liver homogenate from four patients with Hunter's or Hurler's syndromes a striking increase in β -galactosidase was seen, up to a CPC concentration of about 25 μ g per mg liver (Fig. 1). But with still higher concentrations of CPC, enzyme activity was strongly reduced almost to nothing. The maximum activation corresponded only to a partial restoration of the β -galactosidase activity. This can be explained on the assumptions⁷: (1) that CPC dissociates the complex of mucopolysaccharides and β -galactosidase with restoration of enzyme activity; and (2) that CPC itself is a strong inhibitor of free β -galactosidase (that is, without bound chondroitin sulphate). The latter statement is supported by the fact that in the homogenate of a normal liver, strong inhibition of the enzyme activity is seen (Fig. 1).

In order to obviate the CPC drawback, I tried to dissociate the complex using other methods. Addition of albumin in acid medium seemed appropriate because of the possible precipitation of mucopolysaccharides with acidified solutions of albumin⁷. When increasing amounts of albumin were added to the homogenates of the patients' liver, β -galactosidase rose about five- to ten-fold, whereas in a normal liver an activation of only 10% was found. The restoration resulted in an essentially normal activity in the livers from two patients, while for the two other patients a final activity of about 40% of the mean normal value was achieved (Fig. 2). The highest restoration was found in the liver which had been stored for the shortest time.

It seems unlikely that therapeutic applications can result from the data presented here. If the albumin concentration

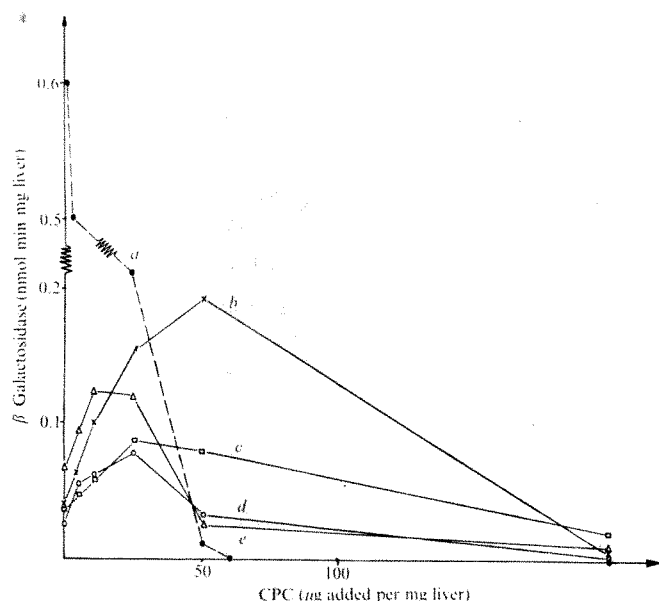


Fig. 1 Influence of increasing amounts of cetylpyridinium chloride on β -galactosidase activity in human liver homogenates of *a*, normal; *b*, Hunter; *c-e*, Hurler individuals. Autopsy material from patient *c* had been stored at -20°C for 4 yr while the three other liver pieces were stored for 7 yr. Liver homogenates (4% v/v) in distilled water were centrifuged after sonicating for 60 s and five cycles of freezing and thawing. Increasing amounts of CPC in distilled water were added to the supernatant of each individual and β -galactosidase activity was then measured as described⁶.

of the *in vitro* experiments reflect the conditions for *in vivo* restoration, high intracellular concentrations of albumin are necessary. Moreover, the restoration of β -galactosidase activity will result in only a minor attack on the stored material. Only the accumulated galactose-containing glycolipids and gangliosides will be broken down, while the fundamental absence of α -iduronidase⁸, sulpho-iduronosulphatase⁹, and N-acetyl- α -glucosaminidase^{10,11} will not be corrected by the albumin treatment.

But, if the mental deficiency of patients with mucopolysaccharidosis is due to the neuronal accumulation of the glycolipids, *in vivo* restoration of β -galactosidase by infusion of large amounts of albumin early in life, might nevertheless be beneficial.

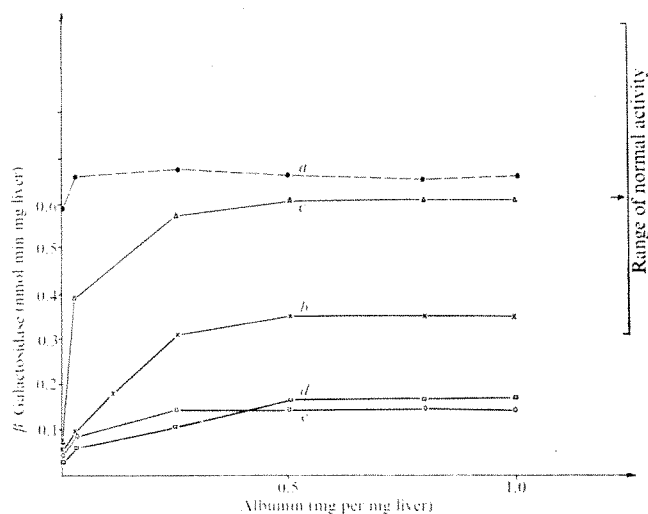


Fig. 2 Influence of increasing amounts of albumin on β -galactosidase activity in liver homogenates from *a*, control; *b*, Hunter; *c-e*, Hurler individuals. Legend and methods as in Fig. 1, but albumin in acetate buffer (0.2 M, pH=4.5) was added to the liver homogenate instead of CPC. The range of normal values is shown at the upper right. The arrow indicates the mean value of the enzyme activity in eight control autopsy livers.

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- ¹ Van Hoof, F., and Hers, H. G., *Eur. J. Biochem.*, **7**, 34-44 (1968).
- ² Öckerman, P. A., *Scand. J. clin. Lab. Invest.*, **22**, 142-146 (1968).
- ³ Mac Brinn, M., Okada, S., Woollacott, M., Patel, V., Ho, M. W., Tappel, A. L., and O'Brien, J., *New Engl. J. Med.*, **281**, 338-343 (1969).
- ⁴ Ho, M. W., and O'Brien, J. S., *Science*, **165**, 611-613 (1969).
- ⁵ Kint, J. A., Dacremont, G., Carton, D., Orye, E., and Hooft, C., *Science*, **181**, 352-354 (1973).
- ⁶ Kint, J. A., *FEBS Lett.*, **36**, 53-56 (1973).
- ⁷ Dorfman, A., and Ott, M. L., *J. biol. Chem.*, **164**, 367-375 (1948).
- ⁸ Mortalon, R., and Dorfman, A., *Biochem. biophys. Res. Commun.*, **47**, 959-964 (1972).
- ⁹ Sjöberg, I., Fransson, L. A., Matalon, R., and Dorfman, A., *Biochem. biophys. Res. Commun.*, **54**, 1125-1132 (1973).
- ¹⁰ Von Figura, K., and Kresse, H., *Biochem. biophys. Res. Commun.*, **48**, 262-269 (1972).
- ¹¹ O'Brien, J. S., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1720-1722 (1972).

Aspirin, indomethacin, catecholamine and prostaglandin interactions on rat arterioles and rabbit hearts

VANE *et al.* have demonstrated that antipyretic, anti-inflammatory drugs can inhibit prostaglandin synthesis and release¹⁻³. We have investigated the effects of aspirin on the responses of rat arterioles to noradrenaline.

Using male, 150 g, Sprague-Dawley rats anaesthetised with ether we cannulated the superior mesenteric artery, dissected out the mesenteric part of its vascular bed and mounted the preparation in an organ bath⁴. We perfused the artery with Krebs bicarbonate buffer at 37°C , bubbled with 5% carbon dioxide in oxygen. After an equilibration period of 30 min we adjusted the flow rate so that the pressure in the arterial cannula had a mean value of about 80 mm Hg. The flow rate was then left constant for the remainder of the experiment. The baseline pressure remained steady (variation less than 10%) for periods as long as 3 h (Fig. 2). The preparation responds to injection of vasoconstrictor drugs by a rise in arterial pressure. The maximal pressor response to constrictor drugs is usually in the region of 80-120 mm Hg above baseline. Noradrenaline solution (0.1 ml) in 0.9% saline was injected into the arterial cannula at regular intervals. Each preparation was tested with a range of noradrenaline concentrations to find one which gave a pressure rise of about 70 mm Hg. This was usually about 1 ng ml^{-1} solution. This concentration was then used for the rest of the experiment. Each injection produced a transient rise in pressure which returned to baseline within about 1 min. Once three pressor responses of constant amplitude ($\pm 10\%$) had been obtained, the formal experiment was started, results being expressed as percentages of the mean initial response.

Aspirin was added to the perfusate in three concentrations 5, 10 and $20\text{ }\mu\text{g ml}^{-1}$ (Fig. 1). The results demonstrate a reversible depression of responsiveness to noradrenaline, the constrictor effect being eliminated by an aspirin concentration of $20\text{ }\mu\text{g ml}^{-1}$. Baseline perfusion pressure was not altered.

The abolition of responsiveness might be explained by pH changes, a direct action of aspirin itself on the interaction between noradrenaline and muscle receptors, on excitation-contraction coupling or on the contractile process,

or an indirect action of aspirin depending on its suppression of prostaglandin synthesis. The $20 \mu\text{g ml}^{-1}$ aspirin concentration produced a pH change of less than 0.1 unit and changes of 0.3 unit produced by adding acetic acid to the buffer caused no significant changes in responsiveness. In five experiments indomethacin, another suppressor of prostaglandin synthesis, in a concentration of $5 \mu\text{g ml}^{-1}$ also produced a complete abolition of responsiveness. We therefore attempted to reverse the effect of aspirin by adding prostaglandin E_2 to aspirin-blocked preparations (Fig. 2).

A prostaglandin E_2 concentration of 1 pg ml^{-1} produced partial restoration of responsiveness. Above 10^3 pg ml^{-1} the relationship between E_2 concentration and responsiveness to noradrenaline flattened and no further increase could be demonstrated above 10^4 pg ml^{-1} . Furthermore at an E_2 concentration of 10^4 pg ml^{-1} the two noradrenaline doses used produced essentially the same responses: sample testing with higher noradrenaline concentrations demonstrated that this was a maximal response under these conditions. Restoration of the control perfusion fluid for 30 min showed that the initial control responsiveness could be nearly restored. Addition of prostaglandin $\text{F}_{2\alpha}$ to the perfusion fluid in concentrations up to 100 ng ml^{-1} failed to restore responsiveness. Prostaglandin E_1 had a weak action, being about 100 times less effective than prostaglandin E_2 .

Possible explanations for the results are that endogenous prostaglandin synthesis may be necessary for the combination of noradrenaline with its receptor site or for the chain of events which occur between catecholamine-receptor combination and contraction. The mechanism is not specific to alpha receptors since we have also shown that the vasoconstrictor effects of vasopressin and angiotensin can also be abolished by either aspirin or indomethacin and restored by prostaglandin E_2 . In contrast in five experiments using a rabbit Langendorff perfused heart preparation the chronotropic and inotropic beta actions of adrenaline were not inhibited by concentrations of indomethacin up to $50 \mu\text{g ml}^{-1}$. This raises the interesting possibility that one difference between alpha and beta effects of catecholamines may be that the former require prostaglandins and the latter do not.

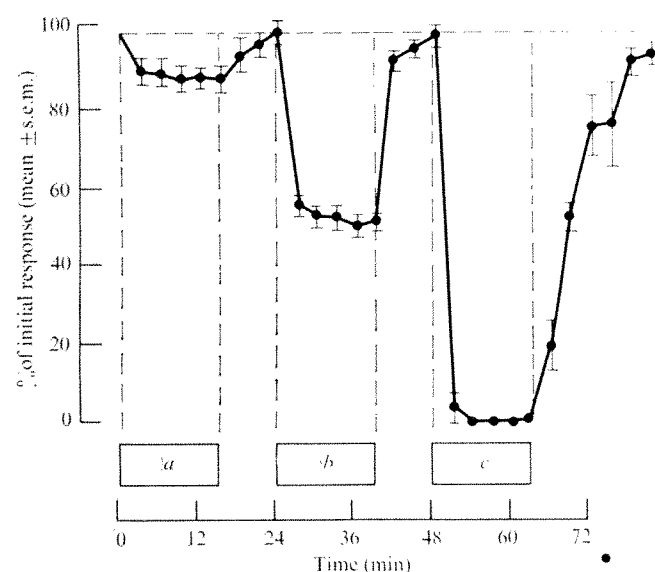


Fig. 1 Changes in responsiveness of the rat mesenteric vascular bed to noradrenaline brought about by adding aspirin to the perfusing fluid. Six experiments were performed. Bars indicate 15 min periods during which aspirin was added to the perfusing fluid; concentrations: a, $5 \mu\text{g ml}^{-1}$; b, $10 \mu\text{g ml}^{-1}$; c, $20 \mu\text{g ml}^{-1}$. Before, between and after the bars the preparation was perfused with Krebs-bicarbonate buffer.

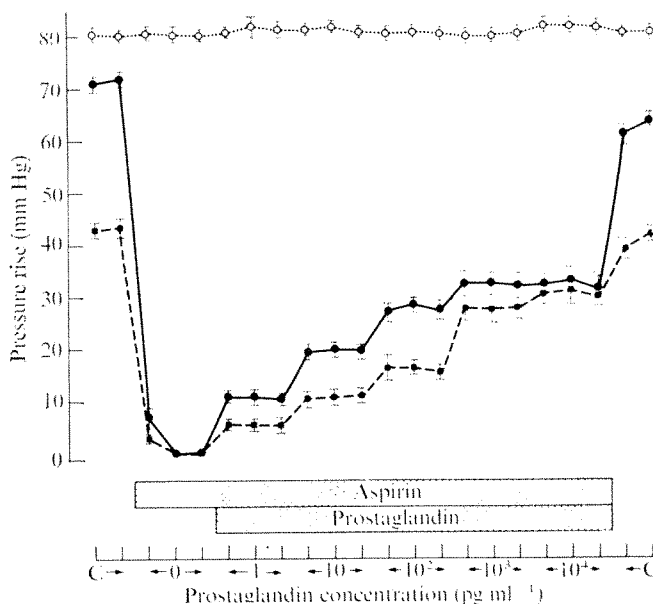


Fig. 2 Responses of the aspirin-blocked rat mesenteric preparation to noradrenaline in the presence of various concentrations of PGE_2 . Closed circles indicate responses to 0.1 ml of a noradrenaline concentration sufficient to produce a pressor response in the initial control period of about 70 mm Hg; closed squares indicate responses to 0.1 ml of a noradrenaline solution of half that concentration. During each test the lower concentration was given first and the higher after the first response was completely over. Open circles indicate the baseline pressure. Five experiments were performed and each point represents the mean pressure rise \pm s.e.m. The pairs of noradrenaline injections were given at five minute intervals. After initial control (C) responses aspirin was added to the perfusate in a concentration of $30 \mu\text{g ml}^{-1}$ in order to block responsiveness completely. While maintaining the aspirin perfusion, progressively increasing concentrations of PGE_2 (Upjohn) were added to the perfusate, perfusion of each concentration being maintained for 15 min. At the end of the experiments Krebs-bicarbonate buffer was perfused through the preparation for 30 min. The last two C (control) responses were the pressor effects obtained 20 and 30 min after returning to the Krebs solution.

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- ¹ Vane, J. R., *Nature new Biol.*, **231**, 232 (1971).
- ² Smith, J. B., and Willis, A. L., *Nature new Biol.*, **231**, 235 (1971).
- ³ Ferreira, S. H., Moncada, S., and Vane, J. R., *Nature new Biol.*, **231**, 237 (1971).
- ⁴ Manku, M. S., Nassar, B. A., and Horrobin, D. F., *Lancet*, **ii**, 991 (1973).

Syntheses of amino acids from aliphatic carboxylic acid by glow discharge electrolysis

Glow discharge electrolysis (GDE)^{1,2} has been studied mainly on inorganic compounds such as water, ammonia,

and metal ions in aqueous solutions. Little study has been done, however, on the chemistry of GDE using organic compounds³⁻⁷ although experimental results indicate that the application of GDE to organic compounds is a promising area in chemistry and is especially interesting in connection with the concept of chemical evolution.

Here we report representative results of amino acid

formation by contact glow discharge electrolysis (CGDE)^{1,2,8} from several aliphatic carboxylic acids by direct amination using ammonia in aqueous solution.

Two types of electrolysis tubes were used for CGDE; one a U-shaped tube, the other a single tube. In the U tube, the cathode and anode compartments were separated by a fine-porosity glass frit and CGDE was carried out in the

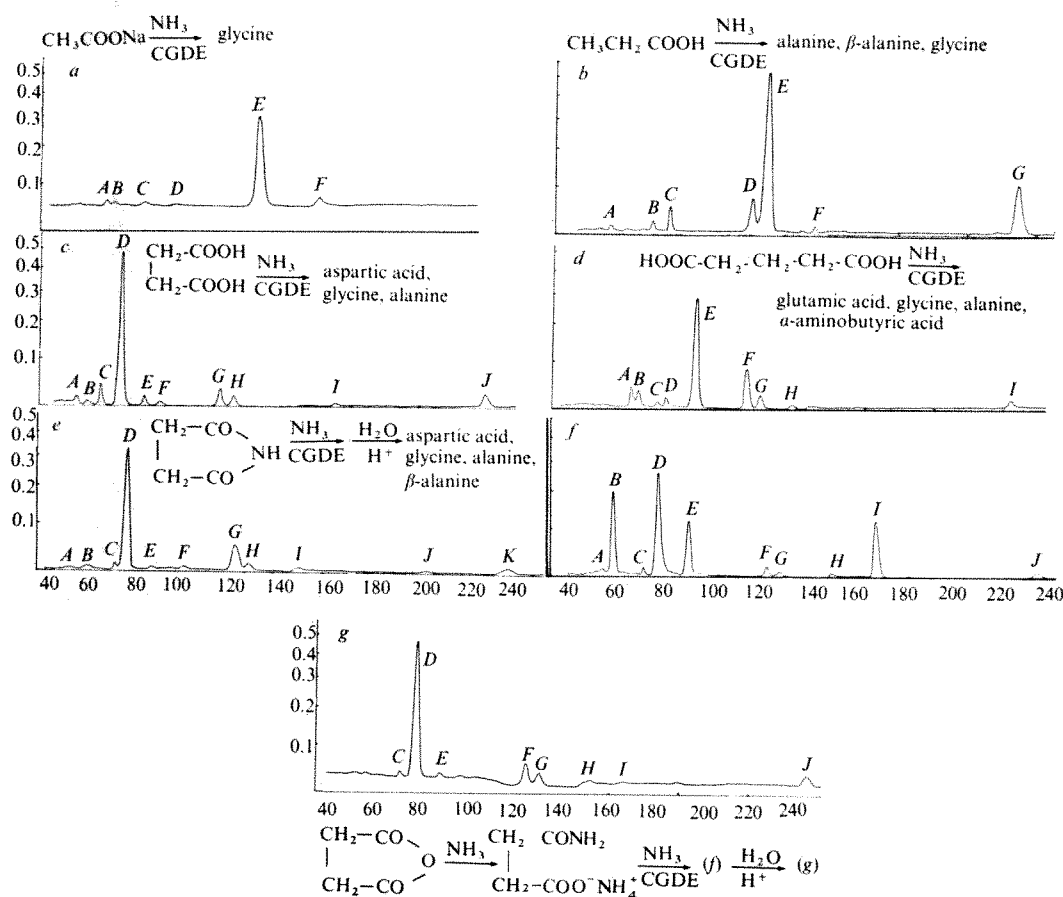


Fig. 1 *a*, A, B, C, D, unknown; E, glycine; F, unknown + buffer change. Reaction conditions: electric current, 65 mA; reaction temperature, 15° C; reaction time, 6 h. The yield of glycine (peak E) was 3.5% from sodium acetate. *b*, A, B, C, unknown; D, glycine; E, alanine; F, unknown; G, β -alanine. Reaction conditions: electric current, 75 mA; reaction temperature, 15° C; reaction time, 3 h. Alanine (peak E, yield 6.9%) was synthesised by amination of the α -carbon of propionic acid. β -Alanine (peak G, yield 5.3%) was formed by amination of the β -carbon. A small amount of glycine (peak D) was formed by the splitting of the α - β carbon linkage. The glycine formation by α - β cleavage of carboxylic acid is a general phenomenon in GDE experiments. *c*, A, B, C, unknown; D, aspartic acid; E, F, unknown; G, glycine; H, alanine; I, unknown; J, β -alanine. Reaction conditions: electric current, 50–70 mA; reaction temperature, 15° C; reaction time, 6 h. The major amino acid product is aspartic acid (peak D, yield 9.1%). Glycine (peak G, yield 0.5%) was formed by α - β bond cleavage. Alanine (peak H, yield 0.3%) was formed by β -decarboxylation, and β -alanine (peak J, yield 1.6%) was formed by α -decarboxylation of aspartic acid. In the several aspartic acid formations, a peak corresponding to β -hydroxy aspartic acid (peak C) was usually found with aspartic acid. The nature of the amino acid at peak C, however, is not yet fully characterised. Similarly, peaks corresponding to threonine and serine (peaks E and F) were commonly found in the reaction products of GDE experiments. These are also not yet characterised. *d*, A, B, C, D, unknown; E, glutamic acid; F, glycine; G, alanine; H, α -aminobutyric acid; I, unknown. Reaction conditions: electric current, 75 mA; reaction temperature, 15° C; reaction time, 3 h. The main

product was glutamic acid (peak E, yield 3.1%). Glycine (peak F, yield 0.8%) and alanine (peak G, yield 0.2%) were also formed. The peak of an expected product, β -aminoglutaric acid, was not identified. *e*, A, B, C, unknown; D, aspartic acid; E, F, unknown; G, glycine; H, alanine; I, J, unknown; K, β -alanine. Reaction conditions: electric current, 75 mA; reaction temperature, 5° C; reaction time, 3 h. After the reaction was over, the ammoniacal solution was evaporated under reduced pressure, and the residue was hydrolysed with 6 N hydrochloric acid for 6 h. The main product was aspartic acid (peak D, yield 3.2%). Glycine (peak G, yield 0.8%) and alanine (peak H) were also found in the hydrolysate. The peak K corresponds to that of β -alanine (yield 0.7%). *f*, A, B, C, unknown; D, aspartic acid; E, glycine; G, alanine; H, buffer change; I, isoasparagine; J, β -alanine. Reaction conditions: electric current, 55 mA; reaction temperature, 5° C; reaction time, 3 h. The amino acids in the reaction mixture were analysed without hydrolysis (*f*). Asparagine (peak E, yield 1.1%) and isoasparagine (peak I) were formed by CGDE. A larger aspartic acid peak however (peak D, yield 2.6%), was found. This suggests that the CGDE process could hydrolyse the amide bond of the asparagine and isoasparagine formed. Glycine (peak F, yield 0.1%), alanine (peak G), and β -alanine (peak J) were also observed. The nature of the large peak B is not known; however, the peak B disappeared after acid hydrolysis (*g*). The yields of aspartic acid, glycine, alanine, and β -alanine after hydrolysis were 4.5, 0.5, 0.3, and 1.2%, respectively.

anodic compartment. In the amination reaction of carboxylic acid (0.0025 mol in 10 ml of concentrated aqueous ammonia), CGDE took place in conditions of ammonia gas saturation and stirring. All reactions were carried out at 1 atm. The reaction temperature was measured by thermometer in the reaction tube which was immersed in the methanol-dry ice bath. The applied electric current (d.c.) was 450–600 V and 20–100 mA. The reaction time ranged from 0.5–6 h. After the reaction ceased, the reaction mixtures were evaporated to dryness under reduced pressure and the residues were appropriately diluted for amino acid analysis. Some of the reaction products were hydrolysed with hydrochloric acid and the amino acids were then analysed. The reaction mixture was also treated with 2,4-dinitrofluorobenzene, and the resulting dinitrophenyl (DNP)-amino acids were separated by column chromatography and thin layer chromatography. The major amino acid products were confirmed as DNP-amino acids.

Figure 1 represents amino acid formation from acetic acid, propionic acid, succinic acid, glutaric acid, succinyl-anhydride, and succinimide. The resulting amino acids, their yields, and the reaction conditions are described. The yields of amino acids in some amination reactions reached up to 13% (Fig. 1b). These amino acid formations by CGDE do not, however, represent optimal conditions.

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- ¹ Hickling, A., and Ingram, M. D., *J. electroanalyt. Chem.*, **8**, 65 (1964).
- ² Hickling, A., in *Modern Aspects of Electrochemistry* (edit. by Bockris, J. O., and Conway, B. E.), **6**, 329 (Plenum Press, New York, 1971).
- ³ Klemenc, A., *Z. Elektrochem.*, **56**, 694 (1953).
- ⁴ Gore, G. H., and Hickling, A., in *Modern Aspects of Electrochemistry* (edit. by Bockris, J. O., and Conway, B. E.), 352 (Plenum Press, New York, 1971).
- ⁵ Brown, E. H., Wilhide, W. D., and Elmore, K. L., *J. org. Chem.*, **29**, 5698 (1962).
- ⁶ Woodmann, J. F., *United States Patent* 2,632,729 (1953).
- ⁷ Denaro, A. R., and Hough, K. O., *Electrochim. Acta*, **18**, 863 (1973).
- ⁸ Hickling, A., and Ingram, M. D., *J. chem. Soc.*, 783 (1964).

Facilitation of lysosome disruption by ATP at low pH

HAYASHI *et al.*¹ reported that ATP stimulates *in vitro* the degradation of proteins at pH 4.5 by lysosomes which were intact at the start of the experiment. In accordance with our previous results, no effect was found at pH 5.0 (ref. 2). Although they found more rapid degradation by disrupted lysosomes, these authors concluded that the effect of ATP was not due to an increased labilisation of the lysosomal membrane, but that "ATP facilitates the pinocytotic engulfment of proteins into lysosomes and thereby stimulates proteolysis". This conclusion was mainly based on experiments on non-sedimentable cathepsin D activity, discussed below. We have reinvestigated the effect of ATP on protein breakdown at different pH values, and found essentially the same results. Our evidence shows, however, that the increase of proteolysis is due to facilitation of lysosome disruption by ATP at pH 4.5.

Figure 1 shows the degradation of haemoglobin using intact

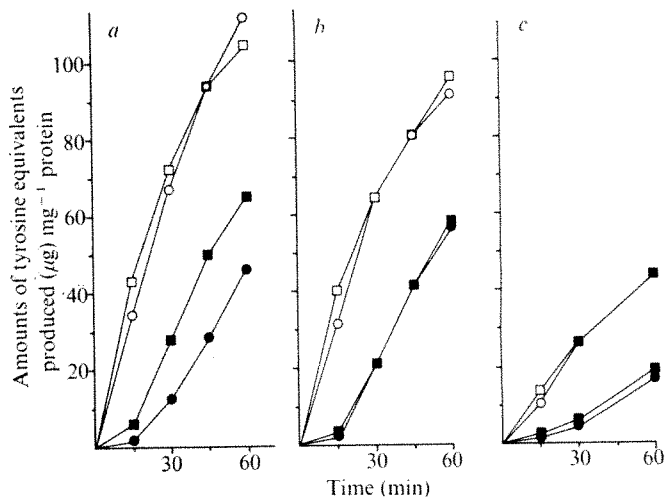


Fig. 1 Haemoglobin degradation by intact and disrupted lysosomes. A mitochondrial-lysosomal fraction was isolated from rat liver according to Sawant *et al.*³. The particle fraction was used either intact, or after disruption by three cycles of freezing in liquid nitrogen and thawing at 38°C. The incubation mixture contained 2.5 mg mitochondrial-lysosomal protein, 10.8 mg haemoglobin, 50 mM Na-acetate buffer and 0.25 M sucrose in a volume of 3.1 ml. The incubation temperature was 38°C. ATP and MgSO₄ were used at concentrations of 10 mM. At the times indicated, samples of 0.5 ml were added to 1.0 ml ice-cold 10% trichloroacetic acid. The mixture was cooled immediately and centrifuged at 0°C. Degradation products were determined in the supernatant by means of the Folin-Ciocalteu reagent, using tyrosine as standard⁴. ●, Intact lysosomes, no additions; ■, intact lysosomes, ATP and Mg²⁺ present; ○, disrupted lysosomes, no additions; □, disrupted lysosomes, ATP and Mg²⁺ present. Degradation at a, pH 4.48; b, pH 4.73; c, pH 5.05.

and disrupted mitochondrial-lysosomal fractions prepared according to Sawant *et al.*³. Similar results were obtained with a mitochondrial-lysosomal fraction prepared according to Bouma and Gruber⁵ and with a fraction isolated from hepatocytes⁶. ATP, in the presence of Mg²⁺ ions, stimulated the degradation of haemoglobin by the intact particle fraction at pH 4.48, but not at pH 4.73 and pH 5.05. During the first 30 min, the stimulation at pH 4.5 was two to three times in several experiments. The same results were obtained for the

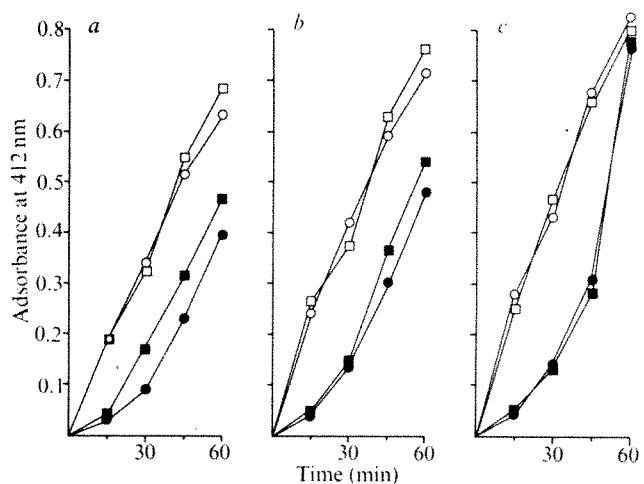


Fig. 2 Hydrolysis of glycyl-phenylalanyl-*p*-nitroanilide by intact and disrupted lysosomes. The incubation conditions are the same as those described under Fig. 1, except for the inclusion of 10 mM NaCl, 9 mM dithiothreitol and 0.315 mM GPNA. The trichloroacetic acid supernatants were added to 0.2 ml 2.0 M Tris-HCl buffer pH 9.0, and the adsorbance was determined at 412 nm. ●, Intact lysosomes, no additions; ■, intact lysosomes, ATP and Mg²⁺ present; ○, disrupted lysosomes, no additions; □, disrupted lysosomes, ATP and Mg²⁺ present. Degradation at a, pH 4.45; b, pH 4.78; c, pH 5.00.

hydrolysis of glycyl-phenylalanyl-*p*-nitroanilide (GPNA), a low-molecular weight substrate of cathepsin C (ref. 7), as is shown in Fig. 2. These findings are most simply explained if one assumes that ATP facilitates the disruption of the lysosomal membrane at pH 4.5.

Hayashi *et al.*¹ rejected this explanation because ATP did not enhance the non-sedimentable cathepsin D activity. Non-sedimentable activity is, however, not always a valid reflection of lysosomal integrity⁸, since at low pH values some enzymes are strongly adsorbed to membranes of disrupted lysosomes⁹. For that reason, we investigated the effect of ATP on the 'free' activity of cathepsin C. The results are shown in Table 1. The presence of ATP clearly accelerates lysosomal disruption at pH 4.5 only. It should be noted, that at the times indicated in the Table, the cathepsin C assay, which itself lasted 15 min, was started. ATP was present in both incubation systems during

Table 1 Influence of ATP on the 'free' cathepsin C activity of a mitochondrial-lysosomal fraction during incubation at pH 4.5, 4.8, and 5.0

Time (min)	ATP	'Free' cathepsin C activity (%)					
		pH 4.48		pH 4.78		pH 5.00	
0	—	—	—	—	—	—	—
10	25	29	24	24	26	26	26
20	58	78	41	43	42	37	37
30	86	87	65	66	59	54	54
40	94	96	82	89	82	79	79
60	97	102	91	89	100	98	98

A mitochondrial-lysosomal fraction was prepared according to Sawant *et al.*³ and incubated as described under Fig. 1. At the times indicated, samples of 0.5 ml were added to 0.5 ml buffered isotonic GPNA solution (final concentrations: 50 mM sodium acetate buffer, 10 mM Cl⁻, 9 mM dithiothreitol, 0.315 mM GPNA and 0.25 M sucrose). The chloride ions and the thiol were added to activate and stabilise cathepsin C (ref. 10). To those samples which had been preincubated without ATP and MgSO₄, these substances were added together with the GPNA solution to give the same final concentration of 5 mM. After 15 min at 38°C, the incubation with GPNA was stopped by adding 0.2 ml trichloroacetic acid to a final concentration of 10%. The trichloroacetic acid supernatant was added to 0.2 ml 2M Tris-HCl buffer pH 9.0, and the absorbance was determined at 412 nm in 2 cm cells. Blanks were prepared by adding trichloroacetic acid before the enzyme. The 'free' cathepsin C activity, determined in this way, is expressed as a percentage of the value obtained after three cycles of freezing-thawing (see text under Fig. 1). Three representative experiments are shown; +, the presence of 10 mM ATP and 10 mM Mg²⁺; —, their absence during incubation.

the assay. Thus, the relative increase in lability at pH 4.5, due to the presence of ATP during the incubation, is larger than that indicated by the data of Table 1.

At pH 4.8 and 5.0 ATP has no effect on lysosomal membrane stability. At pH 7.0, ATP even stabilises the lysosomal membrane^{11,12}. Thus, the direction and the magnitude of the effect of ATP on the lysosomal membrane is strongly pH dependent.

This is reflected in the absence of a stimulating effect of ATP on the degradation of haemoglobin and GPNA at pH 4.75 and 5.0. The pH dependence explains why Hayashi *et al.*¹ found no effect on acid phosphatase and β -glucuronidase activity measured at pH 4.8 and 5.2 respectively.

The second argument of Hayashi *et al.*¹ for the hypothesis that ATP stimulated the uptake of macromolecules into lysosomes, was the finding that ATP increased the amount of non-washable cytosol protein in the mitochondrial-lysosomal pellet slightly. A control experiment with a disrupted fraction was, however, not carried out. We found that even at neutral pH adsorption of cytosol proteins was as good to a disrupted as to an intact mitochondrial-lysosomal fraction.

Thus the effects of ATP can be explained by its strongly pH-dependent influence on the stability of the lysosomal membrane. No valid evidence for the uptake of macromolecules into lysosomes at pH 4.5 exists.

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- ¹ Hayashi, M., Hiroi, Y., and Natori, Y., *Nature new Biol.*, **242**, 163–166 (1973).
- ² Huisman, W., Bouma, J. M. W., and Gruber, M., *Biochim. biophys. Acta*, **297**, 93–97 (1973).
- ³ Sawant, P. L., Desai, I. D., and Tappel, A. L., *Biochim. biophys. Acta*, **85**, 93–102 (1964).
- ⁴ Gianetto, R., and de Duve, C., *Biochem. J.*, **59**, 433–438 (1955).
- ⁵ Bouma, J. M. W., and Gruber, M., *Biochim. biophys. Acta*, **113**, 350–358 (1966).
- ⁶ Hommes, F. A., Draisma, M. I., and Molenaar, I., *Biochim. biophys. Acta*, **222**, 361–371 (1970).
- ⁷ Planta, R. J., and Gruber, M., *Analyt. Biochem.*, **5**, 360–362 (1963).
- ⁸ Baccino, F. M., Rita, G. A., and Zuretti, M. F., *Biochem. J.*, **122**, 363–371 (1971).
- ⁹ Henning, R., Plattner, H., and Stoffel, W., *Biochim. biophys. Acta*, **330**, 61–75 (1973).
- ¹⁰ Gorter, J., and Gruber, M., *Biochim. biophys. Acta*, **198**, 546–555 (1970).
- ¹¹ Malbica, J. O., *Proc. Soc. exp. Biol. Med.*, **137**, 1140–1144 (1971).
- ¹² Mego, J. L., Farb, R. M., and Barnes, J., *Biochem. J.*, **128**, 763–769 (1972).

Inhibition of high-affinity glial uptake of ¹⁴C-glutamate by folate

EVIDENCE is accumulating to support the suggestion¹ that folic acid (pteroyl glutamic acid) may play a role in epileptic phenomena. *Grand mal* epileptic patients undergoing anti-convulsant drug therapy are likely to develop megaloblastic anaemia as a result of disturbances in folic acid metabolism¹, and it has been reported that correction of the folic acid deficiency by folate therapy results in an exacerbation of the frequency and intensity of epileptic episodes^{2,3}. It is also known that folate and related pteridines possess convulsant properties in their own right when administered in high intravenous doses to rats, or given intracortically⁴.

In a recent examination⁵ of the effects of iontophoretically applied folate or folinate (N⁵-formyl-5, 6, 7, 8-tetrahydro-folate) on the firing rate of neurones in the cat pericruciate cortex, these substances were found to have powerful facilitatory effects on the firing of spontaneously active, or glutamate-activated cells though they possessed only weak excitatory actions on quiescent neurones. The mechanism of the facilitatory action was not clear, but the authors provided some evidence for a possible blockade of inhibitory γ -aminobutyric acid (GABA) receptors. Similarly, it has been found that folate will antagonise presynaptic inhibition in the cuneate nucleus⁶; however, no antagonism of GABA-induced inhibition was demonstrable⁷.

Glutamate is likely to be an important excitatory neurotransmitter in the CNS⁸, and the major means of terminating the synaptic action of this amino acid is considered to be by re-uptake, largely into glial cells⁹. The rat dorsal root ganglion provides a convenient model system for studying the glial uptake of amino acids and has been previously used¹⁰ to demonstrate an active, energy-dependent, high-affinity transport process ($K_m = 2 \times 10^{-5}$ M) for glutamate.

Here I examine the possibility that folate might interfere with this glutamate uptake mechanism.

Rat dorsal root ganglia were isolated and desheathed, and transferred in pairs to 0.75 ml of Krebs-bicarbonate medium (pH 7.4) and preincubated for 10 min at 27°C under O₂–CO₂ (95 : 5). L-U-¹⁴C-glutamate (Amersham) was then added to give a final concentration of 10⁻⁶ M, and the incubation con-

tinued for a further 15 min. At the end of this time ganglia were rapidly rinsed in ice-cold Krebs medium, blotted on filter paper and weighed individually. The tissues were transferred to liquid scintillation vials, solubilised with 400 μ l Protosol (NEN Chemicals) and total radioactivity assayed by liquid scintillation counting. Uptake of ^{14}C -glutamate was found to be linear with time. In each series of experiments ganglia were included which were incubated throughout at 0°C . The radioactivity in these blanks, which enabled a correction to be made for the ^{14}C -glutamate localised in the extracellular water, and for uptake due to diffusion, was subtracted from the values in the corresponding experimental sample (in each case the radioactivity in the blanks was less than 16% of the control). ^{14}C -glutamate was accumulated by dorsal root ganglia at a rate of 5.86 ± 0.33 pmol per mg tissue per 15 min (means \pm s.e.m. of 10 independent observations).

The effects of folate (Sigma, UK), the related pteridine, folinate (calcium salt; Lederle) and the dihydrofolate reductase inhibitor, methotrexate, 4-amino- N^{10} -methylpteroyl glutamic acid (Lederle) were examined at a single test concentration of $5 \times 10^{-4}\text{M}$, on the uptake of 10^{-6}M ^{14}C -glutamate. Each of the three compounds tested produced an inhibition of $38.8 \pm 4.9\%$, $35.5 \pm 6.4\%$ and $27.2 \pm 1.9\%$ respectively (means \pm s.e.m. of eight independent observations).

The Michaelis-Menten kinetics were determined for folate, using a limited range of folate concentrations ($2.5 \times 10^{-4}\text{M}$ – 10^{-3}M). The values are presented as a Dixon plot (Fig. 1) which indicates that folate inhibits competitively the high-affinity uptake of ^{14}C -glutamate with a $K_i = 5.64 \times 10^{-4}\text{M}$.

The effects on the uptake process of a clinically useful anti-convulsant, diphenylhydantoin, were examined at a concentra-

tion of $5 \times 10^{-4}\text{M}$. The compound itself was without effect on the uptake of ^{14}C -glutamate, nor did it affect the inhibition of uptake by folate.

The significance of the inhibition of glutamate uptake by folate seen in these experiments, in relation to the epileptic seizure, and to the effects of the therapeutic administration of folate, is not clear. It seems unlikely that extracellular folate concentrations obtained following even prolonged therapy would be sufficient to markedly influence the re-uptake of glutamate from the synapse. But folate is largely localised in synaptosomal fractions¹¹, and it has been suggested¹² that focal epileptic discharges may result from an accumulation of folate in the extracellular space around the focus.

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- 1 Reynolds, E. H., in *Antiepileptic Drugs* (edit. by Woodbury, D. M., Penny, J. K., and Schmidt, R. P.), 247–262 (Raven Press, New York, 1972).
- 2 Reynolds, E. H., *Lancet*, **i**, 1086–1088 (1967).
- 3 Reynolds, E. H., *Lancet*, **i**, 1376–1378 (1973).
- 4 Hommes, O. R., and Obbens, E. A. M. T., *J. neurol. Sci.*, **16**, 271–281 (1972).
- 5 Watkins, J. C., and Davies, J., *Biochem. Pharmacol.*, **22**, 1667–1668 (1973).
- 6 Hill, R. G., and Miller, A. A., *Br. J. Pharmacol.* (in the press).
- 7 Hill, R. G., Miller, A. A., Straughan, D. W., and Webster, R. A., in *The Natural History and Management of Epilepsy* (edit. by Harris, P., and Mawdsley, C.) (Churchill-Livingstone, Edinburgh and London, in the press).
- 8 Johnson, J. L., *Brain Res.*, **37**, 1–19 (1972).
- 9 Van den Berg, C. J., and Garfinkel, D., *Biochem. J.*, **123**, 211–218 (1971).
- 10 Roberts, P. J., and Keen, P., *J. Neurochem.* (in the press).
- 11 Bridgers, W. F., and McClain, L. D., *Adv. Biochem. Psychopharmacol.*, **4**, 81–92 (1971).
- 12 Obbens, E. A. M. T., *Experimental Epilepsy Induced by Folate Derivatives* (University Press, Nijmegen).

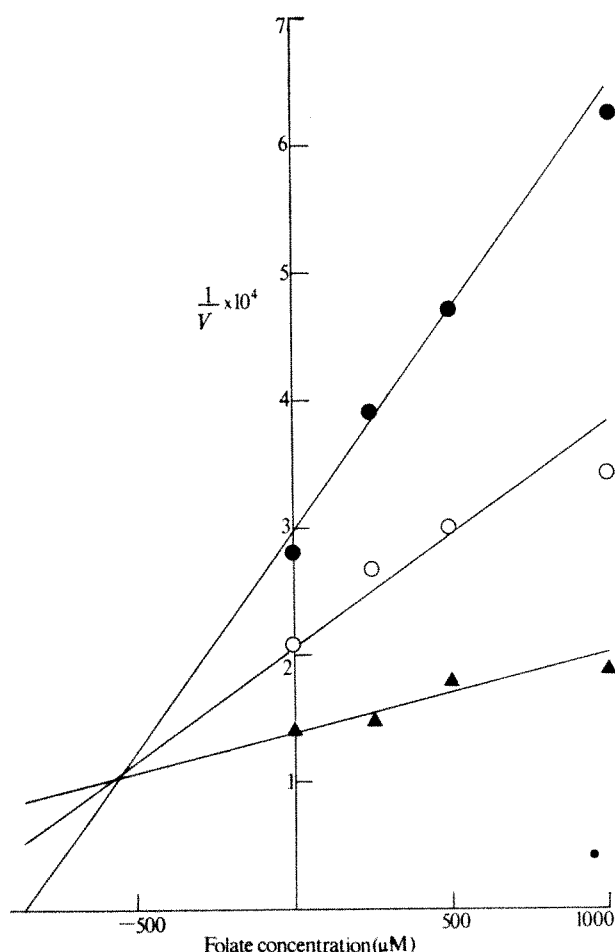


Fig. 1 Dixon plot for the effect of folate on uptake of ^{14}C -glutamate by dorsal root ganglia. V = d.p.m. per mg per 15 min. ^{14}C -glutamate concentrations: \bullet , 1 μM ; \circ , 1.5 μM ; \blacktriangle , 2 μM .

Presynaptic inhibition at mammalian peripheral synapse?

STIMULATION of sympathetic nerves leading to the intestine causes a reduction in intestinal motility¹. Norberg² showed that these nerves ramify mainly in the enteric plexuses and has suggested that inhibition may be exerted at a neural rather than at a muscular level but this suggestion has been disputed³. Neural inhibition has been shown to occur by the postsynaptic action of transmitter⁴ but at some synapses it has been established that transmission may be inhibited presynaptically⁵.

Here we describe experiments directly supporting Norberg's suggestion and which suggest that sympathetic inhibition of synaptic transmission in the myenteric plexus of the guinea pig may be mediated presynaptically rather than by the postsynaptic action of inhibitory transmitter.

Intracellular recordings were made from myenteric neurones in flaps of plexus attached to the anal end of an adjacent segment of guinea pig mid small intestine⁶. The periarterial nerves leading to the intestinal segment were drawn into a suction electrode and stimulated electrically. Synaptic activity was evoked in the myenteric plexus by use of a pair of fine platinum electrodes, one under and the other just above the plexus flap⁷. In some experiments synaptic activity was evoked by distending a balloon placed inside the lumen of the gut segment⁸. Preparations were bathed in modified Krebs solution maintained at 35°C ; atropine ($2 \times 10^{-7}\text{ g ml}^{-1}$) was present during each experiment to prevent cholinergic excitation of the smooth muscle⁹.

In most experiments, trains of 10–100 stimuli (pulse width 0.1 ms, frequency 0.1–50 Hz) applied to periarterial nerves

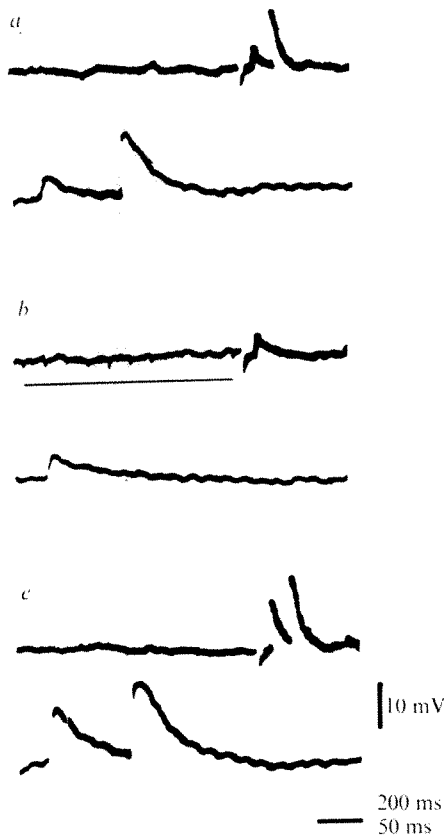


Fig. 1 Effect of periarterial nerve stimulation on transmission in the myenteric plexus of guinea pig small intestine. The upper trace in each illustration records the membrane potential of a myenteric neurone at slow scan speed (horizontal calibration 200 ms); the lower traces show EPSPs evoked by single transmural stimuli applied across the plexus flap close to the recording site (horizontal calibration 50 ms). In *a* and *c*, recorded respectively 15 s before and 15 s after *b*, it can be seen that such stimuli each evoked two EPSPs. But when the periarterial nerves leading to the plexus flap were stimulated (solid bar, 10 Hz for 1 s) just before a transmural stimulus (*b*) only a single EPSP was evoked. Vertical calibration bar (10 mV) applies to each trace.

produced no detectable change in the electrical properties of the myenteric neurones. Transmural stimulation of the flap of myenteric plexus never evoked inhibitory postsynaptic potentials; moreover Nishi and North⁸ have shown that noradrenaline, the presumptive transmitter released by periarterial nerves to the intestine, has no effect on the sensitivity of myenteric neurones to acetylcholine (ACh). In two out of more than 40 experiments, short trains of impulses (10 Hz for 1 s) applied to periarterial nerves caused a persistent discharge of excitatory postsynaptic potentials (EPSPs). Such EPSPs were identical to those that could be evoked by close transmural stimulation of a plexus flap and are presumably mediated by the release of ACh^{7,8}. We take this observation to support the view that periarterial nerves to the small intestine contain a small proportion of vagal parasympathetic fibres⁹.

An intermittent discharge of EPSPs was recorded from some neurones in the absence of any apparent stimulus⁶. Trains of stimuli (2 s duration, 5–10 Hz) applied to the periarterial nerves frequently caused either a reduction in the frequency of occurrence of EPSPs or totally abolished the discharge of EPSPs for several seconds (2–7 s). Distension of the intestinal segment by inflation of the intraluminal balloon increased the frequency of discharge of EPSPs or evoked a discharge of EPSPs in quiescent neurones. Such a discharge of EPSPs was reduced or abolished by a concomitant train of stimuli to the sympathetic nerve. These myenteric neurones mediate either inhi-

bition or excitation of adjacent smooth muscle layers¹⁰; evidently sympathetic nerve stimulation may depress transmission in both inhibitory and excitatory pathways.

Close transmural stimulation evoked a single EPSP or a cluster of EPSPs; a preceding train of stimuli applied to periarterial nerves either abolished the synaptic response or reduced the number EPSPs in the cluster. If the EPSPs were sufficiently synchronous to evoke an action potential, preceding sympathetic nerve stimulation reduced the synaptic response so that only subthreshold EPSPs were recorded. In some experiments, it was possible to evoke two EPSPs and to selectively abolish one of these by sympathetic nerve stimulation. Such an experiment is illustrated in Fig. 1. It can be seen that the second EPSP was abolished in an 'all-or-none' manner whilst the first EPSP was unaffected. In other experiments early EPSPs were abolished rather than late EPSPs. The scatter of latencies for EPSPs could result from delays due to transmission through interneurons but this seems unlikely as EPSPs were repeatedly evoked during repetitive transmural stimulation (10 Hz); polysynaptic nerve pathways in the myenteric plexus often failed to transmit at frequencies as low as 0.2 Mz (ref. 6).

When intracellular recordings were made from longitudinal or circular muscle, trains of stimuli (20 Hz for 5 s) applied to periarterial nerves produced no detectable change in membrane potential of cells of longitudinal layer. They did, however, produce a detectable hyperpolarisation (5–10 mV) of circular muscle membrane potential; frequencies less than 20 Hz only occasionally produced a small hyperpolarisation (1–3 mV) of this layer.

The simplest explanation for our findings is that sympathetic inhibition is mediated presynaptically. In most experiments repetitive sympathetic nerve stimulation reduced the number of EPSPs in a cluster of synaptic potentials. As the amplitude of the EPSPs which persisted during periarterial nerve stimulation was not reduced, it seems unlikely that inhibition is mediated by a conductance change in the postsynaptic membrane. The possibility that inhibition is mediated postsynaptically at restricted areas some distance from the cell soma, however, cannot be completely excluded. It would also seem from these experiments that the mechanism of sympathetic inhibition is somewhat different to that observed at the crayfish neuromuscular junction, as EPSPs were abolished in an 'all-or-none' manner rather than by a reduction in quantal content⁸. Nevertheless, these experiments show that sympathetic nerve stimulation may inhibit transmission in the myenteric plexus and may block local reflexes activated by distension of an intestinal segment.

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- ¹ Kosterlitz, H. W., *Handbook of Physiology* section 6, IV 2147–2171 (American Physiological Society, Washington DC, 1968).
- ² Norberg, K. A., *Int. J. Neuropharmac.*, **3**, 379–382 (1964).
- ³ Gershon, M. D., *J. Physiol. Lond.*, **189**, 317–327 (1967).
- ⁴ Coombes, J. S., Eccles, J. C., and Fatt, P., *J. Physiol. Lond.*, **130**, 326–373 (1955).
- ⁵ Dudel, J., and Kuffler, S. W., *J. Physiol. Lond.*, **155**, 543–562 (1961).
- ⁶ Hirst, G. D. S., and McKirdy, H. C., *J. Physiol. Lond.*, **238**, 129–143 (1974).
- ⁷ Hirst, G. D. S., Holman, M. E., and Spence, I., *J. Physiol. Lond.*, **236**, 303–326 (1974).
- ⁸ Nishi, S., and North, F. A., *J. Physiol. Lond.*, **231**, 471–491 (1973).
- ⁹ Finkleman, B., *J. Physiol. Lond.*, **70**, 145–157 (1930).
- ¹⁰ Hirst, G. D. S., Holman, M. E., and McKirdy, H. C., *J. Physiol. Lond.* (in the press).

Conductance increase by adrenaline in guinea pig taenia coli studied with voltage clamp method

Using the double sucrose gap method, Bülbring and Tomita² have shown that adrenaline hyperpolarises the membrane and reduces the electrotonic potential produced by constant current pulses in the guinea pig taenia coli muscle. They concluded, from the effects of membrane polarisation and changing external ionic composition on the adrenaline action, that an increase in the K and Cl conductances of the membrane is responsible for the hyperpolarisation.

This agrees with the results obtained from ionic flux experiments³. In contrast, however, it has been recently reported, on the basis of voltage clamp experiments, that adrenaline increases the negativity of the potassium equilibrium potential without causing significant changes in the membrane conductance⁴. As the voltage-current relation is roughly linear^{1,3,4}, it is difficult to reconcile these different conclusions.

We therefore examined the effect of adrenaline using the current clamp and voltage clamp methods in the same preparation. The double sucrose gap apparatus was essentially the same as that previously used², although of slightly smaller dimensions, that is, the centre pool where the test solution flowed, was 700 μm in width. The vertical amplifier of the oscilloscope (Textonix, 3A7) was used as a feedback amplifier for the voltage clamp experiments and electrical responses were recorded with a rectilinear pen recorder (Nihon Kohden, PMP-3004).

Figure 1 shows effects of adrenaline (10^{-6} g ml⁻¹) injected into the stream of Krebs solution flowing through the centre pool, in current clamp (Fig. 1a) and voltage clamp (Fig. 1b) conditions. In Fig. 1a, a hyperpolarising current pulse (2 s duration) of constant intensity (5×10^{-7} A) was applied every 8 s. The previous observation², that the membrane was hyperpolarised and the electrotonic potential was concomit-

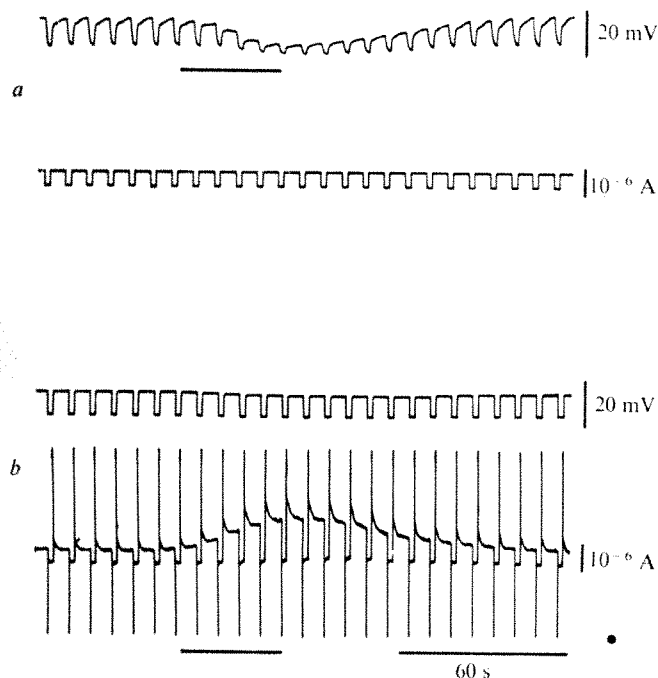


Fig. 1 Effects of a short application of adrenaline (10^{-6} g ml⁻¹) on the guinea pig taenia coli. Adrenaline infusion is indicated by the horizontal bar. Top trace, membrane potential, downward deflection indicating hyperpolarisation; bottom trace, membrane current, downward deflection indicating inward current. *a*, Current clamp; *b*, voltage clamp.

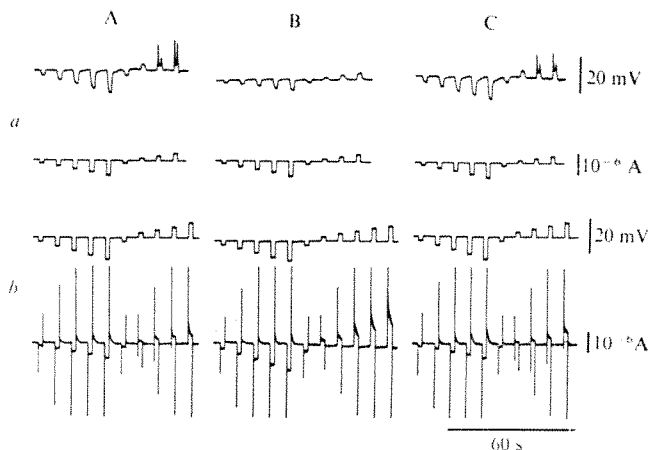


Fig. 2 Effects of a prolonged application of adrenaline (B) and its recovery (C). Compare with control experiments (A). *a*, Current clamp; *b*, voltage clamp. Note decrease in electrotonic potential (top trace) in *a*, and increase in membrane current flowing during the voltage pulses (bottom trace) in *b* during adrenaline application. Also note that an early transient inward current produced by depolarising voltage pulse was abolished in B.

antly reduced by adrenaline, was confirmed. When adrenaline was applied in the voltage clamp condition, an outward current was observed and in response to hyperpolarising voltage pulses the membrane currents were increased (Fig. 1b). Thus, it is clear that adrenaline increases the membrane conductance and produces an outward current.

This was also confirmed by studying the current-voltage relationship (Fig. 2a) and the voltage-current relationship (Fig. 2b) in the absence and presence of adrenaline (5×10^{-7} g ml⁻¹). After taking control records in current clamp and voltage clamp conditions in normal Krebs solution, adrenaline was continuously administered and 10 min later the same experiments were repeated in the presence of adrenaline. Records were taken 10 min after washing out the adrenaline. There was some gradual recovery in membrane potential and membrane resistance during prolonged exposure to adrenaline, however, it was clear that the membrane was still hyperpolarised, the membrane resistance reduced, and the spike or the early transient inward current blocked. Thus, the results were similar to those obtained by a short application of adrenaline.

It was also demonstrated that the outward current produced by adrenaline was increased during conditioning depolarisation and reduced during conditioning hyperpolarisation. When the membrane potential was clamped at a hyperpolarised level of more than 15 mV beyond the resting potential, adrenaline produced an inward current.

Our results support the idea that adrenaline increases the membrane conductance, and hyperpolarises the membrane by increasing the outward current. As no careful examination was made of tail currents following repolarisation from a large depolarisation to different voltage levels, a shift in the potassium equilibrium potential is not ruled out by the present experiments. As far as the conductance change is concerned, however, our results conflict with the data obtained by Inomata and Kao⁴. The reason for their failure to demonstrate the conductance change by adrenaline is not clear.

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- ¹ Abe, Y., and Tomita, T., *J. Physiol., Lond.*, **196**, 87–100 (1968).
² Bülbring, E., and Tomita, T., *Proc. R. Soc. Lond.*, **B172**, 89–102 (1969).
³ Tomita, T., *J. Physiol., Lond.*, **183**, 450–468 (1966).
⁴ Inomata, H., and Kao, C. Y., *J. Physiol., Lond.*, **226**, 53–54P (1972).
⁵ Jenkinson, D. H., and Morton, I. K. M., *J. Physiol., Lond.*, **188**, 373–386 (1967).

Development of polycystic ovaries in rats actively immunised against T-3-BSA

THE aetiology of the polycystic ovary syndrome of Stein-Leventhal¹ is still unknown. Histologically, the ovaries from Stein-Leventhal patients are comparable in many respects to polycystic rat ovaries which have been observed in sexually mature rats following the administration of testosterone propionate during the neonatal period or continuous exposure of adult rats to a constant light environment². Furthermore, increased *in vitro* conversion of progesterone to androgen and oestrogen has been described in incubations of both human and rat polycystic ovaries³. A clear insight into altered ovarian physiology which may precede the development of polycystic ovaries in the rat might therefore provide a better understanding of the human syndrome.

Mahesh and Greenblatt⁴ have suggested that the Stein-Leventhal syndrome is caused by altered steroid synthesis in the ovary resulting in an imbalance of the pituitary-ovarian axis and subsequent persistent stimulation of the ovary by follicle stimulating hormone (FSH). A previous report from this laboratory has clearly shown that active immunisation of intact male rats against testosterone-3-(O-carboxymethyl) imino-bovine serum albumin (T-3-BSA) leads to a grossly elevated total serum testosterone concentration with a concomitant decrease in the circulating biologically active free testosterone fraction due to high-affinity binding by anti-testosterone antibodies^{5,6}. This situation was reflected in significantly increased serum concentrations of FSH and luteinising hormone (LH). These observations led us to postulate that a similar immunisation of female rats might induce gradual alterations in the endocrine status of the host conducive to the development of polycystic ovaries, thus providing a well controlled model in which aspects of the aetiology of the syndrome can be examined.

Adult female Sprague Dawley rats (18 weeks) used in this study were housed in an air-conditioned room illuminated between 0700 h and 1900 h. Only rats which had shown at least two consecutive 4-d oestrous cycles (determined by daily vaginal smears) were used. Seven experimental animals were immunised against a T-3-BSA conjugate prepared by the method of Erlanger *et al.*⁷. The immunogen (100 µg) was administered

as described previously⁵ at monthly intervals. Controls were immunised against unconjugated BSA. Fifteen weeks after beginning immunisation, vaginal smears were taken on 8 consecutive days. At this time all animals immunised against T-3-BSA exhibited persistent vaginal cornification whereas controls continued to cycle normally. After 17 weeks, all animals were exsanguinated, serum collected⁵ and the ovaries removed and fixed in Bouin's solution. Significant serum antitestosterone antibody titre (greater than 1:20 final dilution)⁵ was detected in three of the T-3-BSA immunised animals, ranging between 1:200–1:700. Ovaries removed from these animals bore massive fluid-filled cysts up to 1.5 cm diameter. Following fixation and dehydration the ovaries removed at autopsy were embedded in paraffin wax and serial 5 µm sections cut and stained with haematoxylin and eosin. Microscopic examination of sections from control ovaries (Fig. 1a) revealed apparently normal follicular development with abundant corpora lutea. Those from animals which produced anti-T-3-BSA antibodies, however, displayed large distended follicular cysts characterised by almost total absence of a granulosa cell layer; corpora lutea were rarely observed (Fig. 1b).

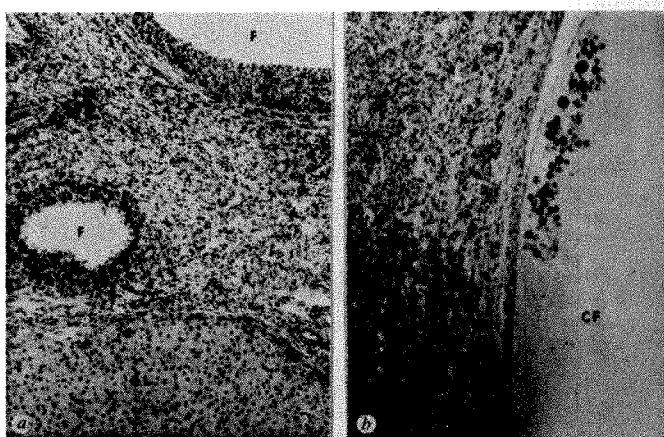


Fig. 1 Photomicrographs of follicles present in, a, ovary removed from a control rat immunised against BSA; b, ovary removed from a rat immunised against T-3-BSA. F, Normal follicle; CF, cystic follicle; CL, corpus luteum.

The total serum testosterone concentration in these animals, as determined by radioimmunoassay⁸ was significantly higher than in controls (Table 1), but using an equilibrium dialysis technique described previously⁵, the circulating biologically active⁹ free testosterone fraction was found to be significantly reduced (Table 1). Serum concentrations of immunoreactive LH, FSH and prolactin were each determined by radioimmunoassay as before⁵ and the values obtained are shown in Table 1. While experimental FSH values were significantly elevated neither LH nor prolactin differed from controls.

These data indicate that a reduction in the circulating free

Table 1 Serum concentration of testosterone, LH, FSH and prolactin in female rats with polycystic ovaries 17 weeks after commencing immunisation against T-3-BSA

	Testosterone (ng per 100 ml)		LH (ng ml ⁻¹)	FSH (ng ml ⁻¹)	Prolactin (ng ml ⁻¹)
	Total	Free			
Controls (immunised against BSA; n = 6)	27 ± 18*	9.8 ± 2.5	75 ± 17	465 ± 186	123 ± 114
Experimental (immunised against T-3-BSA; n = 3)	90 ± 23	1.72 ± 0.68	62 ± 47	1110 ± 127	69 ± 32
P	<0.0025	<0.0025	NS	<0.0025	NS

* Mean ± 1 s.d.

testosterone fraction which occurs in animals immunised against T-3-BSA is reflected in a selectively increased pituitary FSH output which might be a factor in the aetiology of pathological changes observed in the ovaries of animals thus treated. A detailed examination of various parameters of ovarian physiology before the appearance of polycystic ovaries in such animals may be of value in our understanding of the human syndrome.

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¹ Stein, I. F., and Leventhal, M. L., *Am. J. Obst. Gynec.*, **29**, 181 (1935).

² Singh, K. B., *Obst. gynec. Surv.*, **24**, 2 (1969).

³ Weisz, J., and Lloyd, C. W., *Endocrinology*, **77**, 735 (1965).

⁴ Mahesh, V. B., and Greenblatt, R. B., *Nature*, **191**, 888 (1961).

⁵ Hillier, S. G., Cole, E. N., Groom, G. V., Boyns, A. R., and Cameron, E. H. D., *Steroids*, **22**, 227 (1973).

⁶ Hillier, S. G., Groom, G. V., Boyns, A. R., and Cameron, E. H. D., *J. Endocr.*, **61**, 11 (1974).

⁷ Erlanger, B. F., Borek, F., Beiser, S. M., and Lieberman, S., *J. biol. Chem.*, **228**, 713 (1957).

⁸ Hillier, S. G., Brownsey, B. G., and Cameron, E. H. D., *Steroids*, **21**, 735 (1973).

⁹ Vermeulen, A., and Verdonck, L., *J. Steroid Biochem.*, **3**, 421 (1972).

Influence of NAD⁺ on development of mouse blastocysts *in vitro*

THE metabolites pyruvate and lactate are of central importance for the development of preimplanted mouse embryos¹. One-cell ova cannot develop when lactate is the only energy source², but the necessary pyruvate can apparently be provided for the first cleavage division when the incubation medium contains NAD⁺ as well as lactate³. Cross and Brinster have reported that the development of the mouse embryo was optimal after the first cleavage division when both lactate and pyruvate were present in the medium⁴. Recent biochemical investigations have demonstrated that NAD⁺ metabolism can interfere with DNA synthesis: this effect is independent of the function of NAD⁺ as a coenzyme of dehydrogenases^{5,6}. We have investigated whether NAD⁺ supports the development of mouse blastocysts from the two-cell stage embryos by converting lactate to pyruvate, or whether it inhibits this development by interfering with DNA synthesis. •

Sexually mature virgin female white inbred mice (8–10 weeks old) were mated for 2–3 h with sexually mature males of the same strain (two females per cage). Females with vaginal plugs were segregated, and the embryos were obtained from these animals 28–30 h later by flushing Culture Medium BMOC-2 (ref. 7) through the oviduct. The embryos were kept during the collecting period in Culture Medium

Table 1 Effects of NAD⁺ on development and NADH content of two-cell embryos

NAD ⁺ (mM)	Development to blastocyst stage (%)		NADH (mM)*
	BMOC-2(+pyruvate)	BMOC-2(-pyruvate)	
0	92 (178†)	40 (35)	0
0.23	65 (26)	81 (21)	0.005
1.0	52 (71)	24 (29)	0.04
5.0	28 (50)	10 (20)	0.12

* Concentration after 66 h incubation with BMOC-2 (without pyruvate) with various NAD⁺ concentrations.

† Numbers of embryos.

BMOC-2 with lactate and pyruvate at 30–35° C; afterwards they were washed twice with 0.9% NaCl and 0.1% Ficoll (Pharmacia, Sweden) and transferred to 100 μ l Culture Medium BMOC-2 which contained lactate or lactate and pyruvate. The medium also contained NAD⁺ in various concentrations and was overlaid with silicone oil Tegiloxan 50 (Goldschmidt, Essen) for the cultivation period. The embryos were cultivated essentially as described by Brinster⁷.

NAD⁺ and NADH were measured with alcohol dehydrogenase and lactate dehydrogenase⁸ by fluorescence spectrometry (Zeiss ZFM 4C).

As seen from Table 1 optimal development of the mouse embryos was obtained using the complete Culture Medium BMOC-2 which contained 0.25 mM pyruvate. After 66 h incubation, 92% of the embryos isolated at the two-cell stage had developed to blastocysts but when pyruvate was omitted this rate was only 40%. In medium containing pyruvate, even small NAD⁺ concentrations inhibited embryonic development (Table 1). With increasing NAD⁺ concentrations this inhibition became stronger and no blastocysts were observed when the culture medium contained 12.5 mM NAD⁺.

But, for embryos cultured without pyruvate, development was stimulated by low NAD⁺ concentrations (Table 1). With higher NAD⁺ levels (1 mM and higher) the formation of blastocysts from the two-cell stage embryos was inhibited as in the pyruvate-containing medium. No development to blastocysts was observed with a NAD⁺ concentration of 12.5 mM in the culture medium.

Table 1 also shows that the NADH content of the culture medium at the end of an incubation without pyruvate is dependent on the NAD⁺ concentration. The measured NADH concentration increased almost proportionally with the NAD⁺ concentration in the investigated range although we can say nothing about the turnover of the NADH. These data clearly demonstrate that lactate can be metabolised to pyruvate under these conditions. We do not know, however, where these reactions could occur: by intracellular dehydrogenases, by dehydrogenases attached to the zona pellucida, or by dehydrogenases released from the embryos into the medium. This is especially problematical since the pyridine nucleotides cannot usually penetrate mammalian cell membranes⁹.

Under our experimental conditions the NAD⁺ as a coenzyme of dehydrogenases can apparently provide the pyruvate which the embryos require for their development. But, with higher NAD⁺ concentrations the second metabolic effect of NAD⁺ overcomes this stimulation and its inhibitory effect on proliferation becomes predominant.

This inhibitory effect may be connected with the formation of adenosine diphosphoribose (ADPR)⁴ and its polymer and with the binding of ADPR to the chromatin^{5,6}. An NAD glycohydrolase (EC 3.2.2.5) activity is found in the preimplanted mouse embryo¹⁰ which could be of significance in this connection. It is also possible that metabolic breakdown products of NAD⁺ are responsible for the inhibitory effect. Adenosine is found to act in this way on the development of the mouse embryo (unpublished results). Nolde and Hilz¹¹ reported that adenosine caused a reduction

in the proliferation of HeLa cells. We are investigating these problems and that of the uptake and metabolism of NAD⁺ by the embryo.

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Received April 16, 1974.

- ¹ Biggers, J. D., *J. Reprod. Fert. Suppl.* **14**, 41–54 (1971).
- ² Biggers, J. D., Whittingham, D. G., and Donahue, R. C., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 560 (1967).
- ³ Sorensen, R. A., *Biol. Reprod.*, **7**, 139 (1972).
- ⁴ Cross, P. C., and Brinster, R. L., *Exp. Cell Res.*, **77**, 57–62 (1973).
- ⁵ Sugimura, T., in *Progr. Nucleic Acid Res. molec. Biol.*, **13**, 121–151 (Academic Press, New York and London, 1973).
- ⁶ Streffer, C., and Scholz, G., *Z. physiol. Chemie*, **353**, 1855–1862 (1972).
- ⁷ Brinster, R. L., in *The mammalian oviduct* (edit. by Hafez, E. S. E., and Blandau, R. J.), 419–444 (University of Chicago Press, Chicago and London, 1969).
- ⁸ Klingenberg, M., in *Methoden der enzymatischen Analyse*, (edit. by Bergmeyer, H.-U.), 1975–1990 (Verlag-Chemie, Weinheim, 1970).
- ⁹ Liersch, M., Grotteluschen, H., and Decker, K., *Z. physiol. Chemie*, **352**, 267–274 (1971).
- ¹⁰ Streffer, C., and van Beuningen, D., *Dev. Biol.* (in the press).
- ¹¹ Nolde, S., and Hiltz, H., *Z. physiol. Chemie*, **353**, 505–513 (1972).

Evidence for sexual fusion and recombination in the dinoflagellate *Cryptocodinium (Gyrodinium) cohnii*

SEVERAL cytological and biochemical features of dinoflagellate chromosomes, such as low histone content^{1–3}, threadlike appearance under the electron microscope^{1,4–6}, absence of centromeres⁶, the condensed state persisting throughout interphase^{1,4–6}, unusual DNA⁷ and the controversial possibility of multistrandedness^{8,9}, suggest that the genetics of these organisms might be unusual. We report here initial experiments on the genetics of the heterotrophic marine, coccoid dinoflagellate *Cryptocodinium cohnii*¹⁰ previously believed to be asexual¹⁰. We have evidence of sexuality, complementation, recombination and a complex life cycle.

Axenic cultures of Puerto Rican and Woods Hole strains of *C. cohnii* (gifts from Drs K. Gold and L. Provasoli, respectively) and a strain which we isolated from Mattapoisett Bay, Massachusetts, were cloned by isolating single cells on agar by micromanipulation. Several clones of each strain were maintained on Gold's medium (GM)¹¹, either liquid or solidified with agar (1.0–1.5%).

We found that growing cultures have, in addition to reproductive cysts and coccoid individuals of many sizes and stages of development, motile flagellated cells—swarmers—of various sizes⁸ (Fig. 1a). DNA content per nucleus, also varies widely (Fig. 1b) as previously reported briefly, but within one cyst, the two, four or eight nuclei appear to contain equal amounts. Cysts with eight nuclei are rare and have minimal amounts of DNA per nucleus. Cysts with four nuclei also usually contain the minimal amounts while nuclei of binucleate cysts contain either minimal DNA or twice this amount. These results agree with present and previous observations^{10,11} of cysts producing one, two, four or eight cells. An average of such widely divergent amounts of DNA, as determined chemically^{3,8} is obviously too large if used to indicate a basal DNA content per genome. As we show later, cells of different size and DNA content result, at least in some cases, from cell fusion.

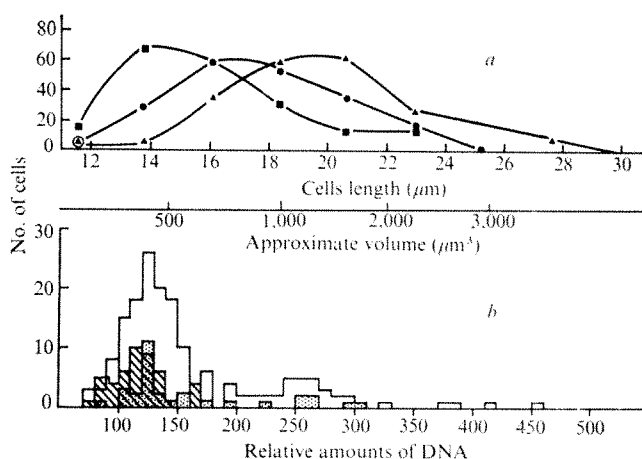


Fig. 1 *a*, Distributions in size of swimming cells of three representative cultures of *C. cohnii*, fm 1 mutant: ■, cells from bottom of log phase liquid culture; ●, cells from top of same culture; ▲, cells from bottom of stationary phase culture. *b*, Measurements of relative amounts of Feulgen dye bound to individual nuclei. A Leitz microspectrophotometer and the two wave length method^{14,15} for eliminating distributional error were used. □, Cells of wild type in log phase; ■, in stationary phase; ▨, nuclei of different reproductive cysts containing two, four, and eight nuclei.

We used easily distinguishable motility mutants for microscopy and for genetic experiments; one mutant occurred spontaneously and the other was isolated after ultraviolet irradiation. Following *Chlamydomonas* nomenclature^{16,17}, they were designated fm 1 and fm 2. Motile cells of fm 1 swim at a rate of 50 μm s⁻¹, about one-fifth the normal speed. The swarmers of fm 2 have flagella that appear normal under the light microscope, but locomotion is negligible, with most cells twitching only occasionally.

Extended microscopy of individual normal cells of *C. cohnii* is hampered by their speed. Examination of cultures of fm 1, however, especially cells from the bottom of log phase cultures, revealed motile cells adhering in pairs, moving characteristically with two nonsynchronously beating trailing flagella. (C. K. Franker has observed pairing in other cultures of *C. cohnii*.) With this slow moving strain, it was possible to photograph the pairing of cells and their gradual fusion into a single cell indistinguishable, except for two trailing flagella, from a normal individual of about double the size. (Similar behaviour has been described in other dinoflagellates¹⁸.) Figure 2 shows typical sequences for cells (gametes) of different sizes. Usually fusions involved cells of the same size, but occasionally cells of different sizes fused (Fig. 2*m*, *n* and *s*), and very rarely more than two cells fused. Smaller individuals usually paired side by side, commonly followed by a cruciform juxtaposition of the cells (Fig. 2*a* and *b*). Larger cells frequently completed the process end to end (Fig. 2*j*, *k* and *i*). Fusing cells, watched on open wet mounts until they dried on the glass, and then stained, showed that nuclear fusion closely follows that of the cytoplasm (Fig. 2*q*, *r* and *s*).

Examination of normal cells of *C. cohnii* also revealed pairing and fusion in all clones observed, including those of the Puerto Rican, Mattapoisett and McLaughlin strains. Since all clones represent single cell isolates, it seems likely that the sexuality of *C. cohnii* does not depend on different mating types (homothallism).

In genetic experiments cells of fm 1 were mixed with cells of fm 2 in liquid cultures. After about 10 h normally swimming cells were observed. This was remarkable since mutant cells have never reverted in culture for more than 400 generations, and, since they grow less vigorously than wild type, they would probably soon be overgrown by a back mutation. Fusion of an fm 1 cell and an fm 2 cell could be observed since the trailing flagellum of each could be recognised: that of fm 1 appeared to push both cells while that of fm 2 was ineffectual. Control

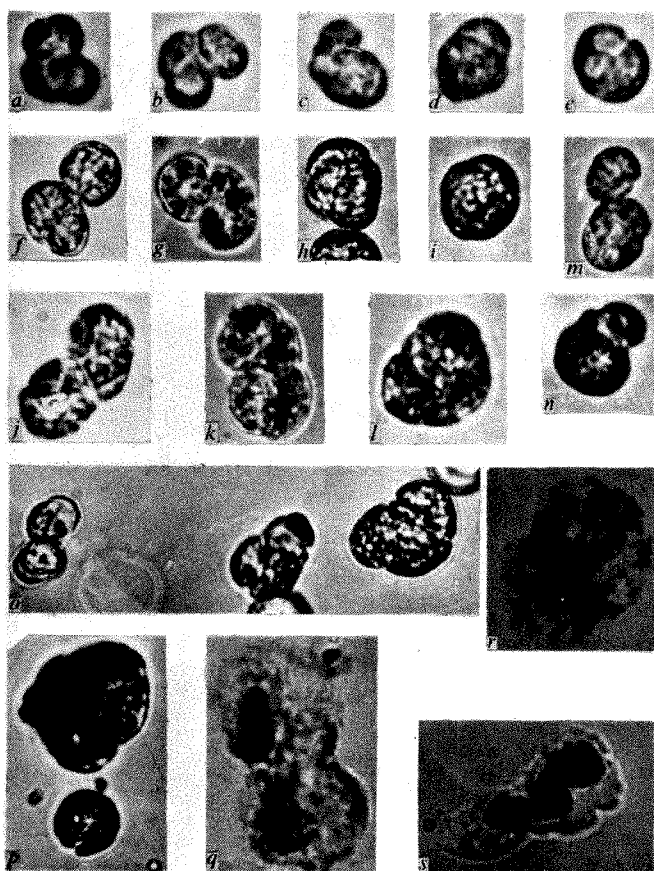


Fig. 2 Pairs of flagellated cells of *C. cohnii*, mutant fm 1. Magnification for living cells, $\times 528$; Feulgen-stained, $\times 1190$. a-e, Small cells (length about $14\ \mu\text{m}$) total time about 35 min; f-i larger cells (about $18\ \mu\text{m}$), total time about 90 min; j-l, large cells (about $23\ \mu\text{m}$), total time more than 12 h; m and n, anisogamous fusion; o, fusions of different sizes occurring at the same time; p, fusion product of largest size (about $32\ \mu\text{m}$); q, Feulgen-fast green-stained pair corresponding to (a) or (f); r, similarly stained fusion product corresponding to (e) or (i); s, anisogamous fusion corresponding to (r).

experiments in which cells of each mutant were incubated in culture filtrates of the other indicated that no diffusible substance was involved.

In mixtures of fm 1 and fm 2 some normally motile large cells appeared after 6 h, apparently too soon for completion of fusion, encystment, cell division and release of progeny, and we believe that they represent complementation in the 'zygote' itself. Since many fusions are complete within 1 h, the 6 h delay of restored function suggests that complementarily deficient organelles are not contributed by the 'gametes', but that new organelles are elaborated by the 'zygote'. Since cysts can give rise to single motile products, the 'zygote' might encyst for a time during which complementation is effected, after which it emerges as a normally swimming cell. Similar encystment of zygotes has been reported for other dinoflagellates¹⁸.

Only after a minimum of 14 h were the smallest rapidly swimming cells seen in the mixtures. These were too small to be the immediate products of fusion and are thought to be the products of reproductive cysts in which recombinational events occurred. This view is supported by the following genetic evidence. Early appearing large, fast swimming cells ('zygotes') were isolated by micromanipulation on agar. These soon rounded up, grew and ultimately produced four or eight progeny. These were in turn isolated and transferred to liquid medium. After several generations the cells were classified as to their type of motility and their ability to generate normal swimmers in mixtures with their parents (back crosses). Several types of segregations ('tetrads') were observed: in

fourteen zygotes analysed four contained only parental mutant types, four showed recombination—each contained two normal swimmers and two double mutants—and the rest appeared to yield parental types phenotypically, but each segregant showed recombination either with both or with neither parent. All segregants have been maintained for many generations in culture without alteration of phenotype, indicating the absence of 'post-meiotic' segregation.

The fact that the mutant characteristics are recoverable from complementing fusion products is consistent with Mendelism, as is the occurrence of normal recombinants with their double mutant counterpart. However the absence of tetrads showing four types of cells—parental and recombinant—is unusual, and suggests that recombination occurs before chromosome replication.

Our evidence indicates: (1) unusual homothallic sexuality with 'gametes' and 'zygotes' in a wide range of sizes, DNA content and chromosome numbers; (2) complementation in 'zygotes' after the fusion of two different mutants; (3) reduction in the first one or two divisions of the complementing 'zygote'; (4) that these reduction division(s) produce either: (a) both parental mutant types, (b) recombinants, both normal and double mutant, or (c) apparent parental types that display unexpected behaviour. These findings suggest that the chromosomes of this organism are functionally unineme, as has been proposed⁹ but that they behave unconventionally in several respects.

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- ¹ Dodge, J. D., *Arch. Protistenk.*, **106**, 442-452 (1963); *Arch. Microbiol.*, **48**, 66-80 (1964); in *The chromosomes of the algae* edit. by Godward, M. B. E., 96 (Arnold, London, 1966); *Bot. Rev.*, **37**, 481-508 (1971).
- ² Stewart, J. M., and Beck, J. S., *J. Protozool.*, **14**, 225-231 (1967).
- ³ Rizzo, P. J., and Nooden, L. D., *Science*, **176**, 796-797 (1972); *J. Protozool.*, **20**, 666-672 (1973).
- ⁴ de Haller, G., Kellenberger, E., Rowiller, C., *J. Microsc. Paris*, **3**, 627-643 (1964).
- ⁵ Grasse, P. P., Hollande, A., Cachon-Enjumet, M., *C.r. hebd. Séanc. Acad. Sci.*, **260**, 6975-6978 (1965).
- ⁶ Kubai, D. F., and Ris, H., *J. Cell Biol.*, **40**, 508-528 (1969).
- ⁷ Rae, P. M. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1141-1145 (1973).
- ⁸ Haapala, O. K., and Soyer, M. O., *Nature new Biol.*, **244**, 195-197 (1973).
- ⁹ Roberts, T. M., Tuttle, R. C., Allen, J. R., Loeblich III, A. R., and Klotz, L. C., *Nature*, **248**, 446-447 (1974).
- ¹⁰ Javornicky, P., *Preslia*, **34**, 98-113 (1962).
- ¹¹ Gold, K., and Baren, C. F., *J. Protozool.*, **13**, 255-257 (1966).
- ¹² Himes, M., Beam, C. A., and Berman, S., *J. Cell Biol.*, **59**, 143a (1973).
- ¹³ Franker, C. K., Smith, L. F., and Sakhrini, L. M., *Arch. Microbiol.*, **90**, 255-262 (1973).
- ¹⁴ Ornstein, L., *Lab. Invest.*, **1**, 250-262 (1952).
- ¹⁵ Patau, K., *Chromosoma*, **5**, 341-362 (1952).
- ¹⁶ Lewin, R., *J. gen. Microbiol.*, **6**, 233-248 (1952).
- ¹⁷ Rubin, W. R., and Filner, P., *J. Cell Biol.*, **56**, 628-635 (1973).
- ¹⁸ von Stosch, H. A., *Br. phycol. J.*, **8**, 105-134 (1973).

Aetiology of Down's syndrome inferred by Waardenburg in 1932

It is not widely known, and should be of historical interest, that in 1932 P. J. Waardenburg¹ suggested, in a monograph on the human eye, that Down's syndrome resulted from a chromosomal aberration due to non-disjunction. This was 5 yr after the first report of a chromosomal aberration in a mammal². In 1952, Mittwoch³ reported studying the meiotic chromosomes of a mongoloid patient and judged them to be normal. Waarden-

burg's suggestion was rarely cited, and the eventual discovery of trisomy-21 in 1959 (ref. 4) was generally received with surprise.

The relevant passage in Waardenburg's book appeared on pages 47 and 48 near the end of a chapter on mongolism. After enumerating the possible aberrations in several sentences, he went on to discuss the significance of the hypothesis for human genetics. He began as follows:

"Ich möchte die Zytologen anregen zu untersuchen ob es nicht möglich wäre, dass hier beim Menschen ein Beispiel einer bestimmten Chromosomenaberration vorläge. . . . Man sollte einmal beim Mongolismus untersuchen ob hier vielleicht 'chromosomal deficiency' durch 'non-disjunction' oder das umgekehrte 'chromosomal duplication' vorliegt."

(I should like to suggest that the cytologists investigate the possibility that we have here in man an example of a specific chromosomal aberration. . . . Mongolism should be studied to determine whether there may be a 'chromosomal deficiency' resulting from 'non-disjunction' or the converse, a 'chromosomal duplication'.)

Waardenburg is a Dutch ophthalmologist, formerly a practitioner in Arnhem and Lecturer in Medical Genetics at the universities of Utrecht and Leiden. He is now retired in Oosterbeek.

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¹ Waardenburg, P. J., *Das Menschliche Auge und seine Erbanlagen* (Martinus, Nijhoff, Den Haag, 1932).

² Painter, T. S., *Genetics, Princeton*, 12, 379-392 (1927).

³ Mittwoch, U., *Ann. Eugen.*, 17, 37 (1952).

⁴ Lejeune, J., Gautier, M., and Turpin, R., *C. r. hebdomadaire Séances Acad. Sci., Paris*, 248, 1721-1722 (1959).

Electrophysiological evidence for colour channels in human pattern vision

LIGHT striking the eye is absorbed by three types of pigment preferentially sensitive to either red, green or blue light. The resulting neurophysiological signals are ultimately pooled before they activate the neural mechanism that underlies the perception of photometric brightness. On the other hand, the role of colour might be different when viewing coloured scenes containing fine detail (pattern vision) compared with standard photometric stimulus fields; colour signals may not be pooled as when perceiving brightness^{1,2}. There is evidence that the human visual system handles red patterns in parallel with green and blue patterns^{1,2}. I have attempted to determine spectral sensitivity curves for the red-sensitive and green-sensitive channels of human pattern vision. This was done objectively by measuring brain activity electrophysiologically.

When a subject viewed a pattern of red and black checks that exchanged places seven times per second, the visual area of the brain generated a 7 Hz alternating electrical waveform called an evoked potential (EP). Superposed on the red pattern was an unpatterned disk of desensitising light whose wavelength was λ . Evoked potential amplitude was progressively reduced by increasing the intensity of the superposed disk of light. The principle of this experiment was to compare disks of different wavelengths in their ability to attenuate EPs produced by the red pattern.

The intensity of the disk was logarithmically increased with time for 28 s and then abruptly restored to its initial value. The total intensity change was 1.9 log units. While the disk intensity was slowly changing, the red pattern,

which was oscillating at 7 Hz, was continuously eliciting EPs whose moment-to-moment amplitudes were recorded as a function of time.

This was done by a Fourier analyser that operated as a narrow-band EEG filter centred on 7 Hz; its output was the running average of the EP amplitude³. In this way a plot of EP amplitude against disk intensity was directly obtained over the 28 s stimulus period⁴. Irregularities were reduced by electronically averaging four such samples of the required plot, allowing 20 s rest between each sampling. The technical details and experimental advantages of this new rapid technique are discussed elsewhere^{4,5}.

Figure 1 shows how the amplitudes of the red pattern's EPs were altered by increasing the intensity of the superposed disk of light. It should be noted that identical red stimulus patterns elicited all the EPs shown in this figure. Only the superposed unpatterned disk of light differed. Figure 1 also shows that as the desensitising disk's intensity was increased, EP amplitude was at first unaffected or even rose a little and then decreased roughly proportionally to log intensity. These two regions were separated by a 'knee' that was often clearly defined (Fig. 1 arrows). The trace marked *a* shows the noise level; it was recorded with the red pattern occluded. The evoked potential amplitude fell to noise level at an intensity close to that for which the pattern disappeared. These last findings are similar to the results of changing stimulus contrast at constant intensity^{2,6-8}.

Curves such as those of Fig. 1 were similar in their main features whatever the colour of the desensitising light; thus, to a first approximation, the only effect caused by changing the disk's colour was to displace the plot bodily along the intensity axis. This indicated that the EPs produced by the red pattern could, in the first instance, be treated as though the differently-coloured disks of desensitising light would have identical effects when appropriately balanced for intensity.

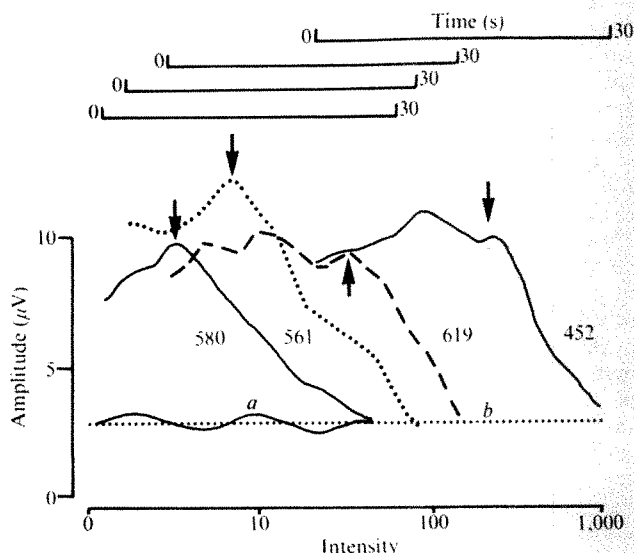


Fig. 1 Effect of desensitising light on evoked potentials elicited by a red checkerboard pattern that oscillated at 7 Hz. The $2^\circ \times 2^\circ$ pattern of 676 nm, 9 min checks was viewed centrally. Ordinates: amplitude of pattern evoked potentials. Superposed on the pattern was a 6° patch of desensitising light. Intensities of desensitising light are plotted along abscissa for desensitising wavelengths of 452 nm, 561 nm, 580 nm and 619 nm. Four samples averaged for each plot. *a*, The noise level recorded with the red pattern occluded. *b*, Mean noise level. Arrows indicate positions of 'knees'. The time axes show, for each separate plot, the timecourse with which the desensitising light's intensity was changed. Half-widths of filters were 10 nm. Bipolar recordings between electrodes on theinion and 9 cm anterior along the midline. The left mastoid was grounded.

The spectral sensitivity of the red pattern EPs was found as follows: Clearly, the red pattern EPs were most sensitive to the wavelength which gave a plot at the extreme left hand side of Fig. 1. This was not, as might be supposed, when the pattern and disk had the same wavelength (676 nm); in fact the plot was shifted most to the left when the disk's wavelength was approximately 590 nm. The 590 nm curve was arbitrarily taken as reference. Some other curve (501 nm) was then shifted to the left by an amount I_{501} log units until the linear portion from the 'knee' downwards coincided with the corresponding part of the 590 nm curve. The relative sensitivity of the red pattern EPs to light of wavelength 501 nm, was then directly calculated from I_{501} . This procedure was repeated for all disk wavelengths. The filled circles in Fig. 2a plot relative spectral sensitivity against disk wavelength λ . Similar results were obtained by the criterion response method using evoked potential feedback²; these have been added in Fig. 2a. Peak sensitivity lies between 580–600 nm.

The range of desensitising intensities for which this spectral sensitivity curve applied was calculated as follows. When the wavelengths of the disk and of the pattern were the same (676 nm), the 'knee' was found to mark the point where the disk intensity was approximately the same as that of the pattern's bright checks (that is stimulus contrast was 33%). As mentioned above, spectral sensitivity was estimated for the sections of the plots of Fig. 1 that lay between the 'knee' and the lowest EP amplitude. Thus, the spectral sensitivity curve of Fig. 2 held from the 'knee' (where only little desensitising light had been added so that chromatic adaptation was slight) to an adapting level roughly 1.5 log units higher, where the red pattern could no longer be seen.

This use of the 'knees' illustrates how evoked potential recording can offer the possibility of quantitatively studying visual processes at everyday suprathreshold levels of sensation where available psychophysical methods may be uncertain or ineffective.

The dotted line in Fig. 2a shows the relative spectral sensitivity curve for luminance (brightness) perception measured by the method of heterochromatic flicker photometry. A $2^\circ \times 2^\circ$ unpatterned patch of wavelength 676 nm and 122 trolands was flickered against a comparison patch of wavelength λ . There was a $36^\circ \times 36^\circ$ white surround of 250 trolands. I have previously reported that this luminosity curve agrees closely with the spectral sensitivity curve of EPs elicited by rapidly flickering a spatially-unpatterned patch of light⁹. On the other hand, Fig. 2a shows that the dotted luminosity curve is quite different from the spectral sensitivity curve for red pattern EPs. The luminosity curve peaks near 555 nm. In contrast, the sensitivity of red pattern EPs is displaced markedly towards the red end of the spectrum, and peaks at 580–600 nm. This means that for pattern EPs, visual effectiveness is not directly proportional to luminance; for example Fig. 2a shows that red and green lights that were equally effective for the red pattern channel might differ in luminance by 0.5 log units.

Figure 2b shows the result of repeating these EP experiments, but with the red pattern replaced by a green (544 nm) pattern. When Fig. 2b and 2a are compared, it is clear that the green pattern's spectral sensitivity curve peaked at much shorter wavelengths (540–560 nm) than the red pattern's curve. The wavelength of the green peak was also estimated by fitting a displaced rod sensitivity curve to the data of Fig. 2b after allowing for prereceptor absorption¹⁰. The rod curve fitted quite well with its peak displaced to 530–540 nm. The green pattern peaked at roughly the same wavelength, or even at a lower wavelength, than the luminosity curve (Fig. 2a, dotted).

To what extent do the EP curves of Fig. 2 represent the action spectra of red-sensitive and green-sensitive channels in pattern vision? Isolation of colour channels

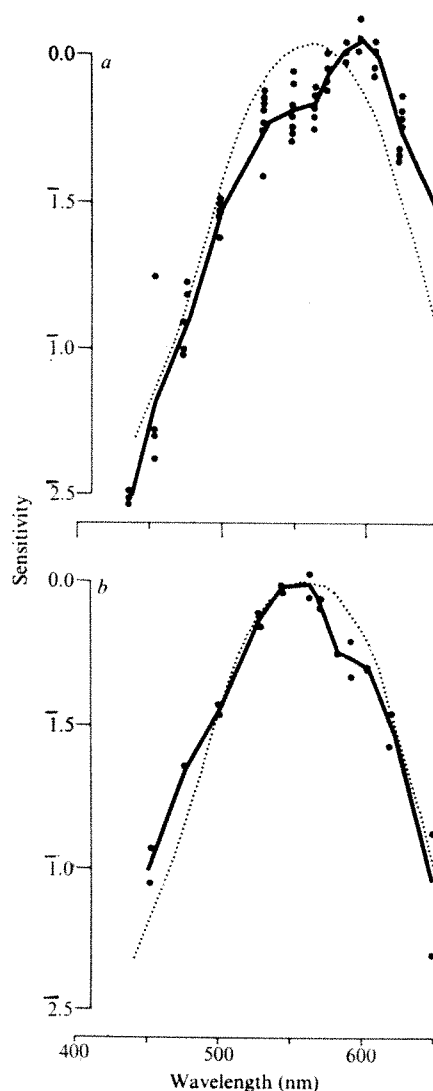
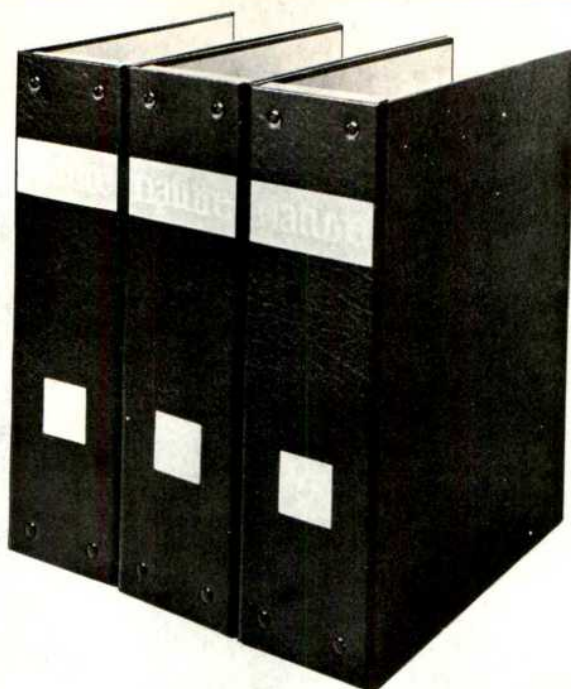


Fig. 2 a, —, The relative spectral sensitivity for brain potentials evoked by a red (676 nm) pattern; ●, individual points; . . . , relative luminosities of spectral lights determined by heterochromatic flicker photometry. b, As for a except that the pattern was green (544 nm).

is notoriously difficult, as the action spectra of different photopigments overlap. Thus, even if each colour channel subserving pattern vision were fed exclusively by a single type of photoreceptor, then even monochromatic lights would stimulate all channels to some extent. In the present case the blue-sensitive pigment was most probably unimportant, not only because its spectral sensitivity is well separated from the red and green-sensitive pigments, but also because a fine stimulus pattern was used (9 min checks). It is well known that the spatial resolving power of the blue-sensitive visual mechanism is coarse¹¹. The shapes of the spectral sensitivity curves were consistent with some minor contribution from green receptors in Fig. 2a and some minor contribution from red receptors in Fig. 2b. Furthermore, the EP curves of Fig. 2 peak at wavelengths that agree with estimates for the peak sensitivities of the red and green photopigments^{12,13} or colour mechanisms¹⁴⁻¹⁹. All this indicates that Fig. 2b to a fair extent represents the action spectrum of the green photopigment and that Fig. 2a describes, though with rather less precision, that of the red pigment.

The data reported above extend the findings of previous experiments in which subjects viewed an equiluminant pattern of adjacent red and green checks (or bars). Large pattern-specific EPs were recorded each time that the



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red and green checks (or bars) exchanged places^{2,7,20,21}. I pointed out that such EPs could not exist unless red and green signals were still to some extent segregated on arrival at pattern-sensitive neurones^{2,7}. Furthermore, chromatic segregation might even be complete²¹. This explanation would also be consistent with the McCollough effect¹ and with evidence that lateral interaction occurs within each class of cone²²⁻²⁴. It is important, however, to distinguish this suggestion from the colour segregation observed when monochromatic patterns stimulate the chromatically-adapted eye (for example, as above and in ref. 22). The latter conditions would reveal peripherally-determined red, green and blue colour channels even if colour signals were no longer segregated when they reached pattern-sensitive neurones.

I thank Dr F. W. Campbell, Dr H. Spekrijse, and Professor R. A. Weale for their comments; Robert E. Cartwright for invaluable technical assistance; H. Wardell and workshop staff for constructing equipment; and the Medical Research Council for supporting this work.

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- ¹ McCollough, C., *Science*, **149**, 1115 (1965).
- ² Regan, D., *Vision Res.*, **11**, 1203 (1971); in *The Visual System* (edit. by Arden, G. B.) (Plenum Press, New York, 1972).
- ³ Regan, D., *Electroenceph. clin. Neurophysiol.*, **20**, 238-248 (1966); *Med. Elect. Biol. Eng.*, **4**, 169-177 (1966); *Evoked Potentials in Psychology, Sensory Physiology and Clinical Medicine* (Chapman and Hall, London, 1972).
- ⁴ Regan, D., *Invest. Ophthalm.*, **12**, 669-679 (1973).
- ⁵ Regan, D., *Vision Res.* (in the press).
- ⁶ Campbell, F. W., and Maffei, L., *J. Physiol., Lond.*, **207**, 635-652 (1970).
- ⁷ Regan, D., *Vision Res.*, **13**, 2381-2402 (1973).
- ⁸ Spekrijse, H., Tweel, L. H., van der, and Zuidma, Th., *Vision Res.*, **13**, 1577-1601 (1973).
- ⁹ Regan, D., *J. opt. Soc. Am.*, **60**, 856-859 (1970).
- ¹⁰ Wyszecki, G., and Stiles, W. S., *Colour Sci.*, (Wiley, New York, 1967).
- ¹¹ Brindley, G. S., *Physiology of the Retina and Visual Pathway*, 2nd ed., (Arnold, London, 1970).
- ¹² Brown, P. K., and Wald, G., *Science*, **144**, 45-52 (1964).
- ¹³ Marks, W. B., Dobel, W. H., and MacNichol, E. F., *Science*, **143**, 1181-1183 (1964).
- ¹⁴ Baker, H. D., and Rushton, W. A. H., *J. Physiol., Lond.*, **176**, 56-72 (1965).
- ¹⁵ Weale, R. A., *Optica Acta*, **6**, 158-174 (1959).
- ¹⁶ Wald, G., *Science*, **145**, 1007-1017 (1964).
- ¹⁷ Pitt, F. H. G., *Proc. R. Soc. B*, **132**, 101-117 (1944).
- ¹⁸ Thomson, L. C., and Wright, W. D., *J. opt. Soc. Am.*, **43**, 890-894 (1953).
- ¹⁹ Stiles, W. S., *Proc. R. Soc. B*, **133**, 418-434 (1946); *Docum. Ophthalm.*, **3**, 138-163 (1949); *Proc. natn. Acad. Sci. U.S.A.*, **45**, 100-114 (1959).
- ²⁰ Regan, D., and Sperling, H. G., *Vision Res.*, **11**, 173-176 (1971).

- ²¹ Regan, D., and Spekrijse, H., *Vision Res.*, **14**, 89-95 (1974).
- ²² Green, D. G., *J. Physiol., Lond.*, **196**, 415-429 (1968).
- ²³ McKee, S. P., and Westheimer, G., *J. Physiol., Lond.*, **206**, 117-128 (1970).
- ²⁴ Kelly, D. H., *J. Physiol., Lond.*, **228**, 55-72 (1973).

Prehistoric human activity and blanket peat initiation on Exmoor

It now seems that the shallow tracts of peat which are so abundant in western parts of the British Isles began their development at about the time when the first signs of human activity became evident in the pollen record^{1,2} contained in the peat. These blanket mire complexes or "Terrainbedeckende Moore"³ have received relatively little attention from palaeoecologists and radiocarbon-dated pollen profiles are few; those which exist refer mainly to deposits from Ireland^{4,5} and the Southern Pennines^{6,7}. We now present a radiocarbon-dated pollen diagram, from a blanket mire on Exmoor, Somerset, which throws further light on the coincidence of prehistoric human activity and the initiation of blanket peat formation.

The deepest blanket mires found on Exmoor were on an upland plateau site termed The Chains. A monolith of peat from the mire was excavated and this was used both for palynological work and for radiocarbon dating. Pretreatment of the samples for dating was carried out according to the specification of the Palaeoecology Laboratory, Belfast, where the dating was performed. In effect this pretreatment involved the extraction of the fine particulate matter from the peat by a process of KOH digestion followed by sieving. The dated material thus consisted largely of pollen and spores together with fine fragments of fungal origin. Contaminant root material penetrating the profile from above was thus eliminated.

Selected results of pollen analysis together with the radiocarbon dates are shown in Figs 1 and 2. The diagram has been zoned on the basis of the characteristics of the pollen assemblage (see Table 1); in Table 1 the probable corresponding archaeological or historical period is also suggested.

The diagram bears a strong similarity to those published from mid-Wales⁸ and the Southern Pennines⁷. The dating of the basal layers at about the time of the arrival of farming cultures lends further support to the suggestion that the introduction of grazing animals² or the ploughing of marginal land⁹ may have assisted in the initiation of these deposits.

Shallow blanket peats of about 1 m in depth are more

Table 1 The Chains pollen zones and their interpretation

Pollen Zone	Major characteristics	Interpretation and date (radiocarbon yr)
EC1	Relatively high <i>Ulmus</i> and <i>Pinus</i>	Undisturbed woodland before arrival of farming cultures
EC2	Decline in <i>Pinus</i> and <i>Ulmus</i> . Increase in <i>Alnus</i> at the expense of <i>Quercus</i> . Increase in <i>Pteridium</i> ; <i>Plantago lanceolata</i> is consistently present	Arrival of farming cultures and the response of the woodland to their activities. Extrapolation from dates, assuming an even rate of peat formation suggests the period covers about 5,000-3,800 b.p. (Neolithic)
EC3	Gradual recovery in <i>Ulmus</i> to a maximum at end of zone. No recovery in <i>Pinus</i> . <i>Plantago</i> sporadic and <i>Pteridium</i> erratic	An unsettled period with no permanent human settlements. Dates between about 3,800 and 2,300 b.p. (Bronze Age)
EC4	Abrupt decline in <i>Ulmus</i> and increase in <i>Betula</i> , <i>Pteridium</i> and <i>Plantago</i> . A decline in <i>Tilia</i> precedes that of <i>Ulmus</i>	Increased activity in terms of farming, woodland clearance and settlement. Dates, about 2,300-1,500 b.p. (Iron Age-Roman)
EC5	A sudden decline in <i>Pteridium</i> and <i>Plantago</i> . An increase in <i>Betula</i>	Reduced human activity. Period begins at about 1,500 b.p. (end of Roman), but is of uncertain duration
EC6	Gradual rise in <i>Pteridium</i> and <i>Plantago</i> to a maximum at the end of the zone. Decrease in <i>Alnus</i> , rise in <i>Ulmus</i> and <i>Pinus</i>	Increased settlement and deforestation, especially of lowlands and valleys. Uncertain date, but possibly culminating in the Napoleonic Wars, about 150 yr ago
EC7	Decrease in <i>Pteridium</i> and <i>Plantago</i> . <i>Pinus</i> reaches maximum	Reduced intensity of farming. Possibly past 150 yr

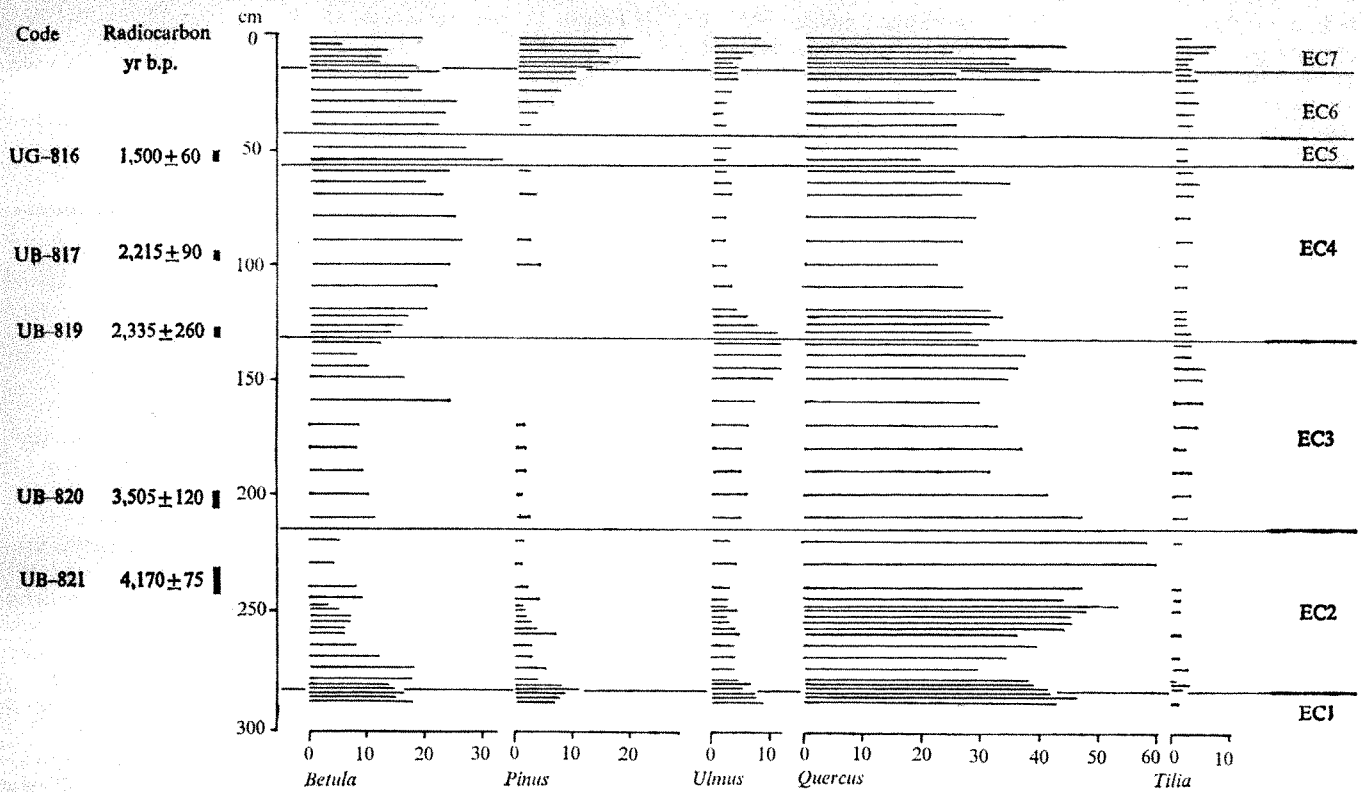


Fig. 1 Selected pollen data and radiocarbon dates from the peat profile at The Chains, Exmoor. (% Arboreal pollen)

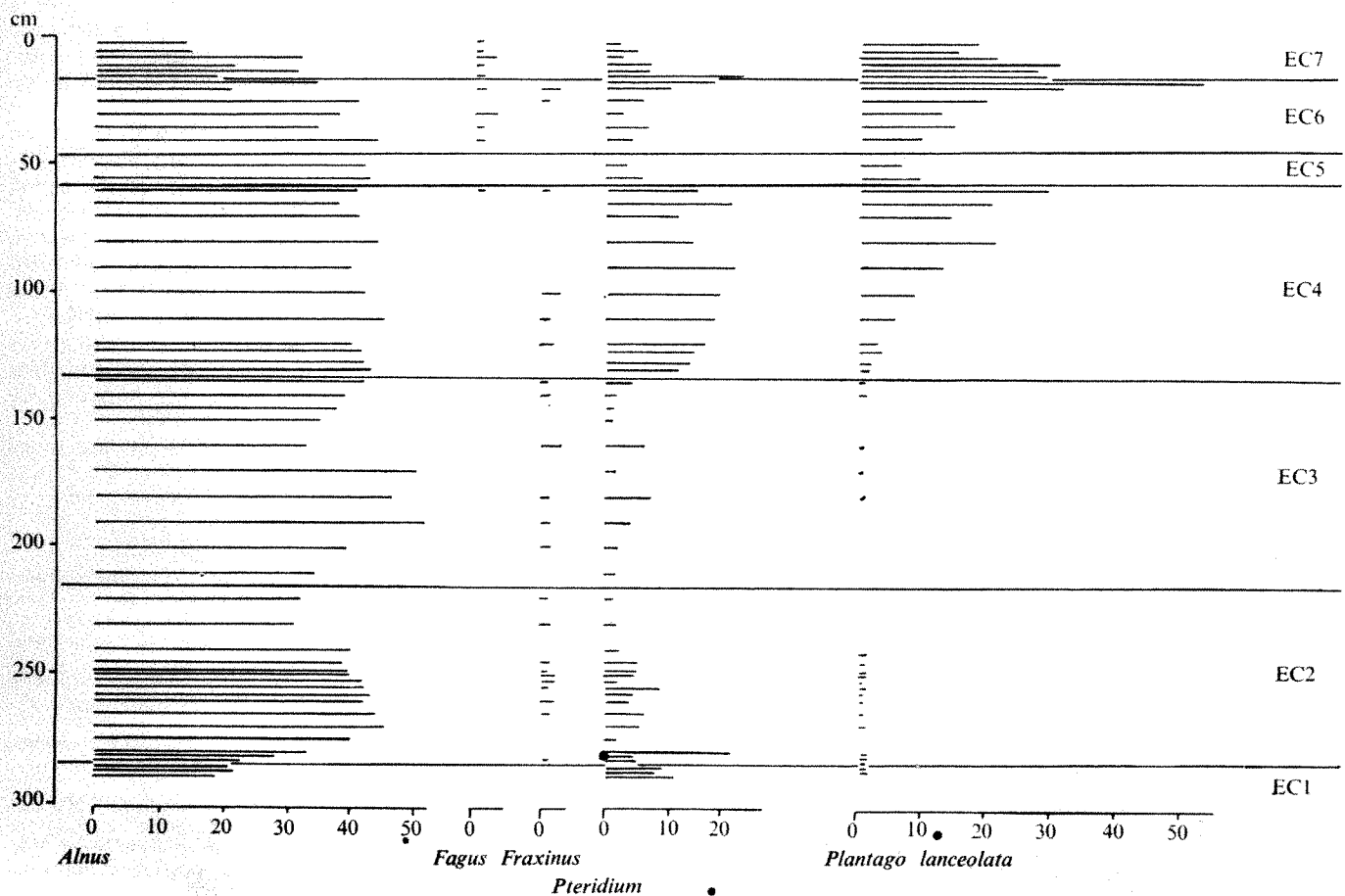


Fig. 2 Further pollen data from The Chains, Exmoor.

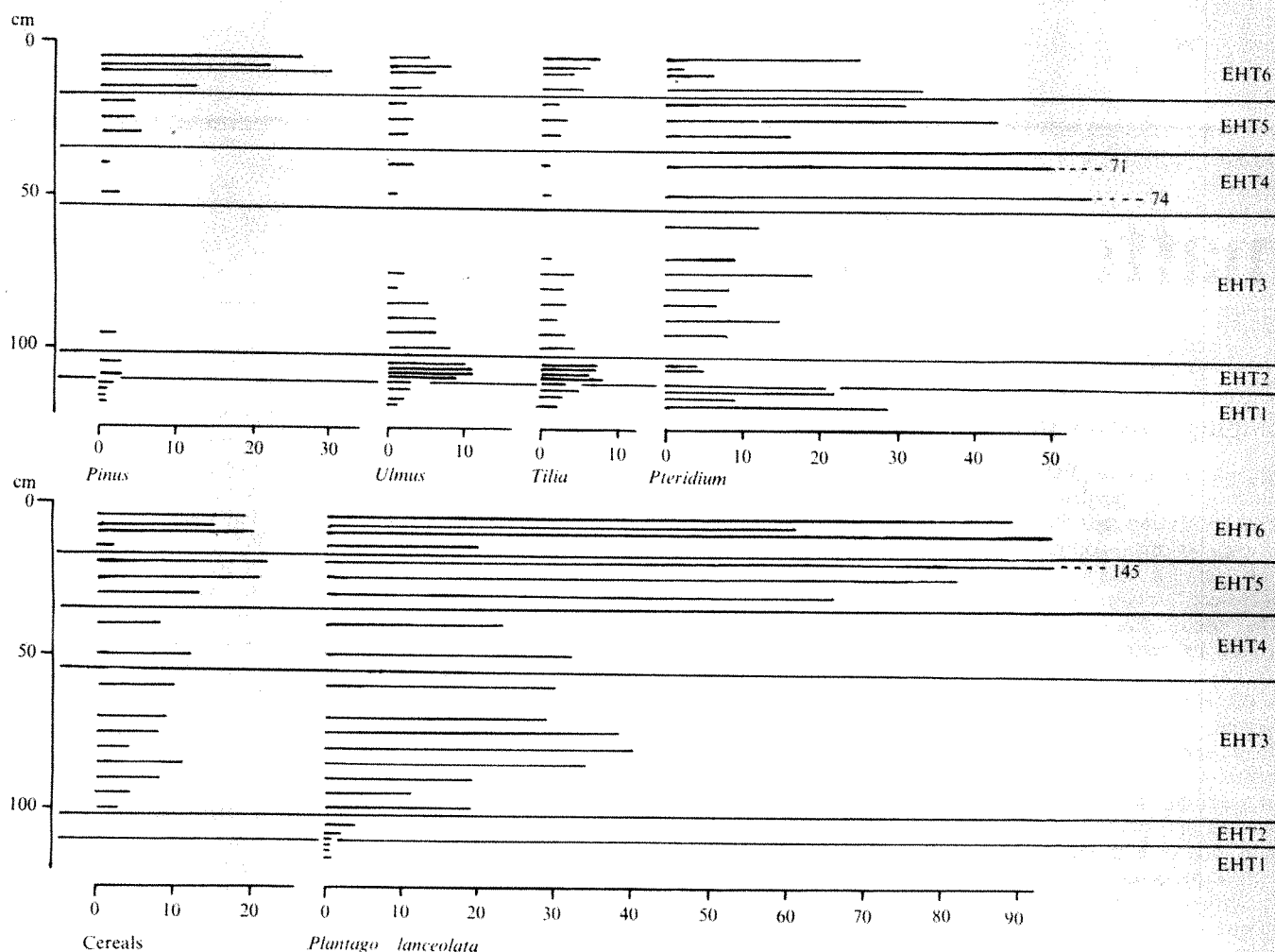


Fig. 3 Selected pollen data from the peat profile at Hoar Tor, Exmoor. (% Arboreal pollen)

common on Exmoor than are deep peats. Figure 3 shows a pollen diagram from a profile of one of these sites at Hoar Tor. Once again there is an *Ulmus* decline close to the base of the diagram, but the other features of the contemporary pollen assemblage, for example, low *Pinus* and high *Tilia*, suggest that this fall in elm corresponds to the second (zone EC4) *Ulmus* decline of The Chains diagram. This would mean that EHT3 = EC4 (Iron Age–Roman); EHT4 = EC5 (? Dark Ages); EHT5 = EC6 (Mediaeval–Napoleonic Wars); EHT6 = EC7 (past 150 y). The absence of radiocarbon dates makes it impossible to confirm these tentative correlations.

It has been pointed out² that the basal peats of blanket mires often contain evidence of human activity. The data reported here endorse that statement but also provide the basis for its qualification. The human activity recorded in the basal peats may date back to Neolithic times (for example, The Chains), or it may represent a more recent interference (for example, Hoar Tor). On Exmoor there are peats which began their formation at both of the major interference periods.

Moore² has suggested that stock grazing in a woodland under climatic and soil nutrient stress (caused by deteriorating climate and prolonged leaching respectively) could reduce regeneration and thereby tip the ecological balance in the favour of peat formation. Mitchell⁹ has described situations in western Ireland where Neolithic ploughing may have assisted the process of podsolisation by liberating iron in the upper soil layers which becomes deposited as a pan beneath the level reached by the plough. Once again the outcome is nutrient depletion and incipient blanket

peat formation. In the Exmoor context we favour the former explanation of how man may have influenced the course of peat initiation. The role of climate in this process must be emphasised. We postulate that man, or his domesticated animals, provided a final stress on an ecosystem already under climatic strain. It is salutary to note that both of the periods of blanket peat initiation on Exmoor correspond to times of deteriorating climate¹⁰.

We thank the Royal Society for grants towards the cost of equipment and radiocarbon dating.

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- ¹ Case, H. J., Dimbleby, G. W., Mitchell, G. F., Morrison, M. E. S., and Proudfoot, V. B., *Jl. R. Soc. Antiq. Irl.*, **99**, 39–53 (1969).
- ² Moore, P. D., *Nature*, **241**, 350–353 (1973).
- ³ Moore, P. D., and Bellamy, D. J., *Peatlands* (Elek, London, 1974).
- ⁴ Smith, A. G., and Pilcher, J. R., *Ulster J. Archaeol.*, **35**, 12–21 (1972).
- ⁵ Pilcher, J. R., *New Phytol.*, **72**, 681–689 (1973).
- ⁶ Hicks, S. P., *New Phytol.*, **70**, 647–668 (1971).
- ⁷ Tallis, J. H., and Switsur, V. R., *J. Ecol.*, **61**, 743–751 (1973).
- ⁸ Moore, P. D., *Nature*, **217**, 1006–1009 (1968).
- ⁹ Mitchell, G. F., *Proc. 24th Int. Geol. Congr. Montreal, Symp.* **1**, 59–68 (1972).
- ¹⁰ Lamb, H. H., in *The Flora of a Changing Britain* (edit. by Perring, F.), 11–24 (Classey, Hampton, Middlesex, 1970).

matters arising

Singing muscles in a katydid

SIR,—Your Insect Physiology Correspondent¹ has perpetrated a common error by stating that the tymbal muscles of cicadas may contract at 4,500 Hz. The song of cicadas, like other types of insect, consists of pulses of sound. The sound frequency, which is controlled by resonance of the abdominal air cavities, was measured by me as 4,500 Hz in some large cicadas in Ceylon, but is considerably higher in small species. The pulse modulation frequency, which may relate to the contraction frequency of the tymbal muscles, has never been observed higher than about 600 Hz, even in species with a myogenic contractile mechanism.

Neither your correspondent, nor Josephson in the article quoted² mention that high contraction frequencies in neurogenic muscles have been measured not only in insects but also in some vertebrate muscles producing only a small output of energy (the cricothyroid muscle of the bat³; and a fish swim-bladder muscle⁴). In all cases in fast neurogenic muscles, there is a correlated development of the sarcoplasmic reticulum involved in calcium transport.

Yours faithfully,

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¹ *Nature*, **248**, 106 (1974).

² Josephson, R. K., *J. exp. Biol.*, **59**, 781 (1973).

³ Revel, J. P., *J. Cell Biol.*, **12**, 571 (1962).

⁴ Fawcett, D. W., and Revel, J. P., *J. biophys. biochem. Cytol.*, **10**, 89 (1961).

Biosynthesis of bacteriochlorophyll

SIR,—Bacteriochlorophyll (BChl) production in nonsulphur purple photosynthetic bacteria growing anaerobi-

cally is inversely related to light intensity, and introduction of O₂ causes a rapid suppression of pigment synthesis. In a recent report¹, Davies *et al.* summarise findings which lead them to conclude "the original suggestion of Cohen-Bazire *et al.*² that the synthesis of BChl is controlled by the redox state of one or more of the components of the electron transport chain, is correct."

In this connection, Davies *et al.* cite experiments³ with intact cells in which various respiratory electron transport inhibitors were found to interfere with the synthesis of magnesium protoporphyrin. Since interruption of electron transport in intact cells can be expected to have numerous ramifying effects, there is reason to question the statement that "one or more of these [electron transport] components has been shown to be directly involved in the insertion of magnesium into protoporphyrin".

Davies *et al.*¹ suggest that "direct" control of BChl synthesis by O₂ (and light intensity) is also effected through regulation of aminolaevulinate (ALA) synthetase, the first enzyme of tetrapyrrole biosynthesis. They assert that ALA synthetase may be regulated by a trisulphide of glutathione or cystine which, in its oxidised form, increases activity of the enzyme. According to their scheme, oxygen (or light) has the effect of oxidising carriers of the electron transport chain, which in turn cause oxidation of an unidentified sulphhydryl compound to its disulphide form; the latter than is presumed to oxidise the "reduced polysulphide activator" to its active (stimulatory) state. On this basis, O₂ might be expected to accelerate BChl synthesis, rather than inhibit, but Davies *et al.*¹ seem to suggest that O₂ also disturbs sulphur metabolism so as to interfere with biosynthesis of the trisulphide activator. Elsewhere⁴, Neuberger *et al.* express the view that "oxygenation may lead to direct inactivation of the activator". We have been unable to rationalise the regulation of BChl synthesis by oxygen in terms of their interpretations.

Our recent experiments⁵ with mutants of *Rhodospseudomonas capsulata*, blocked at different points in

respiratory electron transport, demonstrate that the rapid inhibition of BChl synthesis by oxygen is independent of the cell's metabolic capacity to use O₂ as a terminal oxidant or to deliver reducing equivalents to the aerobic electron transport system. This argues cogently against the "redox carrier governor" postulation for regulation of BChl synthesis, and we have proposed an alternative working hypothesis in the study cited⁵.

Yours faithfully,

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¹ Davies, R. C., Gorchein, A., Neuberger, A., Sandy, J. D., and Tait, G. H., *Nature*, **245**, 15 (1973).

² Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y., *J. cell. comp. Physiol.*, **49**, 25 (1957).

³ Gorchein, A., *Biochem. J.*, **134**, 833 (1973).

⁴ Neuberger, A., Sandy, J. D., and Tait, G. H., *Biochem. J.*, **136**, 491 (1973).

⁵ Marrs, B., and Gest, H., *J. Bact.*, **114**, 1052 (1973).

DR NEUBERGER REPLIES: At the time when the paper by Davies *et al.*¹ was first submitted to *Nature*, all the known facts discovered by others and ourselves were compatible with the proposition first put forward by Cohen-Bazire *et al.* in 1957 that the electron transport chain in some way exerted a direct control over bacterial chlorophyll synthesis. Later experiments by Marrs and Gest published in 1973² and our own work published later in that year³ indicated that the effects of oxygen may be more specific and may be exerted through its action on sulphur metabolism, and in particular may be due to the depletion of the cellular content of a trisulphide activator of the enzyme. In this respect we are in

essential agreement with Gest and Marrs and our current work also is in accord with this general idea. It is, however, still possible that the activation process, which was first described by Marriott *et al.*⁴, involves in some way the electron transport chain. The possibility also exists that similar mechanisms operate *in vivo*, but data at present available do not allow a definite conclusion to be reached.

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¹ Davies, R. C., Gorchein, A., Neuberger, A., Sandy, J. D., and Tait, G. H., *Nature*, **245**, 15 (1973).

² Marrs, B., and Gest, H., *J. Bact.*, **114**, 1052 (1973).

³ Neuberger, A., Sandy, J. D., Tait, G. H., *Biochem. J.*, **136**, 491 (1973).

⁴ Marriott, J., Neuberger, A., and Tait, G. H., *Biochem. J.*, **111**, 385-394 (1969).

Microstructure of magnesium oxychloride cements

SIR,—Matkovic and Young in their article on the microstructure of magnesium oxychloride cements¹ reported some interesting observations, and raised some points for discussion. I would like to discuss those points which have wider implications.

The authors coated their samples with a Au-Pd layer of unstated thickness before examination in a scanning electron microscope (SEM). In the case of the metal coated specimen, SEM only reveals the outer topography of the metal layer. Figure 1 shows a section through three parallel needles each 2,000 Å thick, separated by 500 Å. The volume porosity of this packing is 37%. Assuming that the depositing vapour had a uniform density, any layer more than 250 Å (that is $\frac{1}{8}$ of the needle diameter) thick, will start to coalesce them. If the film thickness is 500 Å ($\frac{1}{4}$ of the needle diameter) the shaded areas of Fig. 1 will receive double the amount of metal necessary to fill them up. This excess metal will pile up locally, making the outer surface slightly undulated (indicated by the broken lines), and will be difficult to resolve into three. A thicker coating will make them appear monolithic. With lower porosity, a lower film thickness will be necessary to submerge them. If the needles are diverging, a metal film of proper thickness will make them appear to intergrow at the points of nearest approach, though in reality they do not. It can be seen that unless the coating thickness is adjusted to take care of particle size and density

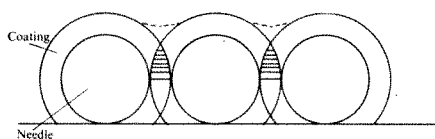


Fig. 1 A section through three parallel needles, each 2,000 Å thick, separated from one another by 500 Å. The broken lines indicate the slightly undulating outer surface formed by excess metal.

of packing in the specimen, quite a misleading topography may result.

Figure 1c of ref. 1 shows that the average apparent diameter of the crystals is about 1,200 Å. For the real diameter, twice the film thickness has to be deducted from that figure. For crystals of 1,200 Å real diameter, a film thickness of 150 Å or less will be required before it can be distinguished whether they are intergrowing or not. The above crystals were, however, the initial reaction products. The subsequent products are expected to be smaller and more densely packed. It is perhaps not surprising that the authors found that in older samples, the crystals were intergrown and the samples had a monolithic appearance. The smoothness of the cracked surfaces in their Fig. 3 can also be explained on this basis, though there are other alternatives.

The term 'intergrowth' has been used rather uncritically. There are two types of crystal-crystal intergrowth. A crystal may intergrow into another crystal without having any structural continuity, like a nail in a piece of wood. This is a special case of mechanical intergrowth. The second type of intergrowth involves structural continuity between the participating crystals. For this type of intergrowth to occur the crystals need to meet each other in definite orientations, satisfying the laws of twinning. Matkovic and Young seem to imply the second type of intergrowth. I wonder if they have tried, on this basis, an order of magnitude calculation on the probability of formation of a three dimensional load bearing structure. The result of that calculation would be quite illuminating.

Note added in proof: Re-examination of Fig. 1c reveals isolated needles whose real diameters are equal to the stated film thickness. How many more of them were in the real sample?

Yours faithfully,
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¹ Matkovic, B., and Young, J. F., *Nature phys. Sci.*, **246**, 79 (1973).

DRS YOUNG AND MATKOVIC REPLY:

It is certainly true that one of the problems of SEM is the need to have a conducting layer on the surface of inorganic materials. We are aware of the inherent problems of metal coatings, but do not think it invalidates the results presented in the original article. We used comparatively light coatings (~250 Å) of gold-palladium, and comparisons of such coatings with pure carbon coatings (which are transparent to secondary electrons) on samples of portland cement compounds, have not shown substantial differences in microstructural details. We therefore have confidence in the qualitative observation described in our article.

The term 'intergrowth' has been deliberately used in a vague sense and we regret any resultant misinterpretations. On the basis of SEM observations alone it is not possible to draw any definite conclusions concerning the exact interactions between crystals. We feel that, in order to explain the high mechanical strengths, these interactions must be stronger than weak Van der Waals surface forces, but may range from strong 'solid-solid contacts' postulated for hydrated portland cements¹, to true structural continuity.

Finally, we emphasise that these results are preliminary observations. We advanced our ideas in the interests of suggesting an alternative approach to the problem of strength development in cementitious systems. We realise that SEM cannot provide all the answers and we feel that, at present, attempts to make quantitative predictions may be premature.

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¹ Feldman, R. F., and Sereda, P. J., *Proc. fifth int. Symp. chem. Cements, Tokyo, 1968*, **III**, 36-44 (Cement Association of Japan, Tokyo, 1969).

Photosynthesis in leaves exposed to SO₂ and NO₂

SIR,—Bell and Clough¹ have reported that the growth of S23 ryegrass was substantially reduced by exposure to 12 p.p.m. (parts per hundred million) SO₂. Bleasdale² grew the same variety of ryegrass in greenhouses ventilated with polluted air from out-of-doors on a suburban site in Manchester and compared the growth with that in a greenhouse ventilated with purified air. There were reductions in the dry weight of the plants amounting to 57% in the polluted atmosphere, even though the SO₂ concentrations were below 3 p.p.m. for 39 out of 42 d and the daily

means did not rise above 9 p.p.h.m. at any time.

Bleasdale's data appear to show that S23 ryegrass is more sensitive to SO_2 than is apparent from the study by Bell and Clough. This discrepancy may be the result of their having performed experiments in different conditions, but it may equally have resulted from Bell and Clough's simulated SO_2 pollution rather than polluted air from outside. In Bleasdale's experiments other air pollutants would also have been present. One such is NO_2 . This is found in polluted atmospheres because NO is produced by combination when fossil fuels are burnt, and is quickly oxidised in air to NO_2 . We have recently completed some experiments in which the combined effects of SO_2 and NO_2 pollution on photosynthesis in the pea,

p.p.h.m. and above of both SO_2 and NO_2 were found to produce visible lesions on intact plants. Visible injury to crop plants after simultaneous exposure to SO_2 and NO_2 has been reported previously³.

Our data suggest that combined effects of SO_2 and NO_2 might explain the growth inhibition caused by urban pollution², and that this possibility should be considered in future studies.

J. N. Bull acknowledges the receipt of a CASE award from the Science Research Council, and we are grateful to ICI Ltd for financial support and Mr J. F. Newman for his advice.

Yours faithfully,

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¹ Bell, J. N. B., and Clough, W. S., *Nature*, **241**, 47-49 (1973).

² Bleasdale, J. K. A., *Environ. Pollut.*, **5**, 275-285 (1973).

³ Tingey, D. R., Reinert, R. A., Dunning, J. A., and Heck, W. W., *Phytopathology*, **61**, 1506-1511 (1971).

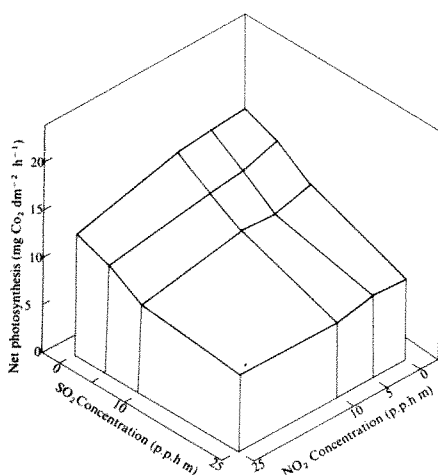


Fig. 1 Effects of simulated SO_2 and NO_2 pollution on net photosynthesis in *Pisum sativum*. Fumigation conditions: 1.20 air changes min^{-1} ; temperature, 21°C ; light intensity, $73\text{ J}^{-2}\text{ s}^{-1}$; water vapour pressure deficit, 995 N m^{-2} .

Pisum sativum, were examined over a concentration range of 0-25 p.p.h.m. for each gas. Initially, exposure to these pollutants increased net photosynthesis, but the effect was short lived and a substantial inhibition soon followed. The magnitude of this inhibition for 15 different treatments is shown in Fig. 1, with the control included for comparison. Analysis of this factorial experiment revealed statistically significant effects of SO_2 and NO_2 ($P < 0.001$ in both cases) but no significant $\text{SO}_2 \times \text{NO}_2$ interaction. It is clear, however, that the effects of the two pollutants are at least additive. These observations were made on detached leaves and no visible injury accompanied the depression of net photosynthesis, but more prolonged exposures to concentrations of 10

Regulation of albumin-bound tryptophan

SIR—Madras *et al.*¹ recently reported on the effect of tryptophan concentrations in serum and brain concerning its conversion to serotonin in rats. This and related work have been summarised by Fernstrom and Wurtman² who suggest that albumin-bound tryptophan is the precursor of serotonin. These authors^{1,2} have found that decreased nonesterified fatty acid content (albumin-bound) in the serum caused by administration of carbohydrate or insulin results in higher concentrations of albumin-bound tryptophan, while fasting (high fatty acid content in serum) results in lower albumin-bound tryptophan levels, suggesting that fatty acids competitively prevent the binding of tryptophan by albumin.

If one assumes a serum albumin concentration of 4 g per 100 ml, the data of Madras *et al.*¹, on the concentrations of serum-bound tryptophan and fatty acids can be converted into moles of tryptophan or fatty acid bound moles of serum albumin (Table 1). Thus conditions which decrease concentrations of fatty acids in the serum increase concentrations of albumin-bound tryptophan. McMenamy and Oncley³ found that bovine albumin bound 1 mol of L-tryptophan per mol of albumin. Addition of fatty acid decreased the binding capacity of the albumin for tryptophan by 0.1 mol for each mol of fatty acid. When 2.0 mol of oleate was present the albumin bound 0.75 mol of tryptophan.

Since 2.0 mol of fatty acid per mol of albumin represents the largest fatty acid ratio in Table 1, I assume that a minimum of 0.75 mol of tryptophan could be bound to the albumin rather than the maximum of 0.135 mol observed if fatty acid competition were the limiting factor.

The free energy (-7.2), enthalpy (-14.5) and entropy (-24) changes determined by Fairclough and Fruton⁴ for the binding of L-tryptophan to serum albumin are similar to those observed for binding of iodide⁵, lysolecithin⁶ and probably dodecyl sulphate⁵ to the high energy site of albumin. Thus such a material, which is strongly bound to the albumin, might decrease the amount of this site available for tryptophan and so function, along with fatty acids, to regulate the amount of tryptophan bound.

Yours faithfully,

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¹ Madras, B. K., Cohen, E. L., Fernstrom, J. D., Larin, F., Munro, H. N., and Wurtman, R. J., *Nature*, **244**, 34-35 (1973).

² Fernstrom, J. D., and Wurtman, R. J., *Sci. Amer.*, **230**, No. 2, 84-91 (1974).

³ McMenamy, R. H., and Oncley, J. L., *J. biol. Chem.*, **233**, 1436-1447 (1958).

⁴ Fairclough, G. F., jun., and Fruton, J. S., *Biochemistry*, **5**, 673-683 (1966).

⁵ Lovrien, R., and Sturtevant, J. M., *Biochemistry*, **10**, 3811-3815 (1971).

⁶ Klopfenstein, W. E., *Biochim. biophys. Acta*, **187**, 272-274 (1966).

Table 1 Effects of glucose and diet on bound tryptophan and fatty acids

	Effect of glucose				Effect of diet	
	Control	Glucose 1 h	Glucose 2 h	Fasted control	Carbohydrate + fat	Carbohydrate
Serum bound tryptophan mol per mol albumin	0.091	0.13	0.135	0.09	0.11	0.135
Non-esterified fatty acids mol per mol albumin	2.0	1.14	1.05	1.46	1.08	0.53

book reviews

De Broglie's philosophy

Louis de Broglie: Sa conception du monde physique, By A. George, et al. Pp. xxviii + 387. (Gauthier — Villars: Paris, 1973.)

LOUIS DE BROGLIE'S 80th birthday (August 15, 1972) came close enough to the 50th anniversary of his three famous notes to *Comptes Rendus* (September–October 1923) and the publication of his doctoral thesis (November 25, 1924) to warrant a double celebration. This collection of articles by his close colleagues, old and young, describes both his long career as a teacher and leader of research and the contributions that he and his pupils have made to "the past and the future of wave mechanics". Handsomely produced, elegantly phrased, and well integrated despite more than twenty different authors, it will prove a valuable source for biographers and for historians of science.

The accuracy of the personal picture cannot be easily estimated in a work of this kind, whose whole emphasis is naturally on the positive and virtuous — the guide, philosopher and friend, the lucid and eloquent teacher, the imaginative scientific intellect, still enthusiastic in old age, still able to state, more clearly than his pupils, the essential stages in his own scientific development and his own analysis of current problems. No man is perfect; but we do well to admire these qualities of greatness.

But the scientific work, being in the public domain, must be appraised more judiciously. Its standing depends not on the fact that it was done by M. de Broglie, but that it conforms to reality and is accepted by other competent scientific experts. This is the harsh law of science which defers not to prince, professor or poet.

Of the thesis of 1924 there can only be the highest praise. The very idea of 'matter waves' is the most revolutionary innovation in the philosophy of nature since the ancient Greeks. This significant step in modern physics was no mere stumble into the unknown, but a tremendous leap, gracefully poised on the classical principles of Fermat and Maupertuis but vaulting beyond Einstein's conjecture on the particulate nature of radiation. The first half of this book deals with the theoretical background of this brilliant paper, and on some of its modern

consequences, in electron microscopy, and so on.

So profound, indeed, was the revolution of thought that the revolutionary has spent the rest of his life trying to resist the further, yet more radical, statistical interpretation of the wave function. The papers in the second half of this volume record the considerable efforts of de Broglie and his school to reconcile waves with particles in a more deterministic scheme. Since this work is largely ignored by other theoretical physicists, one must ask why it has not found wide acceptance.

Nobody who has seriously grappled with (let alone had to teach!) the modern paradigm of quantum theory can feel completely satisfied with its present form. The essential localisation of the particle event is not intuitively consistent with the equally certain evidence of interference between extended wave fields. The fantastically precise discreteness of the rest mass and charge of an electron is a fact of nature that is difficult to swallow philosophically. The operator formalism of Heisenberg and Dirac seems to answer all the practical questions but leaves hollow the interior reality. The extraordinary paradox of a physical quantity that seems to depend upon the state of mind of the physicist is not to be shrugged off without comment.

There is, of course, a vast literature on these problems, to which the de Broglie school make almost no reference. Their theme is the ultimate 'reality' (potentially unequivocal observability?) of wave fields, of which the particles are singularities and by which they are guided. In his work of the 1930s, de Broglie concentrated on a theory of photons, which were assigned a very small rest mass to make them localisable. In the 1950s, he developed the 'double solution' for the particle singularity and its guiding wave, explaining the statistical phenomena of particle physics as manifestations of a hidden thermodynamic ensemble. Are these models of submicroscopic physics convincing, or worthy of further sustained effort?

Despite many elegant results, the physical systems to which they are applied are very simple (for example a few nearly free electrons) and no new phenomena are predicted for experimental verification. Indeed, it is taken

for granted (although with rather meagre proof) that the whole scheme must be capable of reproducing the results obtained by conventional methods for almost all atomic, nuclear and high energy systems. The de Broglie model is scarcely falsifiable, since it is presented as an alternative 'explanation' of quantum physics at the deepest level, incorporating automatically all existing theoretical achievements such as quantum electrodynamics, many body theory, and broken symmetry schemes.

The necessity for such an explanatory scheme thus rests on metaphysical rather than on physical considerations. The practical physicist may continue to solve all his problems by the conventional operator formalism, and need not learn the more complicated mathematical techniques of non-linear field theory. Until experiment, or some astonishing theoretical coup, demonstrates the practical superiority of the de Broglie interpretation, we can safely ignore it.

Does that explain, or justify, the neglect of this work by almost all orthodox physicists? It would be more honest to say that despite all the mathematical effort that has been lavished on it, the whole project rests upon too narrow a foundation of hypothesis and technique. The natural phenomena are richer (for example neutrinos, muons, pions, strong and weak forces) and our mathematical resources more diverse (finite and continuous groups, topology, tensor calculus and so on) than this book seems to imagine. For aesthetic reasons, and in modest deference to the unknown knowledge of future men, we do well to doubt that physics is conceivable only in the manner inherited from Newton, Laplace and Einstein. I would rather live uncomfortably with the paradoxes of quantum physics, hoping that some day they will be resolved, than accept, for the sake of peace of mind, a well meant but essentially backward looking scheme which promises more than it has yet achieved.

JOHN ZIMAN

Fine structure of algae

The Fine Structure of Algal Cells. By John D. Dodge. Pp. xii + 261. (Academic: London and New York, 1973.) £6.

THIS book is well produced and many of the illustrations will doubtless be

turned into lantern slides. The text, on the other hand, reads like a catalogue, with all the disadvantages of this type of format. Moreover there is no flicker of interpretative insight directed towards a reader who may not spontaneously appreciate the scientific interest of the phenomena or structures described.

This is not all the result of compression to reduce costs since, minor errors or omissions apart, some hazards to potential users have been deliberately inserted by the author or publisher without effecting great savings. Thus all too many micrographs are labelled with a generic name only, as if species of algae do not matter. One picture of scales is even labelled "*Chrysochromulina?*". Perhaps some tyro may value this and memorise it as an examination gimmick. An older reader, on the other hand, perhaps a non-botanist hoping to enter the fine structure field personally, must learn at the outset that, especially among algae, species may matter very much and carelessness here can invalidate or render unrepeatable the most elaborate exercises of other kinds. Any book which suggests, even by its format, that this is not so is being less than helpful to a serious and desirable type of reader.

Further, the omission of titles from the papers listed at the end, removes at a stroke the future usefulness of the book as a work of reference while greatly increasing the difficulty of effective proof reading. Thus on page 166 an important topic is said to have recently been summarised by an author dated 1972e while in the literature list one finds only 1972f (subject unknown) following 1972d (subject also unknown).

While, therefore, this book will be useful for a limited time and purpose for which it ought to be bought by libraries, it is not one which I would give as a present to a friend, colleague or student. Considering the intrinsic interest and beauty of the subject this is disappointing.

I. MANTON

The first clover leaves

The Primary Structure of Transfer RNA. By T. V. Venkster. Translated from Russian by Basil Haigh. Pp. x+296. (Plenum: New York and London, 1973.) \$29.

THIS monograph sets out to cover the entire field of transfer RNA primary structure in textbook form. It is an extended edition of a Russian text first published in 1968 and in this revised version surveys the literature up to July 1971. Each of the 22 transfer RNAs which had been sequenced by that time are described in considerable detail. The constant features of transfer

RNA architecture are also discussed, together with the evidence supporting the clover leaf hypothesis of the base-pairing of transfer RNA sequences. Little serious attempt is, however, made to relate features of individual sequences to the functional centres which determine the acceptor or adaptor roles of transfer RNA, other than in a consideration of the codon recognition properties of different anticodon loop structures. Hence those wishing to comprehend the structure-function relationship of transfer RNA will not find in this monograph a topical account of how the nucleotide sequence of a transfer RNA molecule can determine its function. The three-dimensional structure of transfer RNA obtained from X-ray crystallography is now clearly on the horizon and it should soon be possible to provide a more meaningful topological interpretation of the significance of those regions of transfer RNA sequences which lie outside the anticodon.

Much of this book is given over to a clear and detailed account of how the first few transfer RNA sequences were determined. The author takes us back to the initial attempts to determine nucleic acid primary structures and enables us to re-live that exciting period when the first few transfer RNAs were being sequenced. A considerable proportion of the narrative describes methods for the sequencing of non-radioactive RNA and many of these procedures have been largely supplanted by newer methods of sequence analysis which greatly reduce the work load of the experimentalist. There is little more than passing reference to the methods developed in Sanger's laboratory for sequencing ³²P-labelled RNA, even though most transfer RNA sequences are now derived using these techniques. Furthermore several other publications give a much more comprehensive and useful description of nucleic acid sequencing.

The chapter of this book which discusses the minor nucleotides of transfer RNA is by far the most topical and presents a thorough account of the physicochemical properties of these components. In view of the elaborate system of enzymes which exists for modifying transfer RNA it is hard to conceive that the modifications do not have an important biological function. Nevertheless the more inquisitive reader would probably wish to see expanded the sections of this book which describe the roles of the different isoacceptor transfer RNAs and the functions of the different minor components of transfer RNA.

As a result of the considerable effort now being expended in determining nucleic acid structures it is clearly

almost impossible for any book on transfer RNA to be up to date by the time that it is published. It has taken three years for the English version of this book to appear and during that time the number of sequenced transfer RNAs has increased from 22 to nearly 70. A complete summary of all known transfer RNA structures (*The Handbook of Nucleic Acid Sequences*, by B. G. Barrell and B. F. C. Clark) has recently been published as a loose-leaved folder by Joynson-Bruvver, Oxford. This is to be annually updated and will be rather more realistically priced. T. V. Venkster's book is more of value as a comprehensive account of the beginnings of nucleic acid sequencing.

P. W. PIPER

Evolution of plants

Chromosome Botany and the Origins of Cultivated Plants. By C. D. Darlington. Third (revised) edition. Pp. xvii+237. (Allen and Unwin: London, September 1973.) £4.40.

THE fascination of plant evolution is the great variety of mechanisms that have been involved. Plant species have certainly not plodded dully through time, losing a few genes here, gaining a few genes there: they have flashed into polyploidy, chromosome structural changes, inbreeding, apomixis, hybridity, with almost breathtaking frequency. The plant species which have stuck to an ordinary outbreeding diploid type of evolution are probably fewer than those which have not.

Since so much of this cunning and opportunism is tied up with chromosomal change, a book on chromosomes in plant evolution, by the cytologist to whom much of our understanding of chromosomes is due, is surely to be welcomed and respected, especially since it now reaches the eminence of a third edition. The book is also important because it is one of the very few in which the origins of cultivated plants are discussed, making use of recent evidence.

Alas for high hopes. The trouble is that the author falls a victim of his own enthusiasm. One is given too great an impression that plant evolution can be explained solely in terms of chromosomes: changes at the level of the gene cannot be disregarded. One is given a fascinating picture, but one which is capricious and worrying, and sometimes difficult to understand.

Yet the book contains the distillation of a lifetime: a provocative variety of evidence and conclusions on plant evolution that is unique, and a collection of information on crop plant evolution that is not available elsewhere. This is the reward for the reader.

A. D. BRADSHAW

Sad time for salmon

International Atlantic Salmon Symposium. Edited by Morden W. Smith and Wilfred M. Carter. Pp. xi+504. (Atlantic Salmon Research Trust: Fanham, Surrey; International Atlantic Salmon Foundation; New Brunswick, 1973.) £6.50 boards; £5 paper.

THIS collection of papers is the published proceedings of the "Symposium on the Atlantic Salmon: the Management, Biology and Survival of the Species", held at the Huntsman Marine Laboratory, St Andrews, New Brunswick, in September 1972. It consists of thirty-one major contributions under six main headings; the effects of man and the changing environment, physiology, ecology, aquaculture, environmental engineering and fishery economics, and conservation and fisheries.

With such a wide-ranging approach to the problems which surround the Atlantic salmon it is not surprising that several of the papers seem to have little direct relevance to the main objects of the symposium. One contribution invokes the aid of systems analysis for salmon conservation in a dauntingly titled paper, "the FASTRR concept (Facility for Atlantic Salmon Technological Research and Restoration). A status report". This concludes "the design and construction of an improved intensive culture hatchery depends on the determination of biological and physical needs, their estimated implementation and operating costs as balanced against predicted benefit to the overall project objectives. Elements of this study hopefully will ease the decision maker's task of quantitative initial assessment, choosing between alternates, and managing efficiently...". (One wonders what Frank Buckland, Inspector of Salmon Fisheries, 1867-1879, who managed to hatch salmon and trout eggs in the kitchen of his home in Albany Street, London, would have said of that?)

The salmon, in a unique way, is the focus of emotional attitudes. As an economic, leisure, or food resource it is probably without equal, but its decline in the industrialised and heavily populated North Atlantic countries has been quite dramatic. Reaction to dwindling salmon stocks in many countries suggests that although this may be more or less acceptable, it is clearly not acceptable for other nations to exploit the stocks outwith the national boundaries. Thus, the relatively recently developed high-seas fisheries conducted off the coast of West Greenland, and also in the Norwegian Sea, has resulted in a crisis within the salmon-producing countries, made still more emotional by the fact

that the country mainly operating the fishery is not a salmon producer. This development has placed the conservation of the Atlantic salmon firmly in the field of international relations, and as Dr Wilfrid M. Carter, co-editor of this volume, remarks, "the Atlantic salmon, perhaps more than any other fish has become symptomatic of the unwillingness of the international community to face and to solve critical problems...".

The contributions in this volume essentially fall into three categories (although they are not so divided by the organisers): the biological papers, some of which have direct relevance to the conservation of the species, papers on the management of stocks, and those directly related to the international management of the species. This last category would seem to have the most direct bearing on the major problem confronting the salmon. In this context, the review by B. B. Parrish of the work on the ICES/ICNAF Joint Working Party on the North Atlantic Salmon is a major contribution, summarising the problems involved and distinguishing the areas of biological knowledge that require to be clarified before more precise assessments of their effects can be made. But as D. L. McKernan points out elsewhere in the volume, the adoption of an ICNAF formula to phase out the non-native high seas fishery off Greenland, and to impose a catch limit on the inshore fishery, offers reason to be cautiously optimistic for the future of the Atlantic salmon.

ALWYNE WHEELER

Weather forecast

Radar Observation of the Atmosphere. By L. J. Battan. Pp. x+324. (University of Chicago: Chicago and London, 1973.) £7.15.

THERE has been a considerable increase during the past two decades in the use of electronic techniques for the remote sensing of the atmosphere, with radar playing an important if not dominant part. So much new material had become available that the author has carried out an extensive revision of his earlier work *Radar Meteorology*, first published in 1959. The present volume under its new title is solely concerned with the application of microwave radar in the study of atmospheric phenomena including precipitation, cloud, wind and clear air turbulence. The author has succeeded in covering most of the relevant material in less than 300 pages of text. This should have considerable appeal to a wide scientific readership apart from meteorologists, particularly those who lack a specific background in electronics.

The principles of radar are well illustrated although the author's very brief introduction concerning the properties of electromagnetic waves will hardly benefit a reader who is not already familiar with the subject. The remainder of the book deals with radar applications and the related atmospheric physics in appreciable depth. Although space may have been limited it would have been attractive if the author had covered in the same manner some aspects of upper atmospheric work including chaff and meteor radar as well as incoherent scatter techniques. Numerous technical details of radar installations are given in an appendix and readers will welcome a very comprehensive and up to date list of references. H. G. MULLER

Strange coincidence

The Challenge of Chance: Experiments and Speculations. By Alister Hardy, Robert Harvie and Arthur Koestler. Pp. 280. (Hutchinson: London, November 1973.) £3.

DURING a recent visit to America I was met at Roanoke Airport by my friend the statistician, I. J. Good, of the Virginia Polytechnic. Good excitedly asked me what type of plane I had flown in on and when I told him a Boeing 727 he cried "I knew it. I knew it". He then pointed to the licence plate of his Ford Mustang, which boldly featured the inscription "CRE 727". Permanent testimony to the happy correspondence between my initials, the plane I had flown in and Jack Good's car exists in the form of a licence plate which now adorns my office wall.

If you like stories of this kind you will revel in *Challenge of Chance*, which is described on the blurb as being "the first book to be written jointly by a Fellow of the Royal Society and a Fellow of the Royal Society of Literature—with a psychologist providing the bridge between the two cultures". The interdisciplinary flavour of the work is certainly striking, but the bridge is rather a shaky one. The book falls uneasily into three separate chunks—the first an account by Sir Alister of an "unsuccessful" mass extra-sensory perception experiment, the second a thirty page discussion by Harvie of probability theory and how puzzling it all is; the third a long essay by Koestler on some of the strange things that have happened to him, his friends and his correspondents. The majority of the strange things, I hasten to add, are somewhat more marvellous than the CRE 727 example. Hardy's experiment incidentally—a group ESP test in the Caxton Hall—was only unsuccessful in that no evidence of

telepathy was demonstrated. A subsequent and more elaborate search through the data revealed peculiar similarities in the guessing patterns of people seated near to each other in the group test. There are of course a number of possible explanations for such a finding, but Sir Alister considers it to be an example of "the coincidental coming together in time of similar ideas". But what, he asks, do we mean by coincidence anyway? This leads with a bump into the statistician's contribution, where again the inner meaning of the word coincidence is explored. Here the argument appears to be that if you riffle about long enough with sets of numbers you'll discover peculiar sequences in them—but sooner than you ought to have done by chance. Somewhere about here one crosses the transitional zone between statistics and pyramidology and I must confess to not being absolutely clear on occasions on which side of the zone Mr Harvie lies.

One is left in no doubt where Mr Koestler stands however. He doesn't actually use the word 'pyramidology' but like the pyramidologists he clearly believes that man should be very alert to the hidden meanings buried in the depths of what appear to be fairly straightforward objects or events. His section of the book is a compendium of the bizarre and the unlikely—psychic visions, poltergeist-like raps, unusual dreams, chance meetings in strange places and, of course, correspondences of the mystic car number variety. From here he argues that the sheer weight of human testimony to the vast array of oddities of this kind forces one to dispose of the word 'chance' in an explanatory role. Chance itself is a meaningless word which tells us nothing whatsoever about the universe and merely allows us to duck the issue of why such things happen so that we can preserve the creaky yet primary principle of causality. In his final section, ponderously titled "Speculations on Problems Beyond our Present Understanding", he returns to the theme of his previous work, "Roots of Coincidence", arguing that the peculiar phenomena of parapsychology are no longer peculiar in the light of recent discoveries in quantum physics. "Enlightened physicists" he tells us patronisingly, are now willing to concede this. The book is a frank statement of personal opinion and Koestler's segment in particular is highly readable. There's also not much doubt that it will be sweet music to the ears of thousands of readers, including a fair number of scientists who refish the anti-materialistic backlash which Koestler so heartily champions. Curiously, I think it is the parapsychologists, or at least those who have spent

their lives attempting to trap the alleged and elusive phenomena of ESP by using the methods of experimental psychology, who will find the book most disquieting. For if there is a central argument which emerges it is that there are facets of the Universe—large facets of it—which will resist and even reject study by the traditional methods of science. This, the book implies, is the principal reason for parapsychologists' signal lack of progress in attempting to 'prove' the existence of ESP. A discouraging argument I would have felt, and if anyone cared to apply it to other areas of science, the consequences for research could be pretty peculiar. Most scientists therefore will find this a glum rather than a heartening book. Perhaps 'glumly entertaining' would be the best way to describe it.

CHRISTOPHER EVANS

Thrips

Thrips: Their Biology, Ecology and Economic Importance. By Trevor Lewis. Pp. xv+349. (Academic: London and New York, November 1973.) £7.80: \$22.

THE Thysanoptera are a very interesting and important group of insects which are often ignored, apart from a few economically important species, such as the cereal thrips (*Limothrips cerealium*) or the onion thrips (*Thrips tabaci*). The literature on this group is therefore very scattered, and this book fulfills a long-standing need by bringing together a large part of this information into one place. It is not a book to be read from cover to cover, it is far too concentrated for that, but small doses from parts of it make fascinating reading for the general entomologist. It is a book that would form a valuable addition to the bookshelf of any entomology or ecology laboratory.

It tries to cover a very wide field indeed, often with rather scant information, as this is all that exists. It includes sections on the general biology of the group, rearing and sampling techniques, ecology and economic importance. This wide coverage, while revealing the enormous gaps in our knowledge, does mean that the available information from both the pure ecological literature and the applied literature is brought together, and this in itself makes the book worth having.

On the whole the book is well presented and the numerous appendices are particularly useful. There is however a number of annoying details. For example, I should have liked to see the plates distributed at relevant places in the text, instead of collected at the end, and why put Fig. 47 (a) on page 135 and Fig. 47 (b) and the legend overleaf on page 136? Comparison

would have been very much easier if they had been on facing pages. Errors and inconsistencies inevitably creep into compendia of this sort, but are very few in the present case, for example the corrected catch of thrips at Silwood Park is given on page 205 as "about 18,900 individuals" whereas over the page in Table 24 it is given as 19,900.

The author on the whole has done a good job and this book deserves to be widely used, and will no doubt retain its usefulness for a long time.

S. McNEILL

Marine mathematics

The Structure of Marine Ecosystems. By John H. Steele. Pp. x+128. (Blackwell Scientific: Oxford and London, 1974.) £2.75.

THIS "short work" (the author's words) consists of what, otherwise, might have appeared as two or three research papers, preceded by a general discussion about problems of investigating marine ecosystems and followed by some speculations about the impact of man's activities. The core of the book is the author's mathematical simulation of the pelagic ecosystem in the northern North Sea, presented as typifying open sea environments in temperate or sub-arctic waters.

As Dr Steele says, models of this kind reveal the lacunae in one's knowledge and so determine the kinds of research needed in the field and laboratory. In particular, the model helps to assess the validity of simplifications and identify the points at which greater detail is required.

The book serves these objectives admirably. In addition, it provides growing points for debate on topics such as the differences between terrestrial and marine ecosystems, or the significance of ecologists' icons such as diversity, stability, efficiency, food webs and food chains. In conclusion, Dr Steele argues that the most critical parts of marine ecosystems, and the points at which man's activities impinge most seriously, are at the levels of pelagic herbivores and benthic micro-organisms. He suggests that an increasing effort should be devoted to studies of structure and dynamics at these "lower levels" of the system.

The book is amply illustrated with results of Dr Steele's own research; there is an unexpected and probably unnecessary glossary, a short but valuable list of references and a totally inadequate index. It would be a valuable addition to the personal and institutional libraries of ecologists of all kinds, those who read equations as well as those who do not.

R. S. GLOVER

Stratigraphy

The Boreal Lower Cretaceous. Edited by R. Casey and P. F. Rawson. Pp. 448. (Proceedings of a symposium organised by Queen Mary College, London, and the Institute of Geological Sciences, September 1972; *Geological Journal* Special issues no. 5) (Seel House: Liverpool, 1973.) £9.75.

THIS book has been published with the commendable speed and in the attractive form that one has come to expect from the Seel House Press. Owing to its rather specialised nature it will, however, appeal to a more limited audience than the previous geological volumes in this series. The work is basically a well organised and presented compilation of stratigraphic and palaeontological data for a substantial sector of the northern hemisphere by thirty-two authors, mostly English but with a good representation of leading authorities from North America, France, Germany, Denmark, Poland and the Soviet Union. All the articles are in English, with brief summaries in French and German.

It would be both pointless and beyond the scope of a brief review to comment much on individual articles, which range from syntheses of reconnaissance work in large, relatively unexplored territories to reassessments of classic European localities, but I should perhaps single out Casey's substantial contribution on Jurassic-Cretaceous boundary beds in Europe as being especially welcome, because at last he has been persuaded to publish fully, with good illustrations of ammonites, the data on which are based his interesting conclusions on regional correlation. His views on the position of the Jurassic-Cretaceous boundary are well argued, but he has not managed to convince what one might term the Russian school of workers. A consensus on the subject seems as far away as ever. The overall tone of the articles is one of caution and sobriety, so that one greets with raised eyebrows the bold palaeogeographic reconstructions of H. G. Owen, based on the concept of an Earth expanding rapidly at least from the early Mesozoic to the present day. To paraphrase the molecular biologists, he has thereby offended against the 'central dogma' of current earth science, namely plate tectonics. For those with neither the time nor the inclination to plough through the detail, the editors provide a lucid summary of the proceedings in the concluding article.

The outstanding scientific problem concerning the boreal Lower Cretaceous is the cause of the differentiation of the ammonite, belemnite and to a lesser extent other invertebrate faunas from those of the more extensive

Tethyan Realm. It is welcome to have many pertinent distributional data brought together in one volume, but disappointing that very few authors have properly got to grips with the problem. All too many seem content to present conventional stratigraphic descriptions and discuss matters of correlation, all very necessary as a framework for environmental interpretation but decidedly unstimulating. Those who do try to account for faunal provinces have little new to offer. Most favour some sort of temperature control, while varying salinity and palaeogeographic configurations are proposed as subordinate factors. What I missed is an awareness of the great strides recently made in ecological understanding, with the introduction of new concepts such as environmental stability which may be highly relevant to the matter in question. Furthermore, nobody has made much progress in gathering quantitative data for particular fossil groups on density, diversity and distributional changes in relation to sedimentary facies, palaeolatitude and so on to test particular hypotheses. I sensed that many of the workers in the field are quite satisfied if they can describe and correlate their strata more accurately than before, and that they regard penetrating enquiries into cause and meaning as somewhat beyond their scope. Where problems of environmental interpretation are dealt with it is often in a somewhat token manner, as though they were of secondary importance. I hope that they are deterred by modesty rather than complacency or unimaginativeness. Perhaps the term used to describe their discipline is unduly inhibiting. In the wake of the dramatic advances being made in other branches of earth science there could be today rather less stratigraphy and more stratology.

A. HALLAM

Thin layer chromatography

Inorganic Chromatographic Analysis. By Jan Michal. Translation Editor: Julian F. Tyson. Pp. x+217. (Van Nostrand Reinhold Series in Analytical Chemistry.) (Van Nostrand: London and New York, March 1974.) £9.

THE original Czechoslovak edition of this book appeared in 1970 and the present English translation is therefore some four or five years out of date. With the dramatic advances in high performance column chromatography which have occurred since 1969 a book dealing almost exclusively with paper and thin-layer techniques (PC and TLC), and making no mention of high performance liquid chromatography or gas chromatography is now rather dated. This would not necessarily be

too serious if the author had given an authoritative account of the principles and current equipment, and if he had followed this by a critical appraisal of the application of the techniques in inorganic chemistry. But the treatment of principles is far from adequate: the newcomer would gain little or no insight into the key mechanisms of retention and dispersion of spots or bands in chromatography. He would have to look elsewhere to obtain up-to-date information on equipment; and he would have great difficulty in assimilating the mass of analytical information presented uncritically in the form of a long series of recipes for particular separations, and analyses.

This book cannot be recommended with any enthusiasm, but it may prove useful as a starting point for anyone who wishes to review PC and TLC methods before about 1968, say as a preliminary to devising high performance column methods for some specific separation; but even here its value is prejudiced by the poor subject index and the entire absence of an author index.

J. H. KNOX

Latin American physics

Reaction Dynamics. By F. S. Levin and H. Feshbach. Pp. viii+216. (Documents on Modern Physics.) (Gordon and Breach. New York, London and Paris, July 1973.) £6.65 cloth; £3.50 paper.

I WAS rather disappointed on opening this book to find that it is based on notes taken in some of the lectures of the Latin American School of Physics in 1968. Is it really worthwhile publishing a book in a field of research as rapidly changing as nuclear physics some five years after the event? After reading it I am still not completely convinced.

Levin, whose contribution takes up over three quarters of the book, has helped somewhat by giving a list of supplementary references up to 1972. He makes a survey of many of the "recent" developments in direct nuclear reaction theory, mainly in the description of low energy single particle transfer reactions, such as deuteron stripping. It reads rather like a good review article, with its thorough comparison of theoretical predictions with experiment in over a hundred figures. Most of these predictions were obtained using the distorted wave Born approximation (DWBA), which is the standard way of analysing experimental data, and some space is given to a discussion of the reliability of this as a tool for the extraction of spectroscopic factors. There are ample results on polarisation calculations and the extension of the

DWBA to coupled channel problems. As an alternative theoretical framework, Levin ends with a description of the much more controversial stripping theory of Butler and co-workers, comparing it with both the DWBA and experiment.

The article of Feshbach is very different in character. In just 45 pages it describes some of the features of the theory of nuclear reactions which the author and his collaborators at MIT have developed over the years. Central to this is the interplay between the prompt reactions (the direct reactions discussed by Levin) and the time-delayed ones involving the formation of compound nuclei. By the elimination of the time-delayed components, he shows how an optical model description of the prompt reactions can be formulated, stressing the importance of energy averaging in the scheme. This division into prompt and delayed reactions is of course oversimplistic and Feshbach finishes by considering reactions associated with characteristic times intermediate between these two extremes, such as doorway states. Unlike the Levin work, these feel much more like lecture notes, intended to give the student a broad overall impression of the field. COLIN WILKIN

Light on molecules

Organic Molecular Photophysics. Vol. 1. Edited by John B. Birks. Pp. xvii+600 (Wiley Monographs in Chemical Physics.) (Wiley-Interscience: London and New York, July 1973.) £13.50.

THIS book is the first of two volumes aiming to provide "comprehensive coverage of the field of organic molecular photophysics" and is intended to be complementary to *Photophysics of Aromatic Molecules* written by Birks. This volume contains ten articles, ranging in subject content from organic dye lasers to exciton interactions in organic solids, which have been written, on the whole, by unquestionable experts in their fields.

The quality of individual articles is extremely high. For example, the treatment of radiationless transitions by Henry and Siebrand presents one of the clearest discussions on this complicated topic. The fluorescence characteristics of aromatic molecules is dealt with thoroughly in two chapters, one by Stockburger, considering low pressure environments and the other, by Offen, dealing with fluorescence and absorption effects at high pressures. Triplet-triplet absorptions are discussed from an experimental standpoint by Labhart and Heinzelmann who compile much of the data available up to 1971. There is perhaps some overlap in tabular content between this chapter and "Photo-

physics of Aromatic Molecules". Dye lasers are dealt with from an experimental and occasional mathematical approach by Snavely; it is a pity that there is little mention in this article on their obvious uses in, for example, high resolution spectroscopy or fluorescence studies of vibronic states. Klopffner deals thoroughly with intermolecular exciplexes. The first chapter on the spectroscopy of pi-electronic states (by Birks) is introductory in nature and those familiar with the author's first work in this series will find the chapter less useful than the rest of the book. Other articles are written on diffusion-controlled rate processes (Alwattar, Lumb and Birks), electron photoejection from aromatic molecules in condensed media (Lesclaux and Jousset-Dubien) and exciton interactions in organic solids (Svenberg and Gaecintov).

I found it difficult to regard the collection of articles as "integrated" since the book reads more like a high quality "Advances in . . ." series. It could be argued that most of the review articles are available elsewhere although not in a single volume. I hope that the high standard of individual articles is maintained in volume 2. This volume would be a very useful addition to the library of individual researchers engaged in photochemistry and photophysics although it would not be essential. M. A. WEST

Atmospheric chemistry

Chemistry of the Lower Atmosphere. Edited by S. I. Rasool. Pp. xii+335. (Plenum: New York and London, 1973.) \$26.

THE book is a collection of six chapters on various aspects of atmospheric physics and chemistry by six different authors. Like all such collections it is variable in emphasis, standard and depth of treatment of subject. Because of this it is difficult to decide to what audience the book is aimed.

Dealing with each chapter in turn, the first of about 60 pages entitled "Role of Natural and Anthropogenic Pollutants in Cloud and Precipitation Formation", by Professor H. R. Pruppacher of UCLA, does no more than summarise what can be found in the standard texts on cloud microphysics. Although lip service is paid to the importance of cloud dynamics, that is the air motions leading to cloud condensation, and the interaction between cloud microphysics and dynamics, in fact the chapter is almost exclusively devoted to the microphysics of clouds. There is nothing on the possible modification of precipitation due to anthropogenic pollutants except a brief uncritical reference to some evidence that

urban areas experience more precipitation than their adjoining rural environment.

The next chapter, of about 40 pages on "Particulate Matter in the Lower Atmosphere" by Dr R. D. Cadle of NCAR, is a standard review of the main physical and chemical properties of the tropospheric and stratospheric aerosols. The sections on aspects of the atmospheric sulphur cycle and the scavenging processes for particulates in the troposphere duplicate more extensive treatments of these aspects in later chapters.

The third chapter is on "Removal Processes of Gaseous and Particulate Pollutants" by Dr G. M. Hidy, and is perhaps the best chapter in the book. It contains a scholarly and comprehensive treatment of the atmospheric scavenging processes.

"The Global Sulphur Cycle" is the title of the next section (24 pages) by Dr J. P. Friend. It summarises effectively the current state of knowledge of sources, sinks and reservoirs of sulphur. It is nice to see an author emphasising the limitations of his treatment.

The next contribution of 46 pages is perhaps the one closest to the central theme of the book, in that it deals with "The Chemical basis for Climate Change". The authors are Drs Schneider and Kellogg of NCAR. In it are described the qualitative arguments used to ascribe possible causal relationships between changes in atmospheric composition and climate. It also gives the reader some feel for the vast problems involved in any worthwhile quantitative modelling of climatic change.

The last chapter of 79 pages, by Dr C. D. Keeling, is devoted to the carbon dioxide cycle. It could be argued that the mathematical detail contained in this chapter is out of proportion to that in the rest of the book, but nevertheless it gives a very real insight into the various interacting factors concerned with the atmosphere, the oceans and the biosphere which control the concentration of CO₂ in our atmosphere.

In summary, the book has little to offer those workers actively engaged in the fields of atmospheric chemistry and meteorology. But at a time when more and more scientists outside the fields of atmospheric physics and chemistry are taking an interest in pollution matters, this book provides a useful introduction to some aspects of the subject. P. GOLDSMITH

Erratum

The price given for *Comparative Vertebrate Morphology* by Douglas and Molly Webster (reviewed in *Nature*, 249, 866: 1974) was incorrect; it should have been \$14.95 and £7.

science on television

One giant bore

by John Gribbin

ON JULY 20, just five years to the day since Neil Armstrong and Edwin Aldrin stepped on to the Moon, the BBC broadcast, with much huffing and puffing from its publicity machine, "Moonwalk One"; "... a NASA-commissioned documentary which records the moon landing adventure in minute and breathtaking detail", to quote from the *Radio Times*.

Minute the detail may have been, but the programme was hardly breathtaking, except insofar as the encouragement of yawning could be said to constitute a breathtaking spectacle. This was an opportunity missed on the grand scale; a film which should be preserved as a guide to producers in how not to present exciting science on television.

Shots of Saturn rockets taking off are now something of a cliché, but still sufficiently spectacular to justify inclusion in such a supposedly definitive feature. But there was little further material with a comparable visual impact, in spite of the existence of miles of such film in the NASA archives. The reason for this seems to have been a desire on the part of the makers to use only genuine Apollo 11 material, in line with the programme's title.

That would be laudable enough if the material was any good, but it simply is

not. To me, the most dramatic thing about the first Moon landing was that it was covered live on TV around the world. As far as I know, this was just about the only aspect of the mission not predicted by the science fiction writers.

But in retrospect those first pictures were not at all good. Since Apollo 11, we have become even more blasé about the TV coverage of Apollo, and used to pictures which are not just shaky grey blurs labelled "Live from the Moon", but rather in full colour and coming from a camera which is controlled from Earth so that we can watch the astronauts at work.

The answer, surely, would have been to produce a documentary not just about Apollo 11 but about the whole Moon adventure, using material from the later missions which is so ideally suited to the TV medium. As it was, whether through the paucity of good Apollo 11 material or as policy, "Moonwalk One" fell back on long, boring sections of padding. Shots of row upon row of the 'backroom boys' at Houston no doubt brought a warm glow to the hearts of their Moms and kids back home, but told us little of the excitement and importance of the mission. And views of good American citizens flocking lemming like to the beach to watch the launch will no doubt provide useful information for sociologists studying the phenomenon of how dramatically public interest in the Apollo

programme waned after the first mission. But that, surely, was not what the programme was all about.

Even the genuinely exciting parts of the programme—perhaps one sixth of its ninety-minute length—were marred by obtrusive and inappropriate background music in the sub-2001 tradition, and the few graphic aids used were not in the same league as the famous 'simulation' which filled in the gaps when the live Apollo broadcasts were being made. Some heretics, indeed, have been known to argue that the simulation was, visually, the best part of those missions; they have a point, but perhaps those of them who watched "Moonwalk One" were mollified by the segment of old Buck Rogers' film clips. These provided the dramatic highlight of the programme, which declined monotonically in interest for its final hour.

The tone of the whole proceedings is perhaps best summed up by noting that the *Radio Times* even failed to quote Armstrong's famous and carefully rehearsed *ad lib* correctly: "One small step for man, one big step for mankind", they said. None out of ten for effort; whoever wrote that should be encouraged to take a giant leap.

But faith in the American way of life and a genuinely breathtaking spectacle was provided to soothe the heated brow within an hour of the end of "Moonwalk One". "Sergeant Bilko", starring Phil Silvers—now, that was worth staying up late for.

obituary

L. C. Dunn

LESLIE C. DUNN, one of America's oldest and most influential geneticists, died unexpectedly on March 19, 1974 still active in a scientific career which spanned more than sixty years, encompassing almost all of the development of modern genetics. His early work established some of the first cases of linkage in mammals, and he was the first to demonstrate similar degrees of linkage between what seemed to be the same kinds of mutations in two distinct species, mice and rats. This

led him to recognise that Mendelian analysis could yield clues to genetic homologies and relationships among species, and thus be applicable to understanding the evolution of natural populations, and formed the basis of his life-long interest in what was to become population genetics. His chief work, however, continued to be genetic analysis, and the major efforts of his laboratory centred on the analysis, by both genetical and embryological methods, of mutations expressed in early stages of embryonic life. The greatest part of this work concerned one extensive series of mutations at a

complex region (T) in the mouse, where he and his colleagues analysed a number of mutations which identified genetic factors essential to early embryonic development. After the elements of this system had been identified in laboratory mice, his interest in natural populations led him to examine feral groups of *Mus* for the presence of these mutations. Most of such populations in North America and Europe proved to be polymorphic for variant genes, usually embryonic lethals or semilethals, belonging to the T series. In searching for explanations of how lethal genes could be main-

tained at high frequencies in natural populations, he found that all alleles at this locus found in the wild were transmitted by heterozygous males to 90–99% of their offspring. This evolutionary force—genetic selection—was thus more powerful than the force of natural selection which would eliminate homozygous embryos.

In addition to these purely scientific activities, Professor Dunn was convinced that relations between nations and cultures could be improved by using scientific collaboration as a bridge. As an army officer returning from Europe in 1919, he helped to provide agencies in the Soviet Union with scientific and technical literature useful in the new state which arose from the revolution of October, 1917. He visited the Soviet Union in 1927 at the time of the 10th Anniversary of the Revolution and thereafter remained in close contact with the active school of genetics there. During the Second World War he was President of the American–Soviet Science Society which undertook the exchange of scientific and technical publications. The same interest in international scientific collaboration was responsible for his part in organising facilities in the USA for enabling scholars displaced by the Nazi takeover of 1933 to continue their work in American universities.

What began then as the Emergency Committee for German Scholars became, as fascism spread, The Emergency Committee in Aid of Displaced

Scholars, and he served as a member of its Executive Committee from 1933 until its dissolution in 1947.

Always interested in questions of race, he began in 1933 actively to apply biological ideas to social and political questions, and to speak and write about heredity in relation to racial prejudice in ways designed for the general public. This led, in collaboration with his friend and colleague, Th. Dobzhansky, to the publication in 1946 of *Heredity, Race and Society*, a book which has now appeared in almost all languages, and subsequently in 1951 to a Unesco Report, *Race and Biology*; both of these were fundamental in establishing the then novel view of human races as populations differing in the relative frequencies of genes shared by most populations.

These activities were an expression not only of his interest in science as an instrument of international education, but of his view that, to be effective, efforts of this sort had eventually to achieve a political forum. Other expressions of the latter view can be found in advisory and consultative work beginning in 1940 in aid of efforts to devise a means for the support of science by the United States Government. He was involved in early drafts of legislation which are predecessors of that which finally brought about the creation of the United States National Science Foundation in 1950.

Professor Dunn received the ScD from Harvard University in 1920, and

after a time at the Agricultural Experiment Station in Storrs, Connecticut, joined the Faculty of Columbia University as Professor of Zoology in 1928. At the time of his death he was Emeritus Professor and Senior Research Scientist at Columbia. He was a member of the National Academy of Sciences (USA), the American Academy of Arts and Sciences, the American Philosophical Society, the Norwegian Academy of Arts and Sciences, and the Academia Patavina; he had been President of the Genetics Society of America, the American Society of Naturalists, and the American Society of Human Genetics. He also served on many editorial boards, and was for some years managing editor of *Genetics*, and later of the *American Naturalist*, the oldest biological journal in the United States. In addition to *Heredity, Race and Society*, his books include *Principles of Genetics*, *Heredity and Evolution in Human Populations*, and *A Short History of Genetics*.

His associates in all his various endeavours will remember him for his stubborn integrity, his sure and immediate grasp of the essentials of any problem or situation, and his complete freedom from pretence or self-importance of any kind. Many of us who knew him will continue to measure our own success by the degree to which we live up to the standards which he set.

Announcements

Awards

The **Barclay Prize** of the **British Institute of Radiology** has been awarded to **Godfrey Hounsfield** and **James Ambrose**.

Corrigendum

In the article "Batesian mimicry without distastefulness" by D. O. Gibson (*Nature*, 250, 77; 1974) the quotation in the penultimate sentence should read . . . in the population 'the selective disadvantage of being common begins to outweigh the advantage of being conspicuous' and predation will . . .

Reports and Publications

Great Britain

British Antarctic Survey. Scientific Reports, No. 66: Crustal Structure of the South Shetland Islands and Bransfield Strait. By W. A. Ashcroft. Pp. 43. (London: British Antarctic Survey, Natural Environment Research Council, 1972.) £2.25 net. [136]

The Grassland Research Institute. Annual Report for 1973. Pp. 88. (Hurlay, Maidenhead: Grassland Research Institute, 1974.) £1.50. [176]

The Kent Incorporated Society for Promoting Experiments in Horticulture. East Malling Research Station Report for 1973 (1st October, 1972 to 30th

September, 1973.) Pp. xii + 240. (East Malling, Maidstone: East Malling Research Station, 1974.) £1.50. \$5. [176]

University of Oxford. Tenth Annual Report of the Delegates of the Science Area for the year ending 31st July 1973. Pp. 149. (Supplement No. 4, to the *University Gazette*, April 1974.) (Oxford: The University, 1974.) £1. [176]

Medical Research in too Important to the left to the Researchers. By Professor W. S. Peart. (Lecture given at the Royal Institution, London, on 18th October, 1973, to mark the 25th Anniversary of the Foundation of the Glaxo Volume). Pp. 16. (Greenford, Middx.: Glaxo Laboratories, 1974.) [176]

The British Institute of Radiology. Annual Report 1973/1974. Pp. 10. (London: The British Institute of Radiology, 1974.) [196]

Research Fields in Physics at United Kingdom Universities and Polytechnics. Pp. xv + 343. (London and Bristol: The Institute of Physics, 1974.) £7. \$17.50. [196]

First Report of the Advisory Board for the Research Councils. Pp. 28. (Cmd. 5633). (London: HMSO, 1974.) 20p net. [206]

University Grants Committee. Statistics of Education 1971. Vol. 6: Universities. (Department of Education and Science Series.) Pp. xxxvi + 149. (London: HMSO, 1974.) £2.90 net. [206]

Building Research Establishment Digest No. 166: European Product Approval Procedures—I. Pp. 4. (London: HMSO, 1974.) 5p. [206]

Report on Cetacea Stranded on the British Coasts from 1948 to 1966. By F. C. Fraser. Pp. 65 + 9 maps. (London: British Museum (Natural History), 1974.) £3. [206]

Research and Development in France. Pp. 36. (London: Ambassade de France, Service Scientifique, 1974.) [216]

Other countries

World Health Organization—International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol. 4: Some Aromatic Amines, Hydrazine and Related Substances, N-nitroso Compounds and Miscellaneous Alkylating Agents. Pp. 286. Sfr. 18. Vol. 5: Some Organochlorine Pesticides. Pp. 241. Sw. fr. 18. (Geneva: WHO; London: HMSO, 1974.) [186]

Smithsonian Contributions to Botany, No. 13:

Swollen-Thorn Acacias of Central America. By Daniel H. Janzen. Pp. 131. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$2.35. [186]

Smithsonian Studies in History and Technology, No. 24: Wheels and Wheeling—The Smithsonian Cycle Collection. By Smith Hempstone Oliver and Donald H. Berkebile. Pp. v + 104. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.90. [186]

Onchocerciasis: Symptomatology, Pathology, Diagnosis. Edited by A. A. Buck. Pp. 80. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 12. [186]

Guidelines for the Laboratory Diagnosis of Cholera. Prepared by the WHO Bacterial Diseases Unit. Pp. 23. (Geneva: WHO; London: HMSO, 1974.) Sw. fr. 5. [186]

Alfred P. Sloan Foundation. Report for 1973. Pp. viii + 79. (New York: Alfred P. Sloan Foundation 630 Fifth Avenue, 1974.) [186]

Smithsonian Contributions to Zoology, No. 156: A Revision of North American *Capitophorus* Van der Goot and *Plectrichophorus* Börner (Homoptera: Aphididae). By Leonila Alzate Corpuz-Raros and Edwin F. Cook. Pp. iv + 143. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$2.25. [196]

World Meteorological Organization. Technical Note No. 133: An Introduction to Agrotoclimatology. By L. B. MacHattie and F. Schnelle. Pp. xii + 131. (Geneva: WMO, 1974.) [196]

Office de la Recherche Scientifique et Technique Outre-Mer. Collection Travaux et Documents de l'ORSTOM. No. 28: Kinkala—Etude d'Un Centre Urbain Secondaire au Congo-Brazzaville. Par Alain Auger. Pp. 132. (Paris et Bondy: ORSTOM, 1973.) 42 francs. [196]

Australian Academy of Science. Science and Industry Forum. Report No. 8: The Future Education of Scientists. (Papers delivered at the 13th Forum Meeting, 11 February 1973.) Pp. 53. (Canberra: Australian Academy of Sciences, 1973.) [206]

World Health Organization. Technical Report Series, No. 546: Assessment of the Carcinogenicity and Mutagenicity of Chemicals—Report of a WHO Scientific Group. Pp. 19. Sw. fr. 4. No. 548: Planning and Organization of Geriatric Services—Report of a WHO Expert Committee. Pp. 46. Sw. fr. 5. (Geneva: WHO—London: HMSO, 1974.) [216]

Australian Academy of Science—Descriptive Brochure. Pp. 27. (Canberra: Australian Academy of Science, 1974.) [216]

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APPOINTMENTS VACANT
**UNIVERSITY OF ALBERTA
DEPARTMENT OF ENTOMOLOGY**

Applications are invited for the position of ASSISTANT PROFESSOR, effective April 1, 1975. Qualifications are Ph.D. with postdoctoral experience in insect physiology and interest in aspects of this field applicable to agricultural or forest entomology. Duties include teaching courses in general or applied entomology and insect physiology, the development and direction of a research program and supervision of graduate students in insect physiology and in application of some aspect of physiology to agricultural or forest entomology. Maximum starting salary \$14,043.

Please send full curriculum vitae and names of 3 referees by October 31, 1974 to: Dr George E. Ball, Chairman, Department of Entomology, 260 Agriculture Building, University of Alberta, Edmonton, Alberta T6G 2E3. (297)

**NATIONAL VEGETABLE RESEARCH
STATION**
PLANT PATHOLOGIST

There is a vacancy for an officer to assist with research on vegetable virus diseases. Appointment will be as a Scientific Officer (£1,702-£2,675) or Higher Scientific Officer (£2,461 to £3,371), grade and starting salary being determined according to qualifications and experience. Superannuation scheme with allowance to offset personal contributions. The minimum qualification is a degree (or equivalent) in Botany, or horticultural or agricultural science. Candidates for appointment as 'H.S.O.' will normally be expected to have at least five years' postgraduate experience, including experience in plant virus techniques: training in such work will however be given to a less-experienced appointee. Full particulars and application form (to be returned by August 22, 1974) from the Secretary, N.V.R.S., Wellesbourne, Warwick CV35 9EF. (417)

**SIR GEORGE WILLIAMS
UNIVERSITY**

Montreal, Quebec, Canada

**DEPT. OF BIOLOGICAL SCIENCES
THREE FACULTY POSITIONS**

Three faculty positions available, July 1, 1974: 1 Associate Professor (\$15,400 to \$17,000), 2 Assistant Professors (\$12,000 to \$15,000) depending upon experience and qualifications. Research orientated persons qualified to teach in one or more of the following areas will be considered: Embryology, Plant Ecology, Biostatistics, Introductory Biology. Ph.D. required. Send resume and names of 3 references to:

Dr. H. Enesco, Chairman, Dept. of Biological Sciences, Sir George Williams University, Montreal, Que. H3G 1M8. (429)

UNIVERSITY OF OXFORD**DEPARTMENT OF ZOOLOGY**

There is a vacancy for a research assistant to work on some aspects of insect neurophysiology with Dr. P. L. Miller for one year from October 1974. Applicants should have a good Honours Degree in Zoology. Salary in accordance with age and experience. Applications, with names of two referees, to Dr. P. L. Miller, Dept of Zoology, South Parks Road, Oxford. (481)

Animal Studies Diagnostics Research Radio Pharmaceuticals

Our client is a world leader in research based materials for medicine, research and industry. Continuing commercial success has created the need for two additional research and development scientists. Each should be capable of designing, executing and analysing experiments and of leading a small team. Pension; housing assistance. Salary up to £3,500. Please telephone (01-629 1844 at any time) or write - in confidence - for information to Dr. Clive Jones quoting appropriate reference:

Animal Autoradiography

To establish an effective animal testing unit and to take responsibility for individual research projects. Expertise at both microscopic and macroscopic levels is called for together with a proven record of scientific ability in research or research development. The successful candidate, 25 to 35, could come from a number of disciplines but biological-biochemical expertise would be particularly welcome. Ref. U.5593.

Animal Physiology

Leading a small team he would initially strengthen and extend existing facilities and procedures. Candidates, aged between 25 to 35, will ideally have, in addition to a degree in Pharmacology, experience in metabolism and appreciation of surgical techniques or a biochemical background. Ref. U.5594.



Management Selection Limited

17 Stratton Street, London, W1X 6DB.

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(504)

Food Research Biochemist

RHM Research Limited at High Wycombe is the central research and development unit of the Ranks Hovis McDougall Group. A large team of scientists, accommodated in modern and well-equipped laboratories and pilot plants, is working on a wide variety of projects covering the investigation and evaluation of new food sources and the development of new food products and processes.

A vacancy has arisen in the Biochemistry Department for a scientist to work on a wide range of research and development projects in the field of food protein biochemistry and technology, with special emphasis on vegetable proteins. The successful applicant will also be expected to be aware of development in these areas and those peripheral to them.

Applicants should be completing, or have recently completed, a Ph.D. in food vegetable protein technology, food protein processing or fat-protein interactions in food-stuffs. They should possess a degree of creative flair along with the ability to accept commercial and practical constraints. They should also be willing to work as a member of a team and be able to communicate effectively both verbally and in writing.

This position will carry a salary of around £2,300 per annum along with excellent terms and conditions of employment. Please apply, quoting reference JT/SSO, to:



The Personnel Officer,
The Lord Rank Research Centre,
Lincoln Road,
High Wycombe,
Buckinghamshire HP12 3QR.

(505)

Theoretical Biology Group UNIVERSITY OF UTRECHT

Applications are invited for a lectureship in Theoretical biology. Biologists with strong mathematical background, or mathematicians with strong biological background, could apply at the post on post-doctoral level.

The lecturer will participate in the teaching activities of our group. Research is expected primarily on mathematical models of cellular and multi-cellular processes but other areas of theoretical biology are not excluded.

Appointment by September 1974.

Salary according to qualifications, not less than Pf.2,300, per month. Further information can be obtained from Prof. A. Lindenmayer, Theoretical Biology Group, University of Utrecht, Heidelberglaan 2, Transitorium 11, Utrecht, The Netherlands.

(509)

THE EDINBURGH SCHOOL OF AGRICULTURE AGRICULTURAL ENTOMOLOGIST

Applications are invited for a post in the Crop Health Department from candidates with post-graduate experience in Entomology/Nematology.

Salary scale (under review) Grade III £2,233 to £3,895 plus a cost-of-living safeguard and a non-pensionable supplement of 41% to offset superannuation contributions.

Further particulars and application form from The Secretary, The Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG. (468)

THE ROYAL FREE HOSPITAL HAMPSTEAD DEPARTMENT OF SURGERY

This Department now being established in the New Royal Free Hospital requires the following to participate in projects including studies of blood flow, organ preservation and transplantation, cryosurgery and liver surgery.

BIOPHYSICIST

Whose duties will include supervision and development of all physiological apparatus used in the research projects. Qualifications: Degree in Physics or other appropriate Science subject. Commencing salary £1,923 to £2,820 dependent upon qualifications and experience.

MEDICAL PHYSICS TECHNICIAN III

Whose duties will include maintenance and use of department research monitoring equipment including multichannel recorders, transducers and oscilloscopes, amongst other equipment. Qualifications: Appropriate Science Degree or HNC, ONC or HND. Salary: £1,845 to £2,337 depending on experience and qualifications.

Further details from the Professor of Surgery, The Royal Free Hospital, Pond Street, London NW3 2QG. Tel: 01-794 0500.

Application forms (to be returned by August 22) from the Assistant Personnel Officer 21 Pond Street, London NW3 2PN. Tel: 01-794 0431, Ext. 2. (493)

THAMES POLYTECHNIC SCHOOL OF BIOLOGICAL SCIENCES

Research Technician

Applications are invited for the post of research technician in the Division of Physiology and Cell Biology. The successful applicant will be expected to work with Dr. D. J. Beadle on the distribution of lysosomal enzymes in normal and virus-transformed cell lines. Experience in cell culture methods would be an advantage. The appointment will be for one year in the first instance with a salary of £1,478 per annum.

Further particulars and form of application may be obtained from the Secretary, Thames Polytechnic, Wellington Street, London, SE18 6PF, to whom completed applications should be returned by August 13, 1974. (498)

APPLIED SCIENTIST

The Radiochemical Centre is a leading World supplier of radioactive products many derived from reactors.

We have a vacancy for a qualified Scientist to join the Isotope Production Unit at Harwell. The work will involve the development of methods for the production and control of radioactive materials in high-flux reactors, and for their subsequent control and use.

The successful candidate should have a graduate level qualification in physics or engineering and a practical approach to problem solving. The salary will be at least £2,600 and is subject to a Threshold Agreement. Generous leave allowance and contributory superannuation scheme. Access to the staff canteen, social clubs and other facilities of the large UK AEA site at Harwell. Assistance with housing may be available.

For further details of the post dial 100 and ask the operator for FREE PHONE 3557 or write with brief details of career and qualifications to Mr. Martyn Bishop,



The Radiochemical Centre

Amersham

Bucks

GEOPHYSICIST

The Lamont-Doherty Geological Observatory of Columbia University announces a position with the seismology group as a Research Associate or Senior Research Associate. Candidates must have a strong background in seismic instrumentation and data analysis and will be expected to lead existing and initiate new programs of research involving observations of earth strain, tilt, and long-period seismic waves. Qualified candidates are invited to submit a resume of their qualifications, publications, names and addresses of three references by August 15, 1974. (460)

UNIVERSITY OF SURREY DEPARTMENT OF CHEMISTRY POSTDOCTORAL/POSTGRADUATE APPOINTMENT

Applications are invited for a post available from October 1, 1974 to work on the influence of iron salts on the products and mechanism of the nitrosation of organic compounds. Depending on the qualifications of the successful applicant, the appointment may be made at postdoctoral level, or at postgraduate level permitting study for a higher degree. A postdoctoral appointment would be made at the lowest point of the university lecturer scale with F.S.S.U., and a postgraduate appointment at S.R.C. rates (£695 plus fees). The minimum qualification for the latter appointment is a lower Second Class Honours Degree. Applications with a brief curriculum vitae and the names and addresses of two referees should be sent as soon as possible to Dr L. F. Larworthy, Department of Chemistry, University of Surrey, Guildford, Surrey, GU2 5XH. (494)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH DEPARTMENT OF BOTANY AND MICROBIOLOGY

RESEARCH ASSISTANT required for quantitative studies on growth and photosynthetic activity of individual leaves of plants during vegetative growth. Graduates in Botany, Agricultural Botany and related subjects, or Mathematics or Statistics considered. Appointment for one year, renewable up to three years.

Commencing salary: £830 per annum.

Further particulars and forms from the Registrar. (497)

THAMES POLYTECHNIC SCHOOL OF BIOLOGICAL SCIENCES DIVISION OF ENVIRONMENTAL BIOLOGY

Research Assistant

Applications are invited for a RESEARCH ASSISTANTSHIP in this Division to undertake studies on the microbial dissimilation of the halogenated phenoxyacetate herbicides.

Applicants should hold a good honours degree in Microbial Biochemistry, Biochemistry or Chemistry. Industrial experience and some training in Microbiology, are preferable though not essential.

The successful candidate will receive a starting salary of £1,544 per annum, plus payments under the threshold agreement, and will be expected to register for a higher degree.

Further particulars and form of application may be obtained from the Secretary, Thames Polytechnic, Wellington Street, London, SE18 6PF, to whom completed applications should be returned by August 20, 1974. (499)

Workshop Technician

Applications are invited for an experienced technician to service existing equipment and to help in the development and construction of new apparatus; applicants should hold an ONC or HNC or be an ex-Services Artificer and be familiar with electro-mechanical instruments for atmospheric sampling and for physiological measurement, as well as general laboratory apparatus; he should be able to work independently and would be expected to work on various materials and do his own machining and sheet metal fabrication and to work on instruments associated with a large exposure chamber and sampling sites away from the workshops. We offer 3 weeks and 3 days annual leave plus privilege and public holidays and salary according to age and experience rising to £2,451 per annum.

Applications to The Director, Medical Research Council Air Pollution Unit, St Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ, Tel. No. 01-253 1537.

MRC

Medical Research Council

(510)

Bass Production Limited

A member of the Bass Charrington Group

require an

Analytical Chemist

The successful candidate will assume responsibility for analytical work in the Brewery. This key position involves the development of the analytical section, monitoring its performance, and managing a number of staff in a shift orientated department.

Although responsibility primarily will be of a routine nature, adaptability of approach will be sought, as continually developing production processes will require modification of existing methods.

Applicants must possess a degree in chemistry or equivalent qualifications and a knowledge of biochemistry would be an advantage. Management or supervisory experience in quality control laboratories in the brewing industry is equally important as formal qualifications.

The position offers a first class opportunity to acquire experience in the latest developments in brewing plant and production techniques.

In addition to an attractive salary and fringe benefits, help may possibly be given in certain cases for housing to be available in the Runcorn New town.

Please write giving brief details, of age and experience and present salary to:

**The Recruitment Manager,
Bass Production Limited,
Whitehouse Industrial Estate,
Runcorn, Cheshire**

(553)



Resource Economist FISHING INDUSTRY

As part of a general review of the fishing industry in the Northern Territory, the Dept. of the Northern Territory requires the services of a

Consultant Resource Economist

To examine data on existing and potential fish resources around the Northern Territory and to advise on future development strategies for the industry in the following situations:

- (a) Maximum development within a framework of specified controls, such controls to include current Northern Territory Legislation and Policy, together with other controls imposed on the industry by Australian Government policies in respect of Taxation, Immigration, Transport, Education, etc.
- (b) Unrestricted maximum development.

The Study is to be restricted to resources for which background information has been collated and does not extend to Aquaculture. In particular, the Consultant should be prepared to comment on:-

- Desirable rates of development to a given level of availability of particular resources.
- Management strategies to achieve these rates.
- Incentives if any, which may help achieve these rates or ultimate levels.
- The economic advantages or disadvantages of diversification within the fishery.
- Existing wage structures and modifications which may arise under guidelines proposed from the study.

Applicants should be prepared to commence study in 1974 for completion over a period of approximately three months, and it is expected that the successful applicant will spend an appropriate amount of time in the Northern Territory in consultation with local industry.

Further details including a synopsis of the fishing industry of the Northern Territory and a detailed list of all available source material are available from:-

The Executive Member, Northern Territory Fishing Industry Review Committee, P.O. Box 2783, Darwin Northern Territory, Australia 5794.

Applications should include appropriate personal details, summary of experience and any relevant publications, period available, together with full estimate of fees and costs including costs of travelling to Darwin in the Northern Territory of Australia, living expenses, Office accommodation and transportation.

Closing date: Friday 20th September 1974.



Address Proposals:
Assistant Director (Supply),
Department of the Northern Territory,
P.O. Box 4075,
Darwin Northern Territory, Australia 5794.

Telephone No: 819122
Telex Code NTASTOR 85062 Australia.

All enquiries will be accepted as potential proposals.

THE UNIVERSITY OF NEWCASTLE UPON TYNE CHAIR OF GEOGRAPHY

Applications are invited for the post of Professor of Geography. The vacancy arises as a result of the resignation from September 30, 1974, of Professor J. W. House, to take up the Halford Mackinder Professorship of Geography at the University of Oxford. Salary in accordance with the Professorial Scale (£5,973 by £96 to £6,069 by £195 to £6,849 per annum).

Further particulars may be obtained from the Registrar, the University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, should be submitted not later than September 14, 1974. (495)

THE UNIVERSITY OF SHEFFIELD SENIOR LECTURER IN CHEMICAL PATHOLOGY

Applications are invited from medically or non-medically qualified persons for the above post, tenable from a date to be arranged. Research interests of the department include: peptide hormone interaction with cell receptors and subsequent cyclic nucleotide-mediated effects; secretion and action of parathyroid hormone; and certain aspects of the endocrinology of cancer. It is anticipated that the successful candidate will have a background in one of these areas or appropriate basic experience which could be applied. An appropriately qualified person may be considered for honorary consultant status by the Area Health Authority. Salary ranges (non-clinical) £4,707 to £5,844; (clinical without honorary consultant status) £4,917 to £6,213; (clinical with honorary consultant status) £5,085 to £7,599. Further particulars from the Registrar and Secretary, the University, Sheffield S10 2TN to whom applications (8 copies) should be sent by September 9, 1974. Please quote reference R 120/G. (500)

UNIVERSITY OF LEEDS DEPARTMENT OF PURE AND APPLIED ZOOLOGY

Applications are invited from graduates for a S.R.C. RESEARCH ASSISTANT-SHIP to work on the control of ventilation in dragonflies. This post is tenable for a period of up to three years from October 1, 1974. The starting salary will be £1,449 per annum. Applications should be sent to Dr. P. J. Mill, Department of Pure and Applied Zoology, University of Leeds, Leeds LS2 9JT, from whom further particulars may be obtained. Closing date August 17, 1974. (503)

UNIVERSITY OF HULL

A GRADE 4 RESEARCH TECHNICIAN is required for work in the Biomedical Unit. The successful applicant will help to set up a new laboratory and thereafter assist with: care of animals of various species; operative procedures; fixation of specimens; dissection and sectioning for gross anatomical study and optical and electron microscopy. Experience in the histology of central nervous tissue would be an advantage. The post is tenable for three years from October 1. Salary on scale £1,848 to £2,163 p.a. plus £104 p.a. threshold payment. Applications, giving details of age and experience and the names of two referees to the Technical Staff Officer, University of Hull, by Friday, August 9, 1974, quoting Ref. TBU/1. (501)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY TECHNICIAN

Applications are invited for this new post. The successful candidate will be required to take special responsibility for the running and maintenance of the Department's research apparatus (and if possible help design and build new equipment), to organise and supervise the development, costing and preparation of undergraduate classes in physiology and biochemistry, and to deputise for the Chief Technician. The post offers plenty of scope for initiative.

Candidates should be qualified to H.N.C. or degree level in a Biological subject or chemistry. Salary within a range up to £2,874 per annum (plus threshold payments) according to qualifications and experience. Application forms which should be returned by September 1 may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX.

Quote ref RV/276150/N/

(502)

THE UNIVERSITY OF SHEFFIELD C.A.S.E. AWARD IN PHYSICAL CHEMISTRY OF MICELLES

Applications are invited from good honours graduates, or those about to graduate, in chemistry or chemical physics, for a CASE award under joint supervision of Dr I. A. McLure (the University of Sheffield) and Dr D. G. Hall (Unilever Research, Port Sunlight). Project will involve initially a study of distribution of suitable solutes between an aqueous solution of a surfactant and an immiscible and unsolubilized solvent with object of gaining improved understanding of nature of interior of micelles. Applications, (curriculum vitae and names of two referees) to Dr I. A. McLure, Department of Chemistry, The University, Sheffield S3 7HF, Tel. 78555 (S.T.D. 0742), from whom further details can be obtained. Please quote reference R119/G. (506)

BERNHARD BARON MEMORIAL RESEARCH LABORATORIES

Queen Charlottes Maternity Hospital
Goldhawk Road, London W6 0XG

DEPARTMENT OF MICROBIOLOGY

Applications are invited from graduates with 1st or 2nd class Honours Degrees for the post of Senior Microbiologist (Terms and conditions of service Whitley Council A Scale, salary £2,964 p.a. to £3,843 p.a. plus £126 p.a. London Weighting plus threshold payments).

Applicants must have previous experience of medical microbiology.

Applications to the House Governor at the above address, naming three referees. (507)

THE GEOLOGICAL SURVEY OF GREENLAND

Geophysicist

A Geophysicist is required for the Geological Survey of Greenland. Candidates should have at least five years' experience in the various branches of exploration geophysics, particularly seismic interpretation, and should have a good command of English. The person appointed will be based in Copenhagen and will be required to plan and to participate in field projects in Greenland.

The appointment will be on renewable contract and will be pensionable. The salary will be according to Danish Government scales.

Applications should be accompanied by a detailed curriculum vitae, an indication of the approximate salary required, and the names of two referees, and should be sent before September 15 to: The Director, The Geological Survey of Greenland, Øster Voldgade 10, DK-1350 Copenhagen K, Denmark. (516)

RESEARCH OFFICER

Applications are invited for the post of Research Officer with the International Project in the Field of Food Irradiation, located in Karlsruhe, Federal Republic of Germany.

The successful candidate will be required to carry out research into the toxicological methodology of wholesomeness testing as applied to irradiated foods. The precise nature of the research will be determined according to the specific areas of expertise of the candidate selected for appointment who will assist in designing the research programme. Of particular interest to the Project are the problems associated with mutagenicity, carcinogenicity and teratogenicity testing.

Qualifications: Ph.D. or equivalent: several years of practical experience in toxicology or a closely related science. Fluency in English is essential and a working knowledge of German and/or French would be an advantage.

Conditions of service: The appointment will be on a yearly contract basis with the possibility of renewal, as a consultant to the Organisation for Economic Co-operation and Development. Salary, depending on qualifications and experience will be within the range US \$14,000 to \$19,000 net per annum (including expatriation and other allowances according to nationality and family circumstances).

Applications, accompanied by curriculum vitae to be made in writing by September 16, 1974 to the Project Director, International Food Irradiation Project, Institut für Strahlentechnologie, 75 Karlsruhe, Postfach 3640, Federal Republic of Germany. (514)



Wellcome

Research Scientist

This is an opportunity for a Ph.D or Good Honours Graduate with research experience in a biological discipline to join a new Research team being set up at The Wellcome Research Laboratories, Berkhamsted.

The work will involve the initiation of a research programme to investigate the effects of formulation and route of administration on absorption and distribution of drugs in animals.

We offer excellent conditions of employment, including help with re-location expenses where necessary, and real career prospects. Our laboratories are modern and well equipped, situated in pleasant countryside close to Berkhamsted, which is a small country town about 35 miles from Euston, London.

If you are interested please write or telephone for an application form quoting reference PA. 60, to:

**Mr. R. P. Woolridge, Senior Personnel Officer,
The Wellcome Foundation Limited,
Ravens Lane, Berkhamsted, Herts. HP4 2DY.
Tel: Berkhamsted 3333.**

(550)

ORGANIC CHEMIST

Industrial Research Chemist required for studies in synthetic chemistry.

Applicants should have First or Upper 2nd class Honours degree or equivalent, and preferably 1 - 2 years relevant experience. The assignment will be for 18 months in the first instance although the appointment is envisaged to be for at least five years, initially in the south-west of England. The salary will probably be in the

range of £2,500 to £3,000 p.a. according to qualifications and experience.

Reply, in confidence, with curriculum vitae, to Position Number ABO 651 Austin Knight Limited, Hagley House, Hagley Road, Birmingham B16 8QG.

Applications are forwarded to the Client concerned, therefore Companies in which you are not interested should be listed in a covering letter to the Position Number Supervisor.

AK ADVERTISING

UNIVERSITY OF DAR ES SALAAM—TANZANIA

Applications are invited for the following posts tenable as soon as possible:—

PROFESSOR IN PLANT BREEDING

Candidates should have considerable research and/or teaching experience in Plant Breeding and Genetics. The appointee will be required to lecture in some aspects of genetics and Plant Breeding to undergraduate and postgraduate students. He will be expected to plan, design and carry out with a team of breeders, selective plant breeding programmes. He should be able to apply advanced statistical and mathematical techniques for detailed genetic analysis and interpretation of results. He will also be required to collaborate with and advise other scientists in the Department.

ASSOCIATE PROFESSOR IN HORTICULTURE

Candidates should have a good honours degree in horticulture and postgraduate qualifications in horticulture or Crop Physiology. They should have considerable teaching and/or research experience in horticultural crops. The appointee will be expected to give lectures to undergraduate and postgraduate students in plantation crops, vegetable and ornamental crops. He will also be required to conduct research and supervise postgraduate students in his area of specialisation.

LECTURER/SENIOR LECTURER IN CROP PRODUCTION

Candidates should have at least an M.Sc. in Agronomy and some research experience and/or university teaching experience. Previous experience in tropical crops (field crops or plantation crops) would be an advantage. The appointee will be required to teach Annual Crops, Climatology, Weeds and Weed Control, to undergraduate and postgraduate students, and conduct research in his area of specialisation.

LECTURER/SENIOR LECTURER IN BIOMETRY

Candidates should have a good degree in mathematical statistics or Agriculture with postgraduate training in applied statistics and computer science. The appointee will be required to teach Biometry to undergraduate and postgraduate students. He will be expected to advise postgraduate students and academic staff in the Faculty on experimental designs, computer programming and utilisation and the interpretation of results.

LECTURER/SENIOR LECTURER IN PASTURE AGRONOMY

Candidates should have a good degree in Agriculture with postgraduate training and research experience in Pasture Agronomy. Experience in University teaching/research on pasture production in sub-tropical or tropical countries would be an advantage. The appointee will be expected to teach undergraduate students in various aspects of pasture production and range management. He will also be expected to undertake research on pastures.

RESEARCH FELLOW/SENIOR RESEARCH FELLOW IN PLANT BREEDING/AGRONOMY

Candidates for this post should be graduates in Agriculture or Botany with postgraduate training in Plant Breeding or Agronomy. Previous experience in Crop Production research would be an advantage. Appointee will be required to conduct research in the breeding or agronomic aspects of sorghum, millets or grain legumes—mainly soyabean, cowpeas, green and black grams.

Salary Scales: Professor TE3,332 to TE3,612 p.a.; Associate Professor TE3,052 to TE3,472 p.a.; Senior Lecturer/Senior Research Fellow TE2,632 to TE3,052 p.a.; Lecturer/Research Fellow TE2,150 to TE2,590 p.a. (TE1=£1.17 sterling). The British Government may supplement salary in range £996 to £2,448 p.a. (sterling) for married appointees or £348 to £1,500 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. F.S.S.U. Family passages; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail by August 27, 1974 to the Chief Academic Officer, University of Dar es Salaam, P.O. Box 35091, Dar es Salaam, Tanzania. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (539)

M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)

Watford Road,

Harrow, Middlesex, HA1 3UJ

TECHNICIAN

required in the new Department of Surgical Oncology (Division of Surgical Sciences) to assist in a programme of research in human and experimental TUMOUR IMMUNOLOGY.

Familiarity with immunological procedures is desirable, including tissue culture, radiolabelling and cytotoxic reactions.

Salary within the range £1,566 to £2,418 plus Phase III Threshold Agreement increases.

Please contact Mrs. J. Tucker-Bull for further details quoting ref. 128/2. (513)

RESEARCH TECHNICIAN

(Grade 3)

required for work on fungal diseases in plants. Previous experience in this field or in biochemical techniques and electron microscopy is desirable but not essential.

Salary £1,650 to £1,920.

Apply: Senior Assistant Secretary, University of Birmingham, P.O. Box 363, Birmingham, B15 2TT. Reference 139/B/303. (522)

UNIVERSITY OF EDINBURGH DEPARTMENT OF CHEMISTRY

Applications are invited for a Post-doctoral Research Fellowship (S.R.C.) to study the synthesis and properties of cyclazines (annulenes containing internal nitrogen atoms) in collaboration with Dr. D. Leaver.

The appointment will be for two years from October 1, 1974 at a salary of up to £2,247 x £165 per annum with superannuation under F.S.S.U. depending on age and experience.

Applications, together with the name of an academic referee, should be sent to the Secretary to the University, University of Edinburgh Old College, South Bridge, Edinburgh EH8 9YL. Please quote reference number 5040. (517)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(University of London)

Keppel Street, WC1E 7HT

MEDICAL MYCOLOGY REFERENCE LABORATORY RESEARCH SCIENTIST

Applications are invited from postdoctoral Biochemists or Immunochemists for appointment, for five years initially, to work on an M.R.C. grant in this Laboratory run by the P.H.L.S. The successful candidate will direct research into the immunology of fungal diseases. Salary, depending on age, qualifications and experience, on scales rising to £2,694 (Post Probationary), £3,843 (Senior Scientific) or £5,175 (Principal Scientific), plus £126 London Weighting, and threshold awards. Superannuable post.

Applications in writing, giving full details of career etc. and naming two referees, should be sent to Dr D. W. R. Mackenzie, N. at the School, by September 30, 1974. (533)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF VETERINARY PATHOLOGY

LIVERPOOL AND WIRRAL

Applications are invited for the following posts:—

A. **TECHNICIAN** to work in the Liverpool diagnosis laboratory. Applicants should have experience in Bacteriology and Clinical Chemistry and hold H.N.C. Medical Science or equivalent I.M.L.T. certificate. Salary on a scale £1,680 to £1,920 per annum plus threshold payments. Quote ref. RV/N/276158(A).

B. **TECHNICIAN** to work in the Clinical Pathology Unit at the Veterinary Field Station, Leahurst, Neston, Wirral. The laboratory deals with bacteriological and parasitological diagnosis. Applicants must possess Intermediate I.M.L.T. or O.N.C. Medical Sciences. Salary on the scale £1,524 to £1,794 per annum plus threshold payments. Quote ref. RV/N/276158(B).

Initial salary will be according to qualifications and experience. Application forms for both posts may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. (557)

Toxicologist

We require a Toxicologist in the Department of Toxicology to participate in the evaluation of new medical substances for possible toxicological and teratogenic potential.

Applicants should have graduated in a biological subject or possess a Higher National Certificate or Diploma in Applied Biology with appropriate experience: a new graduate will be considered.

Please Apply to:

Mrs J. Andrews,
Personnel Officer,

John Wyeth & Brother Ltd,
Huntercombe Lane South, Taplow,
Nr. Maidenhead, Berks BL6 0PH
Telephone Slough 28311.

Wyeth

(569)

**UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE
ON-LINE BIBLIOGRAPHICAL
RETRIEVAL PROJECT IN
BIOMEDICINE**

Applications are invited for the post of RESEARCH ASSISTANT to work with the Lecturer in Medical Information on the above project, financed by the British Library Research and Development (formerly O.S.T.I.). The systems principally involved will be those of the two MEDLARS on-line retrieval systems, MEDUSA and MEDLINE. The person appointed will act as intermediary between customers and systems in on-line formulation and evaluation.

Applicants should be graduates in a science subject, and information science/library qualifications or experience would be an advantage.

The salary will be £2,118 per annum plus F.S.S.U.

The post is available from October 1, and will extend to December, 1975.

Further particulars from Dr E. D. Whittle, University of Edinburgh Medical School, Teviot Place, Edinburgh, EH8 9AG, to whom applications, together with the names of two referees, should be sent by August 12, 1974. Please quote reference number 5039. (519)

UNIVERSITY OF LEICESTER

Applications are invited for the posts of laboratory technician Grade 3(i) and Grade 4(i) in the Department of Physiology.

The Grade 3 post will mainly involve research work into membrane transport and associated topics, also to assist with some practical classes. Applicants should have H.N.C./H.N.D. or equivalent qualifications and have had 3 to 5 years laboratory experience.

The Grade 4 post will be for organisation of practical classes for medical students and will involve the use of up-to-date electro-physiological equipment and therefore requires a knowledge of equivalent qualifications and have had 7 to 9 years laboratory experience.

Salary scales are Grade 3 £1,650 to £1,920, and tronic. Applicants should have H.N.C./H.N.D. or Grade 4 £1,848 to £2,163 plus cost-of-living supplements.

Applications giving full details of educational background, qualifications and experience together with the names and addresses of two referees should be sent as soon as possible to the Chief Technician, Department of Physiology, University of Leicester LE1 7RH. (565)

**UNIVERSITY OF EDINBURGH
DEPARTMENT OF PHYSICS**

Applications are invited for a vacant post of University Demonstrator in the above Department. Teaching duties (laboratory and tutorial work) will not normally exceed 10 hours per week. The successful candidate will be expected to register for a higher degree, if he does not already hold one. Details of research in progress can be obtained on application to the Department.

The appointment is renewable annually up to a maximum tenure of five years, and will be according to qualifications and experience on the scale £1,848 to £2,538 per annum. Superannuation under F.S.S.U.

Applications, by letter, giving full details together with the names of two referees, should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL, not later than September 13, 1974. Please quote reference number 1036. (518)

**UNIVERSITY COLLEGE GALWAY
Professorship of
Experimental Physics**

Applications are invited for the above statutory whole-time post. Salary scale £5,427 x 141 (8) to £6,555, plus Family Allowances. Non-contributory Pension Scheme.

The closing date for receipt of applications is **September 16, 1974**. Prior to application, further information should be obtained from the Registrar of the College. (562)

entomologists

We wish to recruit two Entomologists. Applicants should hold a degree in Biological Sciences specialising in Entomology, and have subsequent experience in either:

(a) the field evaluation of new insecticides or related products and their establishment on the market. For preference the experience should relate to Mediterranean and tropical crops. The person appointed will be expected to travel up to four months of the year in periods of a few weeks at a time.

(b) The evaluation of new insecticides in the laboratory. Applicants with experience in studying the way insecticides act at the whole insect level will be preferred. A knowledge of insecticide chemistry is desirable, together with an acquaintance with the opportunities for new products gained from experience in the field.

**PLANT PHYSIOLOGIST/
GENETICIST**

We have a vacancy for a Technical Officer to initiate a new programme of work on the effect of chemicals on the development and fertility of flowers of important crop species. The work will be particularly directed towards the discovery of chemicals which may assist

in the development of hybrid varieties.

The person appointed will be based at Jealott's Hill Research Station where extensive modern laboratory glasshouse and growth chambers are available. He may, however, be required to spend periods overseas in order to carry out necessary experimental work, supporting technical staff will be available.

Qualifications: Degree in Botany or Agriculture or allied subject preferably with a Ph.D. in Plant Physiology or physiological aspects of genetics. Previous work on the effects of chemicals on plant development or on the production of hybrid varieties would be an advantage.

Salaries will be dependent on qualifications and experience. Fringe benefits include membership of the ICI Pension and Profit Sharing schemes.

After joining married men will receive a reasonable refund of removal (including travel) expenses and, in approved cases, assistance in house purchase. If you are interested in applying please write for an application form to:

**Mr S. R. Stephenson,
Personnel Officer,
ICI Plant Protection Ltd.,
Jealott's Hill Research Station,
BRACKNELL, Berks (555)**



PLANT PROTECTION LIMITED

UNIVERSITY OF DAR ES SALAAM — TANZANIA

Applications are invited for the following posts tenable as soon as possible:—

LECTURER/RESEARCH FELLOW IN CHEMISTRY (Organic). Candidates must be holders of Ph.D. or its equivalent in Organic Chemistry and should be capable of teaching all aspects of Organic Chemistry to undergraduate level students. The appointee will also be expected to teach and supervise at postgraduate level. Research interests in natural products will be an advantage but not essential. An applicant who would like to do more research and only a limited amount of teaching may be considered for appointment as Research Fellow.

LECTURER/SENIOR LECTURER IN MARINE BIOLOGY. Candidates should have a Ph.D. degree in appropriate subjects. Preference will be given to those with teaching experience in aquaculture or ichthyology. The appointee will be attached to the Marine Biology Station, under the Department of Zoology of the University and will be required to participate in team-work research mainly in marine ecology, to supervise postgraduate students in Marine Biology, and to participate in the teaching of undergraduate students in the Department of Zoology.

Salary Scales: Senior Lecturer T£2,632-T£3,052 p.a.; Lecturer/Research fellow T£2,150-T£2,590 p.a. (T£1 = £1.17 sterling). The British Government may supplement salary in range £996-£1,848 p.a. (sterling) for married appointees or £348-£900 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. FSSU. Family passages; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail by August 27 1974 to the Chief Academic Officer, University of Dar es Salaam, PO Box 35091, Dar es Salaam, Tanzania. Applicants resident in UK should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (538)

YORKSHIRE WATER AUTHORITY SCIENTISTS

The work of the Authority extends over an area which coincides very closely to the geographical boundaries of Yorkshire.

Vacancies have now arisen in the Scientific Services section which carries out works in most aspects of the Authority's activities. First class staff are required for the following work in Leeds.

BACTERIOLOGIST — £3,504 to £3,993 plus Threshold Payment. RN 46. The vacancy exists in a small group which is undertaking specialised bacteriological investigations, identifying the virological problems in the Authority's area and giving bacteriological advice to Divisions. Applicants should be graduates with a degree and experience relevant to this area of work.

SCIENTIFIC AND TECHNICAL OFFICER FISHERY SCIENCE — £2,394 to £3,018 plus Threshold Payment.

RN 58. This post is located in Scientific Services at Head Office, Leeds and heads a small team of Fishery Scientists. The duties will involve investigations into causes of fish mortalities, fish population studies and fishery management advice.

With the agreement of the Water Services Staff Commission these posts are not restricted to staff within the Water Industry, although first consideration will be given to applicants so employed.

Applications quoting the appropriate reference number and giving details of age, qualifications, salary and experience should be sent to the Personnel Officer, Yorkshire Water Authority, West Riding House, 67 Albion Street, Leeds LS1 5AA by August 12, 1974.

(568)

YORKSHIRE WATER AUTHORITY

UNIVERSITY COLLEGE LONDON DEPARTMENT OF ANATOMY

RESEARCH ASSISTANTS (2), preferably post-doctoral, required to work on ultrastructural studies of synaptic organisation in vertebrate C.N.S. with Drs K. E. Webster and A. R. Lieberman. Appointment October 1, 1974 or as soon as possible thereafter for a period of up to 3 years. Starting salary up to £2,118 plus £213 London Allowance. Curriculum Vitae and names of two referees, as soon as possible to Dr Webster and Dr Lieberman, Dept. of Anatomy, (N) University College London, Gower Street, WC1E 6BT. (534)

M.R.C. RHEUMATISM UNIT CANADIAN RED CROSS MEMORIAL HOSPITAL TAPLOW, NR. MAIDENHEAD, BERKS.

IMMUNOLOGY

Technician required to take part in immunological research in rheumatism and connective tissue disease.

Modern immunological laboratory procedures are in everyday use, including immunofluorescence, immunoperoxidase and radioisotope methods.

The successful applicant will be offered training in these as required. The post offers excellent opportunities to work for higher qualifications, IMLT or equivalent qualification required.

Applications, with the names of two referees, to the Director, M.R.C. Rheumatism Unit, at the above Hospital. (531)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF DERMATOLOGY RESEARCH STUDENTSHIP

Applications are invited from graduates with an Honours Degree in Biochemistry for a Junior Research Associate appointment to work on research on androgen metabolism in the skin. Candidates should have an interest in steroid biochemistry. The appointment will be for one year in the first instance but renewable up to a maximum of three years in all and the post will be suitable for a student who wishes to study for a higher degree. Allowances equivalent to those in the case of M.R.C. Studentships will be paid.

Applications should be sent as soon as possible to Professor S. Shuster, Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 7RU. (524)

MURDOCH UNIVERSITY Western Australia

SCHOOL OF VETERINARY STUDIES

Applications are invited for several positions in each of the following fields within the Division of Veterinary Biology. Candidates should be capable of relating structural and functional features of domestic animals. They would normally be expected to have a higher degree.

ANATOMY/HISTOLOGY PHYSIOLOGY

BIOCHEMISTRY/NUTRITION

Duties include: **TEACHING**: participation in the first part of the veterinary biology programme leading to an understanding of the animal body. It starts with concurrent themes on the **BODY AS A WHOLE** and **CELL BIOLOGY** followed by an integrated study of **BODY SYSTEMS**. **RESEARCH**: candidates will be expected to have an active research interest either in general cell biology or in one or more of the body systems.

Level of appointment: One of the Anatomy/Histology vacancies is at a senior level. The other appointments will be in the scales of Lecturer or Senior Lecturer. Present salary scales are: Lecturer \$A9,002 to \$A12,352; Senior Lecturer \$12,643 to \$A16,724; Associate Professor \$A16,389.

General Information: 48 veterinary students will enter this phase of the course in March 1976. The Head of the Veterinary School is Professor R. H. Dunlop; The Chairman of the Division of Veterinary Biology will be Dr J. McC. Howell, presently at the University of Liverpool; The Chair in Physiology will be filled by Dr R. G. Wales, presently at Sydney University, who will coordinate the study of the animal body. Appointments are available to commence at various times during 1975.

•Further information is available from the Secretary, Murdoch University, Murdoch, Western Australia 6153 or from the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. (526)

Applications close on September 30, 1974. (526)

Agricultural Chemicals Development

May & Baker Ltd. wish to recruit staff for two new posts in their Agricultural Development Division at Ongar Research Station, Essex.

DEVELOPMENT OFFICER

This Officer will assist the Chief Development Officer in the planning and co-ordination of programmes for the evaluation of agricultural products throughout the world. The successful candidate, male or female, will be responsible for the collection and dissemination of technical data between the Research Station and the Company's Branches and Agents.

Applicants, between the ages of 24 and 30, should have a Degree, preferably with a science as a main subject, and should have a working knowledge of French, German or Spanish. Experience in the pesticides industry, in the fields of technical information or publications, would be an added advantage, and the candidates should have the ability to summarise technical data and to express themselves lucidly in English.

RESEARCH SCIENTIST

He will lead a small team of biologists and will be responsible for all programmes on the field evaluation of herbicides in the U.K. and overseas.

Candidates, between the ages of 28 and 35, should possess an Hons. Degree or M.Sc., in Botany with some specialisation in weed control. They should also have some experience in Research and Development in the pesticide industry.

Reference No. 185/N/1

Reference No. 186/N/1

May & Baker provide first class conditions of employment including a competitive starting salary and contributory pension scheme with free life assurance cover. Assistance towards removal expenses will be considered in suitable cases.

Please apply in writing quoting the appropriate reference number giving details of qualifications and experience to the Personnel Manager, May & Baker Ltd., Dagenham, Essex RM10 7XS. (512)



M&B May & Baker

ROYAL COLLEGE OF
SURGEONS IN IRELAND
DIVISION OF BIOCHEMISTRY

Junior Lecturer

Applications are invited for the above post.
Salary £2,180 plus £134 (4) to £2,716, point of entry to be determined by qualifications and experience.

Preference will be given to applicants with an interest in one of the following fields: Developmental Biology; Brain Biochemistry; Clinical Research.

Applications giving the names of two referees should be received not later than Monday, September 9, 1974.

Further particulars from the Registrar, Royal College of Surgeons in Ireland, Saint Stephen's Green, Dublin 2. (532)

UNIVERSITY KONSTANZ
(WEST GERMANY)

RESEARCH POSITION FOR
ELECTROPHYSIOLOGIST

Applications are invited for a postdoctoral position to electrophysiological investigations of normal and transformed cell cultures in vitro. This work is part of a research project on regulation of cell division sponsored by Deutsche Forschungsgemeinschaft.

Please send your application, curriculum vitae including details of your previous experience to: Sonderforschungsbereich 138, Biologische Grenzflächen und Spezifität, der Universität Konstanz, D-775 Konstanz, Postfach 733. (520)

UNIVERSITY OF SYDNEY
LECTURESHIP/SENIOR
LECTURESHIP IN
AGRICULTURAL GENETICS

Applications are invited for the above-mentioned position in the Plant Breeding Institute of the Department of Agricultural Botany. We are especially interested in persons who have had research and teaching experience in the cytology and cytogenetics of agricultural crops. The person appointed will be expected to teach courses in general genetics and in cytogenetics to third and fourth year students. He will also be expected to supervise postgraduate students and to participate in some phase of crop improvement in association with the University Plant Breeding Institute.

The position advertised is permanent, but the lectureship may be filled for three years in the first instance with possibility of permanency after that time, or in certain cases return fares.

Salary range: Lectureship: \$A9,002 to \$A12,352 p.a. Senior Lectureship \$A12,643 to \$A14,724 p.a.

Applications, including details of qualifications and experience and names of two referees, by August 19, 1974 to the Registrar, University of Sydney, New South Wales 2006, Australia. Information also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (527)

Research in
Instrumentation and
Control Engineering

Applications are invited from graduates with good honours degrees in Physics, Engineering or Mathematics for two posts:

(1) CONTROL AND INSTABILITY OF FLUID DISTRIBUTION SYSTEMS

(2) CORRELATION TECHNIQUES OF FLOW MEASUREMENT

Candidates will register for a higher degree with the CNAA.

Salary: one post £1,500-2x£81-£1,662.

one post SRC Studentship supplemented by some Tutorial work.

Further details and application form from Staffing Section, Department N, Teesside Polytechnic, Middlesbrough, Cleveland TS1 3BA. (537)

Senior Information Scientist (£4550-£5900)

This post, initially based in London will provide a service to all Ministry of Agriculture, Fisheries and Food R & D laboratories, and to its Agricultural Development and Advisory Service throughout the country.

The scientific information field in the Department is extensive and complex, with a variable level of indexing, abstracting and information retrieval in the many relevant disciplines and at a number of levels for the different areas it serves.

Major responsibilities will be to review the existing scientific and technical information systems in the light of the latest advances in information science; to advise on the introduction of new systems; and to liaise with other Departments and non-Government organisations concerned with the storage, retrieval and dissemination of information with special reference to agriculture and allied subjects.

Candidates should normally hold a 1st or 2nd class honours degree in a scientific subject (including information science). Considerable relevant experience at responsible levels is necessary; experience in organising and developing scientific information systems is essential.

Appointment will be as Principal Scientific Officer, with starting salary within the range quoted, and may be permanent and pensionable or, with present employer's agreement, on secondment terms.

For further details and an application form (to be returned by 23 August 1974) write to Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, or telephone BASINGSTOKE 29222 extension 500 or LONDON 01-839 1992 (24 hour answering service). Please quote reference S/8714/3.

A NEW POST IN MINISTRY OF AGRICULTURE, FISHERIES AND FOOD.

(587)

ROYAL PAVILION
ART GALLERY AND MUSEUMS
BRIGHTON

Applications are invited for the following three new posts:

(1) KEEPER OF BIOLOGY
(ref. BM1)

Salary A.P. 4/5 (£2,235 to £2,820), plus current Threshold payment.

(2) KEEPER OF GEOLOGY
(ref. BM2)

Salary A.P. 4/5 (£2,235 to £2,820), plus current Threshold payment.

Applicants should have an appropriate University degree; possession of the Museums Association diploma would be an advantage. Relevant experience is desirable.

Full details of the posts may be obtained from The Director, The Royal Pavilion, Brighton, BN1 1UE, quoting appropriate post reference. Written applications, giving full details, together with names and addresses of two referees, should be received by August 21, 1974. (511)

PRE- or POST-DOCTORAL
BIOCHEMIST

with at least 4 years post-graduate experience, especially in protein fractionation and immunochemistry, required for research in relation to M.R.C. trials in myeloma and macroglobulinaemia for at least two years from October 1, 1974. Salary equivalent to Senior Biochemist, (from £3,090 p.a.). Applications with two referees to Prof. J. R. Hobbs, Department of Chemical Pathology, Westminster Medical School, 17 Page St., London SW1 by September 9, 1974. (551)

EDITORIAL ASSISTANT

A microbiologist with a good knowledge of French and a zoologist with a good knowledge of German are invited to join the Editorial Team of Information Retrieval Limited, a publisher of scientific literature. Starting salary is £2,108 per annum. Please apply to: Dr. E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London W1V 5FG. (563)

UNIVERSITY OF SYDNEY
LECTURESHIP IN AGRONOMY
(CROP BOTANY)

Graduate in Science or Agricultural Science, with Ph.D. or equivalent experience in crop botany. Applicants should have wide experience with Australian Crop plants, including field crops, horticultural crops and pasture species.

The position advertised is permanent but may be filled for three years in the first instance with possibility of renewal after that date, or in certain cases return fares.

Salary range: \$A9,002 to \$A12,352 p.a.

Applications, including curriculum vitae, list of publications and names of three referees, by September 3, 1974 to the Registrar, University of Sydney, NSW 2006, Australia, from whom further information available. Information also available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (528)

university of wales
university
college of
swansea

DEPARTMENT OF
BIOCHEMISTRY

Applications are invited for the vacancy of Postdoctoral Research Assistant in the Department of Biochemistry, to work on an S.R.C. sponsored research programme concerned with the occurrence, metabolism and function of 3':5'-cyclic A.M.P. in the tissues of higher plants.

The appointment, which will be for one year in the first instance, from October 1, 1974, will be on the scale £2,118 to £2,757 per annum, together with F.S.S.U. benefits.

Further particulars and application forms (2 copies) may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea, SA2 8PP, to whom they should be returned by Friday, August 23, 1974. (542)

Thinking ahead
in
Pharmaceuticals

Young Science Graduate for teratological work

We are a major pharmaceutical company and part of the £multi-million international Glaxo Group. Our modern research laboratories are located in pleasant rural surroundings at Ware in Hertfordshire.

We have an interesting opportunity for a recently qualified science graduate to work on the teratological aspects of drug safety evaluation. This position will involve supervising the work of a small section in the Pathology Department and taking part in all aspects of investigations.

Salary and conditions are attractive and include assistance with relocation expenses, where appropriate.

Please write, with brief career details, to:



Glyn Jones,
Personnel Officer,
Allen & Hanburys Research Ltd.,
Priory St., Ware, Herts. SG12 0DJ.



Allen & Hanburys MAKERS OF FINE PHARMACEUTICALS

NATURAL ENVIRONMENT RESEARCH COUNCIL

INSTITUTE OF GEOLOGICAL SCIENCES ANALYTICAL CHEMIST

A vacancy exists at Scientific Officer/Higher Scientific Officer level in the Institute's Hydrogeology Unit in London. Appointment will be on a permanent established basis.

Duties: To work with a small group engaged in research and applied studies concerned with various aspects of natural waters, and based in the Hydrogeological Department. Responsibilities will consist of management of the laboratory, direction of routine analysis and development of new techniques, particularly for trace analysis.

Qualifications: Candidates will be expected to have a Good Honours Degree in Chemistry, or equivalent qualification. Previous analytical training or experience relevant to geochemical/environmental analysis is desirable: familiarity with flame atomic absorption is required and knowledge of flameless techniques and gas-liquid chromatography would be an advantage. Candidates at H.S.O. level should have at least 2 years relevant postgraduate experience.

Salary: Scientific Officer, £1,820 to £2,903; Higher Scientific Officer, £2,689 to £3,599, inclusive of Inner London Weighting.

Starting salaries may be above the minimum.

The staff of the Council are not Civil Servants although their pay and conditions of service are similar to those of scientists in the Civil Service.

Application forms available from Establishment Section, Institute of Geological Sciences, Exhibition Road, London SW7 2DE, quoting ref: SO/HYD/74/3.

CLOSING DATE FOR RECEIPT OF APPLICATION FORMS AUGUST 16, 1974. (560)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING (University of Reading)

A Scientific Officer or Higher Scientific Officer is required to join a group in the Bacteriology Department investigating the intestinal microflora of the neonate with particular reference to the antibacterial activity of milk. Experience with immunological techniques will be an advantage.

Candidates should have a degree, H.N.C. or equivalent qualification. Appointment will be in the grade of Scientific Officer (£1,592 to £2,675) or Higher Scientific Officer (£2,461 to £3,371), with a starting point dependent on qualifications and experience. At least five years' relevant post-qualifying experience is required for appointment in the higher grade. The post is pensionable.

Apply on forms obtainable from the Secretary, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, quoting reference 74/17. (561)

THE UNIVERSITY COLLEGE OF WALES ABERYSTWYTH DEPARTMENT OF PHYSICS

Applications are invited for the posts of:
**RESEARCH ASSOCIATE or
RESEARCH OFFICER**

to work under the following S.R.C. sponsored projects:

1. Ion-Molecular Collisions of ionospheric interest using a temperature-variable afterglow system.
2. Rocket-borne measurements of minority neutral constituents in the D-region of the ionosphere.

Salary range: Research Associate, £2,118 to £2,580 p.a.; Research Officer, £1,662 to £2,550 p.a.

Further details and forms obtainable from Professor N. D. Twiddy, Department of Physics, Penglais, Aberystwyth. (566)

MASSEY UNIVERSITY Palmerston North, New Zealand BIOTECHNOLOGY DEPARTMENT SENIOR LECTURESHIP - BIOCHEMICAL ENGINEERING

Applications are invited for positions of Senior Lecturer, in the Biotechnology Department, concerned with the application of chemical and biochemical engineering to fermentation technology and industrial biological processing. The background sought ideally would include a degree in chemical engineering, Ph.D. or equivalent in biochemical engineering, and industrial experience.

Salary: in the range NZ\$9,503 to \$11,153 (bar) to \$12,142.

Further details may be obtained from the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Application close on **September 9, 1974.**

(583)

UNIVERSITY OF STRATHCLYDE DEPARTMENT OF FOOD SCIENCE AND NUTRITION

RESEARCH FELLOWSHIP

Applications are invited from post-graduates in biochemistry, biological chemistry or molecular biology for the above appointment.

The appointee will be a member of a team investigating the mechanisms of fluorochrome reactions with micro-organisms.

The appointment will be for three years on the salary scale £2,118 to £2,412 per annum with F.S.S.U.

Applications (quoting R28/84) containing the names of two referees, should be sent to Dr. J. Scholefield, Department of Food Science and Nutrition, University of Strathclyde, James P. Todd Building, 131 Albion Street, Glasgow G1 1SD. (530)

UNIVERSITY OF WAIKATO HAMILTON, NEW ZEALAND LECTURERS/SENIOR LECTURERS

The University invites applications for academic positions that will be available from February 1, 1975. The positions which are listed below will be at lecturer or senior lecturer level. The reference numbers for each vacancy are given in brackets.

BIOCHEMIST

(203) with interests in biological chemistry or one of the branches of biology.

BIOLOGICAL SCIENTISTS

(204) cellular biologist with interests in microbial genetics or physiology or virology or cell physiology —(205A) zoologist with interests in invertebrate physiology or vertebrate biology or physiology or animal behaviour—or (205B) plant ecologist.

CHEMISTS

(206) (207) preferred interests in one or more of physical chemistry, natural products or biological chemistry, spectroscopic methods.

COMPUTER SCIENTISTS

specialising in (208) computing machines and communication systems and (209) management data processing and information systems.

GEOGRAPHER

(216) human geographer specialising in quantitative

MATHEMATICIAN

(222) statistics or computing, preferably with experience in design of experiments or sampling for scientific or technological applicants.

PSYCHOLOGISTS

(preferably with cross cultural interests in each case) (224) applied social psychology including measurement (225) clinical psychology (226) interests in research and natural settings including animal behaviour.

The current salary scales run from NZ\$7,361 to NZ\$9,339 per annum for lecturers and NZ\$9,503 to NZ\$11,153 to NZ\$12,142 per annum for senior lecturers.

Applications close on **September 13, 1974** and should be submitted in a prescribed format.

Details and conditions of appointment are available from the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, University of Waikato, Hamilton, New Zealand. (559)



Chemical or Biochemical Engineer and Fermentation Technicians for Fermentation Research and Development

Shell Research Limited have vacancies for one scientist (Chemical or Biochemical Engineer) and four technicians to work in a multi-disciplinary team in the Fermentation Division of their Woodstock Laboratory, Sittingbourne, Kent.

Chemical or Biochemical Engineer

You will join a team concerned with new fermentation projects based on hydrocarbon and petrochemical feedstocks. You should have a good degree in Chemical Engineering and a minimum of three years' research and/or development experience. Ability to furnish evidence of significant contributions to fermentation or other biological processes would be an advantage.

Fermentation Technicians

You will carry out development work concerned with the production of single-cell protein from methane. You should have experience in fermentation processes and be in possession of an HNC or equivalent in Chemical Engineering, Biochemical Engineering or Applied Biology. Experience in pilot plant fermenter and process equipment operation would be an advantage.

The level of appointment will depend on background and experience and the salary offered will be competitive. There is an excellent pension scheme, a good staff restaurant and sports/social facilities are provided. Please write, indicating which position you wish to apply for, giving full details of personal background and experience to:— Shell Research Limited, Recruitment Division (N), PNE/34, Shell Centre, London SE17NA.

UNIVERSITY OF SIERRA LEONE FOURAH BAY COLLEGE

Applications are invited for the following posts:-

1. **SENIOR LECTURER/LECTURER IN BOTANY**, tenable as soon as possible. Applicants should have a good honours degree and post-graduate research experience, preferably in Plant Morphogenesis or Plant Anatomy, but applicants in other fields will be considered. Teaching duties will include participation in elementary courses in Biology and Botany and other courses according to the qualifications and interests of the appointee and the needs of the Department. He will also be expected to carry out research in his specialist field.

2. **LECTURER IN PHYSICS**. Research interests in Geophysics or Theoretical Physics would be considered an additional advantage.

Salary scales (under review): Senior Lecturer Le4,550 to Le5,400 p.a.; Lecturer Le2,400 to Le4,740 p.a. (£1 sterling = Le2). The British Expatriates Supplementation Scheme is unlikely to be applied to these appointments. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than September 2, 1974 to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in the U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (571)

SUB-EDITOR

BUTTERWORTH'S, who publish a wide range of scientific titles, are looking for a sub-editor to assist in the publication of the International Union of Pure and Applied Chemistry (I.U.P.A.C.) series. Previous editorial experience is required, as is a specialist knowledge of organic chemistry.

The offices are situated in pleasant rural surroundings at Borough Green, near Sevenoaks, Kent and company transport is available from certain surrounding districts.

Salary according to N.U.J. scale.

Please write, giving details of age, qualifications and experience to:

Mr F. Langton,
Butterworth & Co. (Publishers) Ltd.,
Borough Green,
Nr. Sevenoaks,
Kent.

(574)

Imperial College

A Postdoctoral Research Assistant is required in the Metal Physics group for experimental work on the Super-Conducting Proximity Effects. Candidates should have a Ph.D. or confidently expect to obtain one shortly, and should have experience of work at low temperatures. Familiarity with Super-Conductivity and Radio-Frequency techniques would be an advantage. The appointment will be for a maximum of three years beginning October 1, 1974.

The starting salary will be £2,118 plus £213 London Allowance and Threshold Payment with F.S.S.U. Curriculum vitae and the names of at least two referees should be sent as soon as possible to:-

Dr J. G. Park
Department of Physics
Imperial College
London SW7 2BZ
Tel 01 589 5111 Ext. 2303

(570)

HERIOT-WATT UNIVERSITY DEPARTMENT OF CHEMISTRY RESEARCH TECHNICIAN

to work on the synthesis and reactions of new penicillins. Applicants should have appropriate qualifications for synthetic organic chemistry.

Salary up to £1,704 by £54 to £1,812 p.a.

Applications, together with curriculum vitae and names of two referees to Dr M. M. Campbell, Department of Chemistry, Heriot-Watt University, Riccarton, Currie, Midlothian. (576)

UNIVERSITY OF MALAWI CHANCELLOR COLLEGE

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN BIOLOGY (PLANT PHYSIOLOGY), tenable as soon as possible. Candidates should have a higher degree, preferably a Ph.D. and some teaching experience in Plant Physiology. An interest in applied Plant Physiology and Biochemistry is required. Appointee will be required to teach Plant Physiology to 3rd and 4th Year degree students and assist with elementary work in 1st and 2nd Year. Salary scales (including expatriate addition): (a) K5,242 to K5,881 p.a. (b) K2,809 to K4,714 p.a. plus either a University Addition of K720 p.a. (taxable in Malawi) or the British Government may supplement salary in range £600 to £1,080 p.a. (sterling) for married appointee or £300 to £800 p.a. (sterling) for single appointee (normally free of all tax) and provide children's education allowances and holiday visit passages. (£ sterling = K1.99). Gratuity of 15% to 25%; superannuation scheme transferable with F.S.S.U.; family passages; various allowances; biennial overseas leave; housing. Detailed applications (2 copies) including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than September 2, 1974 to the Registrar, University of Malawi, University Office, P.O. Box 278, Zomba, Malawi. Applicants by airmail, not later than September 2, 1974 to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (572)

THE UNIVERSITY OF ADELAIDE invites applications for four appointments as LECTURER IN ZOOLOGY

Applications will be welcomed from persons with interests and expertise in one or more of the following fields: educational innovation at the tertiary level; ecophysiology of aquatic animals—freshwater or marine; ethology; parasitology. Applicants with experience in tertiary teaching and with interests in other fields may be considered. It is desired that successful applicants be able to take up their positions early in January 1975.

SALARY SCALE: \$A9,002 by 479 (4) by 478 (3) to \$A12,352; with superannuation on the F.S.S.U. basis.

FURTHER INFORMATION, including list of particulars required in an application, is available from the Registrar of the University or from the Secretary-General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London, WC1H 0PF.

APPLICATIONS should reach the Registrar, G.P.O. Box 498, Adelaide, South Australia 5001, not later than August 23, 1974. (580)

(588)

Lister Institute of Preventive Medicine
ELSTREE, HERTFORDSHIRE
BLOOD PRODUCTS
LABORATORY

Recent graduate required for bacteriological work in connection with human blood products and to assist in research and development. Possible registration for higher degree for appropriate applicant with first or upper second class honours degree. Please apply stating qualifications, age and experience to: The Secretary (Ref: BP), Lister Institute of Preventive Medicine, Elstree, Herts., from whom further particulars can be obtained.

(579)

NATIONAL INSTITUTE FOR MEDICAL RESEARCH
MILL HILL — LONDON

ELECTRON MICROSCOPY TECHNICIAN required, familiar with thin tissue sectioning, to assist in the examination of cells and sub-cellular components during animal development. Experience in electron microscopy would be an advantage. Initial salary, according to age, qualifications and experience, will be on scales £1,683 to £2,577 p.a. or £2,346 to £3,147 p.a. Please write, quoting ref. EM/DB/BL, to J. H. Woodcock, Personnel Officer, National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA, Tel: 959-3666 giving details of age, experience and qualifications

(575)

PORTSMOUTH POLYTECHNIC
BIOPHYSICS LABORATORIES

Two posts of Research Assistant are available in the above laboratories to join a large research team under Dr E. M. Bradbury, working on the problem of the structure of the chromosome. A wide range of physical and biochemical techniques are employed, including X-ray and neutron diffraction, electron microscopy, gel filtration and ultracentrifugation. The successful candidate will probably have an honours degree in either chemistry, biochemistry or physics, together with a lively interest in the control of fundamental biological processes at the molecular level.

The appointments will be for a period of three years and registration for a higher degree is expected.

Salary scale: £1,320 to £1,420 per annum. A threshold payment currently £10.44 per month is also payable.

Application forms and further particulars may be obtained from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth, PO1 2QQ, within 10 days of the publication of this advertisement. (573)

UNIVERSITY OF EDINBURGH
DEPARTMENT OF ASTRONOMY
RESEARCH ASSISTANT IN
INFRARED ASTRONOMY

Applications are invited for a post of Postdoctoral Research Assistant in observational infrared photometry from October 1, 1974. The appointment is for a period of one year, but extension will be considered.

The Research Assistant will continue the development of an existing infrared photometer, and its use in Tenerife and elsewhere for observations of H II regions and of extragalactic infrared sources.

Salary will be in the range £2,118 to £2,931 per annum with superannuation under F.S.S.U.

Applications, including curriculum vitae and the names of two referees, should be sent by August 24, 1974 to Dr M. J. Smyth, Department of Astronomy, Royal Observatory, Edinburgh EH9 3HJ from whom further information about the post can be obtained. Please quote reference number 5041. (591)

LINCOLN UNIVERSITY
COLLEGE OF AGRICULTURE
New Zealand

CHAIR IN AGRICULTURAL
MICROBIOLOGY

The Council of Lincoln College, a University College of Agriculture located in Canterbury, New Zealand, invites applications for appointment to the Chair in the Agricultural Microbiology at this University College.

The successful applicant will be Head of the Department of Microbiology, which is responsible for a major teaching programme from Diploma to Post Graduate level, and which has active research and advisory interests. The present establishment includes 1 Reader, 2 Senior Lecturers, 1 Lecturer, 1 Demonstrator and 3 Technicians.

Applicants should hold an advanced university degree in an appropriate field and it would be expected that they would have had experience in teaching and/or research.

The successful applicant will be appointed to the position within the existing range of professorial salaries, the commencing salary being in accordance with qualifications and experience within the range of \$NZ15,111 to \$NZ19,233 per annum.

Expenses of appointment reimbursed up to specified limits. The appointee will be eligible to join the New Zealand Government Superannuation fund, or if eligible, he may elect to continue for a limited period existing F.S.S.U. policies.

Apply in writing for Conditions of Appointment obtainable from the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, Lincoln College Canterbury, New Zealand.

Applications close on **October 15, 1974.** (582)

THE UNIVERSITY COLLEGE
OF WALES
ABERYSTWYTH

DEPARTMENT OF BOTANY AND
MICROBIOLOGY

RESEARCH ASSISTANTS

required to work on growth and development in grasses and clovers. Applications are invited from graduates in Botany, Agriculture or Biology. Successful applicants will have the opportunity to study for a higher degree.

Application forms and particulars obtainable from the Registrar to whom application should be sent by August 15. (578)

MONASH UNIVERSITY
Melbourne, Australia
DEPARTMENT OF PHYSIOLOGY
 (including Pharmacology)

SENIOR LECTURER OR LECTURER

The Department conducts courses for B.Sc., B.Sc. (Hons) and M.B., B.S. degrees and accepts graduate students for M.Sc. and Ph.D. degrees. Research interests include: brain ultra structure, neurophysiology, biophysics and pharmacology of central and peripheral synapses, muscle biophysics, circulatory physiology and pharmacology, renal and endocrine physiology.

A medically qualified candidate who can take responsibility for the organisation and teaching of a whole unit in one of the courses offered by the department is sought for 1975.

Salary scale (currently under review): Senior Lecturer (medically qualified) \$A15,143 to \$A17,224 per annum, Senior Lecturer \$A12,642 to \$A14,724 per annum, Lecturer (medically qualified) \$A11,502 to \$A14,852, Lecturer \$A9,002 to \$A12,352 per annum with superannuation based on an endowment assurance scheme, the employee and employer contributing 5% and 10% respectively.

Benefits: Travelling expenses for appointee and family; removal allowance; repatriation after three years' appointment if desired; temporary housing for an initial period. Study leave entitlement accumulates at the rate of one month's leave for each six months' service up to six years with provision for financial assistance.

Further general information and details of application procedures are available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria, Australia 3168, or the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. Enquiries about the Department to the Chairman, Professor R. Porter, in the University.

Closing date: **August 30, 1974.**

The University reserves the right to make no appointment or to appoint by invitation. (581)

JUNIOR TECHNICAL OFFICER

required for Chromosome Laboratory in Department of Cytogenetics and Immunology, Royal Marsden Hospital and Institute of Cancer Research. Previous experience of human cytogenetics essential. Work in progress includes studies of chronic and acute myeloproliferative disorders and the effects of treatment on chromosome breakage.

Salary according to qualifications and experience in scale £1,300 to £2,200 plus Stage III and threshold awards. Applications in duplicate to the Secretary, Institute of Cancer Research, 34 Sumner Place, SW7 3NU quoting ref. 301/B/528 (577)

UNIVERSITY OF BIRMINGHAM
SUB DEPARTMENT OF ETHOLOGY
RESEARCH ASSOCIATE
(Readvertisement)

Applications are invited from suitably qualified graduates to carry out research on the source and nature of olfactory signals in mice.

This work will be undertaken in close co-operation with a chemist and is intended to cover a three year period. Starting salary: £1,758 plus F.S.S.U.

Applications by September 10 to Dr J. H. Mackintosh, Sub-Department of Ethology, The Medical School, University of Birmingham B15 2TJ, from whom further details may be obtained. (590)

University of New South Wales
WOLLONGONG UNIVERSITY
COLLEGE
to become the

UNIVERSITY OF WOLLONGONG
1st January, 1975

LECTURER IN BIOLOGY (2 positions)

The appointees will be expected to help in the development of a course in biological energetics. One lecturer will be responsible for an instructional unit in biophysics with emphasis on cellular energy relations. The other lecturer will be responsible for an instructional unit in the physiology of multicellular organism, again with emphasis on their energy relations.

Further information may be obtained from Dr A. D. Brown, School of Microbiology, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033, Australia.

Commencing salary for both posts according to qualifications and experience within the range \$A9,002 to \$A12,352 per annum.

Conditions of appointment and application forms available from Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Applications close September 2, 1974. (585)

POSTDOCTORAL RESEARCH ASSOCIATE to study the molecular mechanism of hormonal activation of adenyl cyclase using both membrane and whole cell systems. Experience in molecular pharmacology, membrane biology or cellular physiology required. Applications with full curriculum vitae and names of two referees to:

Dr R. M. Epand
Department of Biochemistry
Faculty of Medicine
McMaster University
Hamilton, Ontario
Canada.

(596)

THE UNIVERSITY OF MANCHESTER

STATE REGISTERED TECHNICIAN to join team concerned with chromosome and immunogenetic research in the fields of birth defects and leukaemia. Experience in cytogenetics or immunological techniques an advantage.

Salary will be on the Whitley Council Scale for Medical Laboratory Technicians in the National Health Service.

Applications should be sent to Dr R. Harris, Department of Medical Genetics, St Mary's Hospital, Manchester M13 0JH, together with the names of two referees. (592)

Technical Field

Officer

We require a Technical Field Officer, preferably aged between 20 - 25 years, (Male or Female), who has recently graduated with a science degree. His/Her main responsibilities will be to assist in the design, running and evaluation of field trials on current animal health products and the development of new products. There will be some involvement in the area of technical sales support.

This post offers a chance to obtain experience in commercial field research and development within a large international organisation.

The successful candidate will be provided with a company car, there is a non-contributory pension scheme in operation, together with assisted B.U.P. membership and other fringe benefits.

Please apply for an application form to:-

Mr K. G. Usher,
Head of Administration,
Elanco Products Ltd.,
Broadway House,
The Broadway,
Wimbledon,
London SW19 1RR.
Telephone No: 01-542 6600.
(594)

UNIVERSITY OF SIERRA LEONE FOURAH BAY COLLEGE

Applications are invited for posts of (i) **LECTURER IN INORGANIC CHEMISTRY** and (ii) **LECTURER IN PHYSICAL CHEMISTRY**, tenable as soon as possible. For post (i), appointee should be able to give courses to general and honours degree levels in three or more of the following: Th chemistry of non-transition and transition elements; Electron Deficient Compounds; Ionic Solids; Inorganic Stereo-chemistry and Bonding; Analytical Techniques in Inorganic Chemistry. For post (ii), appointee should be able to give courses to general and honours degree levels in three or more of the following: Thermodynamics; Wave Mechanics and Quantum Chemistry; X-ray Crystallography, Molecular symmetry; Chemical Spectroscopy.

Salary scale (under review): Le2,400 to Le4,740 p.a. (£1 sterling=Le2). The British Expatriates Supplementation Scheme is unlikely to be applied to these appointments. F.S.S.U. Family passages; various allowances; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by air mail, not later than September 4, 1974 to the Secretary, University of Sierra Leone, Private Mail Bag, Freetown, Sierra Leone. Applicants resident in UK should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (599)

British Museum (Natural History) Sub-department of Anthropology

Primatologist

■ Undertake research on fossil and Recent primates ■ Curate fossil primate collections ■ Answer enquiries on fossil and Recent primates.

□ 1st/2nd hon's degree in appropriate subject □ At least 2 years' postgraduate experience in fossil primates essential □ Interest in Recent primates desirable □ Age normally under 32 □ Appointment as Senior Scientific Officer (around £3500 - over £4750) or Higher Scientific Officer (around £2800 - £3700) according to age and experience □ Ref: SB/24/DK.

Application forms (for return by 16 August 1974) from Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB, telephone Basingstoke 29222 ext. 500 or London 01-839 1992 (24 hour answering service).

**Science
group
CIVIL SERVICE**

(586)

KENNEDY INSTITUTE

Division of Experimental Pathology

CHIEF TECHNICIAN

Salary range £3000 to £4400 p.a. inclusive.

A Chief Technician is required to organise and administer the technical services of this active research group concerned with the structure and function of lymphoid tissues combining morphological and biochemical studies with particular reference to nucleic acids. Appropriate qualifications for the seniority of this post would be required. It is hoped that the successful applicant will become involved in the research activities of the group.

Experience in any of the relevant techniques which include histology, cytochemistry, autoradiography, immunology and tissue culture would be a considerable advantage.

Written applications naming two referees to the Laboratory Superintendent, The Kennedy Institute, Bute Gardens, London W6 7DW. Tel: 01-748 9966. (597)

UNIVERSITY OF RIYADH MEDICAL SCHOOL SAUDI ARABIA

(In association with the
University of London)

Applications are invited from male honours graduates for the following post at the University of Riyadh Medical School. The vacancy which is for the premedical years, has arisen as a result of the rapid expansion of the School:

DEMONSTRATOR IN PHYSICS

Applicants should have a higher degree and teaching experience.

The University of Riyadh is an independent University established in 1957. In 1968 the University established a Medical School in association with the University of London. The request by the University of Riyadh for assistance in this project is covered by a sponsorship agreement. The University of London advises on the curriculum, the form and conduct of examinations, the physical facilities for teaching, the appointment of academic staff and other matters. From the outset it has been anticipated that this assistance would continue over a period of at least 10 years possibly extending to 15 years. All teaching of medical undergraduates is in the English language.

Salary Scale: Demonstrators: Salary and housing allowance will be negotiable in accordance with qualifications and experience. Note: £1 sterling = Saudi Riyals 8.5.

Appointments are for 1 year or longer; renewable. Secondments would be considered.

Detailed applications (3 copies) including a curriculum vitae and naming three referees should be sent not later than August 16, 1974 to the Inter-University Council for Higher Education Overseas, 90-91 Tottenham Court Road, London W1P 0DT from whom further particulars are available. (600)

ANGLIAN WATER AUTHORITY GREAT OUSE RIVER DIVISION

Appointment of
LABORATORY ASSISTANT (Biology)

Applications are invited for the above appointment, preferably from persons with a degree or H.N.C. in a biological science, based in the Division's modern laboratory in Cambridge. Knowledge or experience of biological water quality survey techniques, especially on a botanical basis would be advantageous.

The salary will be within the A.P.2 grade (£1,644 to £1,926 p.a., under review, plus threshold payments) and the appointment is subject to the N.J.C. conditions of service. Removal expenses and lodging or travelling allowances may be payable.

Application forms can be obtained from the Divisional Scientist, Great Ouse House, Clarendon Road, Cambridge CB2 2BL, telephone: Cambridge 61561, to whom completed forms should be returned by August 16, 1974.

Applicants from within the Anglian Water Authority and the Water Industry will be given preference for this appointment in the first instance. (593)

THE FACULTY OF MEDICINE DIVISION OF BASIC SCIENCES MEMORIAL UNIVERSITY OF NEWFOUNDLAND

invites applications for two positions at Assistant or Associate Professor level in Biophysics/General Physiology. Preference will be given to those individuals whose research interests are in the area of biological membranes. Teaching responsibilities are biophysics or cell structure and function. Send curriculum vitae and names of three referees to Biophysics Selection Committee, c/o Associate Dean (Basic Sciences), Faculty of Medicine. (601)

ENTOMOLOGISTS

Applications are invited from qualified entomologists, for a two year appointment from October 1, 1974 as a Higher Scientific Officer or Senior Scientific Officer. Candidates should have a first or upper second class honours degree, or equivalent, and preferably postgraduate experience in the field of plant virology.

The successful applicant will be required to continue with studies which have been initiated on the incidence of vectors of grass and cereal viruses, the epidemiology and sources of resistance to insect pests. Experience with Aphididae would be advantageous.

Starting salary according to qualifications and experience in scale H.S.O. £2,461 to £3,371 or S.S.O. £3,157 to £4,441.

At least two years postgraduate experience is required for appointment as H.S.O. and four years for S.S.O.

The post is pensionable and a new superannuation scheme is being devised.

Applications by letter, together with the names of two referees, should be sent to The Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Nr Aberystwyth, Dyfed SY23 3EB not later than August 31, 1974. (595)

UNIVERSITY COLLEGE DUBLIN RESEARCH POSTS IN INVERTEBRATE ECOLOGY

A Postdoctoral Fellow or Research Assistant or Postgraduate Student is required for a 3-year project sponsored by the National Science Council on the organisation and dynamics of grassland invertebrate communities. Applicants should hold an initial honours degree in Agriculture or Zoology and preferably have research experience in quantitative ecology. Applicants graduating this autumn will be considered.

Full details from:

Dr J. P. Curry,
Department of Agricultural Biology,
Faculty of Agriculture,
University College,
Glasnevin,
Dublin 9.

(602)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF ZOOLOGY

A technician is required for work involving the routine maintenance of a cell culture laboratory. Experience of tissue culture work and sterile techniques would be an advantage but is not essential. Minimum qualification required is H.N.C. or equivalent. The post, which is supported on outside funds, is in the University Technical Assistant grade £1,599 to £2,022 plus threshold additions (presently under review). Application form available from Secretary, University of Cambridge, Department of Zoology, Downing Street, Cambridge CB2 3EJ. (598)

UNIVERSITY COLLEGE DUBLIN APPOINTMENT IN AGRICULTURAL BIOLOGY

Applications are invited for a teaching post in the Department of Agricultural Biology. Candidates should be honours graduates in Agricultural Science or Botany with postgraduate experience in Plant Physiology and/or Forest Botany.

The current salary scale is: Assistant Lecturer, £2,420 by £129 to £3,323.

Entry point on the relevant scale will be in accordance with qualifications and experience.

A non-contributory pension scheme and family allowances are additional to salary. An alternative contributory F.S.S.U. type pension scheme is also available.

Prior to application, further information (including application procedure) should be obtained from:

Mr J. P. MacHale,
Secretary and Bursar,
University College,
Belfield,
Dublin 4.
Telephone 693244 Extn. 431.

Latest date for receipt of completed applications is Friday, August 23, 1974. (603)

UNIVERSITY OF KEELE RESEARCH FELLOWSHIP IN BIOCHEMISTRY

Applications are invited for post of Research Fellow in Biochemistry Research Unit. The post is made possible by a grant from the Cancer Research Campaign. The appointee will join a team investigating the pharmacology of pinocytosis, and experience of cell culture would be an advantage. Salary on scale £2,118 x 129 x 165, with F.S.S.U. privileges.

Enquiries and applications to Professor J. B. Lloyd, Biochemistry Research Unit, Keele University, Staffordshire, ST5 5BG, to whom applications should be sent by August 31, 1974. (465)

UNIVERSITY OF OXFORD NUFFIELD LABORATORY OF OPHTHALMOLOGY RESEARCH STUDENTSHIP

Applications are invited from graduates in Biochemistry or Chemistry for a studentship to investigate the covalently-bound hexose of corneal collagen, commencing October 1, 1974 for 3 years.

Applications, naming two referees, should be sent to Dr J. J. Harding, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford, OX2 6AW, as soon as possible. (548)

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CHEMISTRY

Applications are invited for an S.R.C. (C.A.S.E.) STUDENTSHIP for a project involving the synthesis of a novel group of heterocyclic compounds. These are designed to possess controlled chemical reactivity and potential herbicidal character. The work will be carried out in conjunction with I.C.I. Plant Protection Limited and part of the programme will be performed at their Jealott's Hill Research Station. Applicants should have (or expect to obtain this summer) a good Honours degree or G.R.I.C. in chemistry and the successful candidate will be expected to register for a Ph.D. Applications including a curriculum vitae and names and addresses of two referees to: Dr. G. M. Blackburn, Department of Chemistry, The University, Sheffield S3 7HF (from whom further information is available). Quote Ref. R.117/G. (543)

QUEEN MARY COLLEGE University of London DEPARTMENT OF CHEMISTRY

Applications are invited for the following posts in a group working with Professor R. Bonnett on aspects of haemoglobin chemistry. Applicants should be well grounded in chemistry, but an additional interest in biochemistry would be an advantage. Starting date: October 1, 1974, or by arrangement.

Postdoctoral Fellowship in Organic Chemistry, tenable for one year in first instance, but extendable to two years. Initial salary £2,118 per annum plus £213 London Allowance.

Research Studentship, tenable for two years, leading to submission for the M.Phil. degree in Organic Chemistry.

Applications in writing (giving qualifications, previous scientific experience and names and addresses of two referees) to the Registrar, Queen Mary College, Mile End Road, London E1 4NS. (546)

UNIVERSITY OF EAST ANGLIA SCHOOL OF CHEMICAL SCIENCES SENIOR RESEARCH ASSOCIATE

required to co-ordinate and develop work on computer applications to Nuclear Magnetic Spectroscopy. The post is held in conjunction with the Science Research Council Atlas Computer Laboratory at Didcot, but the work will normally be carried out at the University of East Anglia. Contact with N.M.R. spectroscopists in other British universities, is involved. The period of appointment is two years and should start for preference on October 1, 1974.

Experience on the part of the candidate in either theoretical chemistry or N.M.R. spectroscopy or computing is desirable. The position may attract a chemist or physicist who wishes to gain full-time experience in computing. The salary will be in the range £2,118 to £2,580 per annum, the initial point depending on the qualifications of the successful candidate, plus F.S.S.U. benefits.

Applications should be sent to Dr. R. K. Harris, School of Chemical Sciences, University of East Anglia, Norwich NOR 88C, as soon as possible. The names and addresses of two referees are requested. (549)

FELLOWSHIPS AND STUDENTSHIPS

BRUNEL UNIVERSITY

(Department of Polymer Science
and Technology)

Applications are invited for the Cementation Chemicals Research Studentship from graduating and recently graduated chemists or material scientists.

Work will be in the field of solid polymer characterisation but aimed at products of civil engineering end use.

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Candidates—Male or Female should apply to:—

Mr. G. R. Southern,

BRUNEL UNIVERSITY

Department of Polymer Science,
Kingston Lane,
Hillingdon, Uxbridge.

(535)

INSTITUTE OF UROLOGY (UNIVERSITY OF LONDON)

in association with St. Peter's Hospitals

Applications are invited for a Research Fellow (with medical and/or scientific qualifications), in the grade of Lecturer (Senior Registrar status), for an investigation into the ultrastructure of the kidney and urinary tract epithelium. The successful applicant will be attached to the Electronmicroscopy Unit in the Department of Pathology, St. Paul's Hospital. Appointment for one year in the first instance, subject to renewal for a further period of two years. Applications to the Pathologist (N), St. Paul's Hospital, Endell Street, London, W.C.2. (390)

INSTITUTE OF UROLOGY (UNIVERSITY OF LONDON)

in association with St. Peter's Hospitals

Applications are invited for a Research Fellow (with medical and/or scientific qualifications), in the grade of Lecturer (Senior Registrar status), for an investigation into the ultrastructure of the kidney and urinary tract epithelium. The successful applicant will be attached to the Electronmicroscopy Unit in the Department of Pathology, St. Paul's Hospital. Appointment for one year in the first instance, subject to renewal for a further period of two years. Applications to the Pathologist (L), St. Paul's Hospital, Endell Street, London, W.C.2. (391)

UNIVERSITY COLLEGE LONDON RESEARCH STUDENTSHIP

Applications are invited from graduates (Class I or 2i) in Biochemistry or Zoology with biochemical interests to participate in a programme of research on haemoglobin and other blood proteins of lower Primates. Apply to Professor N. A. Barnicot, Dept of Anthropology, University College London, Gower Street, London WC1E 6BT. (545)

UNIVERSITY OF WESTERN AUSTRALIA Perth FORESTS DEPARTMENT POSTGRADUATE RESEARCH FELLOWSHIP IN BOTANY

Applications for the above-mentioned appointment are invited from graduates with a first-class or upper division second-class honours degree in Botany, and Forestry or Agriculture graduates whose courses of study have extended over at least four years and who have had appropriate research experience. The Fellowship, financed by the Forests Department of Western Australia, will be tenable for one year initially with prospects of renewal for up to two further years. The value of the Fellowship is \$A3,150 per annum. The Fellow will be required to undertake research in a field covering mycological, physiological or ecological aspects of tree growth in relation to multiple use forestry, or such other fields as may be prescribed.

Further information may be obtained from the Head of the Department, Professor J. S. Pate, in the University.

Applications in triplicate stating personal particulars, academic record, research experience, work proposed, etc., should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by December 31, 1974. Candidates should request two academic referees to write immediately to the Staffing Officer.

Conditions of Appointment obtainable from the Association of Commonwealth Universities, (Appts) 36 Gordon Square, London WC1H 0PF. (544)

UNIVERSITY OF EDINBURGH DEWAR FELLOWSHIP FUND Applications are invited for a

SENIOR RESEARCH FELLOWSHIP

tenable in the Department of Physics for a period of 2 years from October 1, 1974 (or as soon as possible thereafter). Applicants should hold a Ph.D. or equivalent qualification.

Salary will not be less than £2,118 per annum with F.S.S.U. benefits.

The Selection Committee will retain discretion, if a candidate of sufficient distinction is not available, to recommend the award of a

JUNIOR RESEARCH FELLOWSHIP

with salary in the range £1,400 to £1,600 with F.S.S.U. benefits.

Preference will be given to candidates whose intended fields of research lie in domains in which the subjects of physics and chemistry overlap.

Applications, naming two referees in each case, should be lodged with Professor W. Cochran, F.R.S., Physics Department, James Clerk Maxwell Building, Mayfield Road, Edinburgh EH9 3JX, not later than September 6, 1974. Please quote reference number 7009. (547)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY S.R.C. RESEARCH STUDENTSHIPS

Applications are invited for two research studentships within the department. Research will involve study of the metabolism of steroidal and non-steroidal drugs, in which the department already has an active interest.

The proposed programme of study would give the student instruction and experience in a wide range of chemical, analytical and biological techniques which are currently being employed in both industry and academic departments for studies in biochemical pharmacology and drug safety evaluation. Applicants are expected to have a first or upper second class degree in a relevant subject.

Applications, including a brief curriculum vitae and the names of two referees should be sent as soon as possible to:-

Mrs M. L. Whitley (Ref: JC/AS)
Department of Biochemistry,
University of Surrey,
Guildford,
Surrey. (496)

European Molecular Biology Organization (EMBO) Short- and long-term fellowships in molecular biology

The European Molecular Biology Organisation intends to award to scientists working in laboratories within the European area both short-term (from a few days to several weeks) and longer-term fellowships for collaborative research or advanced training in molecular biology.

The Short-term Fellowships are to support visits to other laboratories for the purpose of carrying out experiments with special techniques or of other forms of scientific collaboration or advanced training, and especially to support developments arising at short notice.

The Long-term Fellowships will usually be for a period of one year, but applications for renewal will be considered. They will be awarded upon individual application, at the "junior" level to promising young research workers who may then spend prolonged periods in other laboratories working under the guidance of leaders in the field of molecular biology. At the "senior" level, they may be awarded to enable established research workers to gain experience in new approaches and new problems. Upon application by European Institutions fellowships at the "senior" level may also be awarded to specialists who can assist in the initiation and development of research programmes in the sponsoring Institution. Such "sponsored" awards may be made to established workers at all stages of their career beyond the postdoctoral stage.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organisation, 6900 Heidelberg 1, Postfach 1022.40, Germany. (521)

IMPERIAL COLLEGE

Applications invited for Research Studentship leading to Ph.D., sponsored by the Analytical Chemistry Trust of the Society for Analytical Chemistry, for investigation of the application of the Opto-Acoustic Effect to Trace Analysis. Candidates should possess a good honours degree from a British University or an equivalent qualification acceptable to the trustees. Further information from, and applications to: Dr G. F. Kirkbright, Department of Chemistry, Imperial College, London, SW7 2AY. (508)

QUEEN'S UNIVERSITY, BELFAST

Studentship in Biochemistry

Applications are invited for a research studentship, three years, leading to a Ph.D. degree. The research topic is the significance of immunoreactive secretin in health and disease, which is part of a collaborative research programme of the Departments of Biochemistry and Medicine. The work will involve radioimmunoassays and immuno-affinity chromatography.

Applications including names of two academic referees should be sent to Dr R. F. Murphy, Department of Biochemistry, Medical Biology Centre, Queen's University, Belfast, BT9 7BL. (540)

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CHEMISTRY

POSTDOCTORAL FELLOWSHIP

Applications are invited for a POSTDOCTORAL RESEARCH FELLOWSHIP for studies on models of the iron-binding site of transferrin, tenable from September 1, 1974. The work will involve the isolation and study of iron compounds of multidentate ligands—especially by X-ray crystallographic methods. Candidates should hold a Ph.D. in Inorganic Chemistry and experience in both transition-metal chemistry and X-ray crystallography is desirable. Tenure one year but may be renewed for a second year. Salary up to £2,058 or £2,247 from October 1. Applications, with the names and addresses of two referees, to Dr N. A. Bailey or Dr E. D. McKenzie, Chemistry Department, The University, Sheffield S3 7HF. Reference R 121/G. (552)

RHODES UNIVERSITY GRAHAMSTOWN, 6140 SOUTH AFRICA

HUGH KELLY FELLOWSHIP

The Rhodes University Council announces the establishment of the Hugh Kelly Fellowships. The Fellowships are for senior scientists wishing to undertake or continue their research in any of the departments within the Faculty of Science; the first of these Fellowships will become available in 1975. The Faculty comprises the Departments of:-

Botany, Chemistry, Geography, Geology, Mathematical Statistics, Mathematics (Applied, including Computer Science), Mathematics (pure), Microbiology, Pharmaceutical Sciences, Physics, Psychology, Zoology and Entomology.

Closely associated with a number of these departments are the Leather Industries Research Institute, the Institute for Freshwater Studies and the J.L.B. Smith Institute of Ichthyology.

The Fellowship in 1975 is available for a period of six months, which period shall include two academic terms of the University. The emoluments attaching to the Fellowship include an award of R4 000 (R1—approximately 62 n.p. sterling) and a tourist class return air fare from the Fellow's place of residence.

The Fellow will be required to present the Hugh Kelly Lecture on a subject of his choice during the tenure of the award.

Forms of application and further particulars may be obtained from the Registrar, to whom completed applications should be submitted by October 31, 1974. (515)

LA TROBE UNIVERSITY MELBOURNE, AUSTRALIA RESEARCH FELLOW IN GENETICS AND HUMAN VARIATION

(one or two positions)

Applicants will be appointed for one or two years, with possible extensions up to a total tenure of three years. They will be expected to work in one of the areas of interest of the Department which include behavioural, cellular, ecological, human, microbial, population and quantitative genetics and cytogenetics. Enquiries may be directed to Professor P. A. Parsons, Chairman of the Department, in the University.

Salary: \$A7,545 to 2 by \$A292 to 3 by \$A291 to 4 by \$A479 to 5 by \$A478 to \$A12,352.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, La Trobe University, Bundoora, Victoria, Australia 3083.

Applications close on September 13, 1974. (529)

UNIVERSITY OF LEICESTER

Applications are invited for a CASE STUDENT-SHIP. The Successful applicant will work with professor M. C. R. Symons on a research project entitled "Quantitative study of Autoxidation Reaction with the aid of E.S.R. and kinetic techniques". The research will be conducted in cooperation with Dr N. Uri, and the student will spend a proportion of his/her time at the research laboratories at Waltham Abbey during the 3-year Ph.D. training.

Applications should be made to professor M. C. R. Symons, Department of Chemistry, University of Leicester, Leicester LE1 7RH, from whom further details may be obtained. (525)

UNIVERSITY OF SOUTHAMPTON
DEPARTMENT OF CHEMISTRY
POSTDOCTORAL FELLOWSHIP

Applications are invited from Physicists and Chemists for a postdoctoral fellowship to study some of the unusual properties of liquid crystals. Attention will be concentrated on the behaviour of a liquid crystal when it is subject to a magnetic field and a viscous torque produced by spinning the sample. The response of the system to these two perturbations will be monitored with the aid of electron resonance spectroscopy. Previous experience in this branch of spectroscopy or of liquid crystals would be an advantage.

The fellowship is available for a period of one year with a Salary £2,247 per annum, plus F.S.S.U. benefits.

Applications, including a curriculum vitae, list of publications and the names of two referees should be sent, as soon as possible, to: Dr G. R. Luckhurst, Department of Chemistry, The University, Southampton SO9 5NH. (541)

A small number of FELLOWSHIPS for one, two or three months are available in 1975. The salary will amount to about 4,500£ a month. Travel costs are at visitors own expense. Applications with list of publications should be sent before December 1, 1974 to:

Monsieur le Directeur de l'Observatoire de Nice
Le Mont Gros
06300 - NICE
FRANCE. (584)

THE UNIVERSITY OF
ASTON IN BIRMINGHAM
DEPARTMENT OF BIOLOGICAL SCIENCES
RESEARCH FELLOW
IN FISH BIOLOGY

Applications are invited for a Research Fellow to work in a small research team led by Mr H. A. Hawkes, studying the effects of a sewage effluent on the survival, growth and tissue contamination by metals of fish populations in simulated streams. Experience in fishery biology—involving population studies and/or fish toxicology—involving histochemical techniques is required. The appointment which will date from October 1, 1974 will be for a period of three years.

Commencing salary will be within the range £2,118 to £2,412 per annum on a scale rising to £3,636 per annum.

Requests for further details and application forms (which should be returned not later than August 26.) should be sent, preferably on a postcard, quoting Ref. No. 988/... to the Staff Officer, the University of Aston in Birmingham, B4 7ET. (556)

UNIVERSITY COLLEGE DUBLIN
FACULTY OF AGRICULTURE
POSTDOCTORAL RESEARCH
FELLOW

Applications are invited for a postdoctoral research fellowship to collaborate on a study of factors affecting intake and metabolism of silage by ruminants on a three year project sponsored by the National Science Council.

Starting salary at £2,100 p.a.

Applicants should have a degree in Agriculture or Biochemistry or Microbiology, followed by appropriate research experience.

Enquiries and applications should be sent to:

Department of Agriculture Chemistry,
Dr J. L'Estrange,
University College,
Glasnevin,
Dublin 9. (604)

UNIVERSITY COLLEGE DUBLIN
DEPARTMENTS OF ZOOLOGY AND BOTANY
RESEARCH FELLOWSHIPS IN
RIVER ECOLOGY

Two posts are offered for Research Fellows to cooperate in a "Base-line Survey of the Caragh River, Co Kerry". Candidates should have obtained a Ph.D. or be about to submit; those with an M.Sc. only will be considered if they have appropriate experience. Experience should include the following:

Fellowship I—A general knowledge of stream and river fauna with expert knowledge in at least two of the following orders (Ephemeroptera, Plecoptera, Trichoptera or Diptera-Chironomidae).

Fellowship II—A knowledge of fresh-water algae and techniques for the determination of their productivity.

A knowledge of appropriate physical and chemical techniques is desirable for both applicants.

The salary offered to a suitably qualified applicant is £1,800 to £2,250 depending on qualifications. The position is tenable for one year with a possible extension to a second year. The work will be based in Dublin and an allowance for field expenses will be paid.

It is hoped that candidates would take up the posts on October 1, 1974.

Applications should be forwarded to the undersigned, together with details of qualifications and the names of two referees before August 7, 1974.

Botany Department,
The Secretary,
U.C.D.,
Belfield,
Dublin 4. (605)

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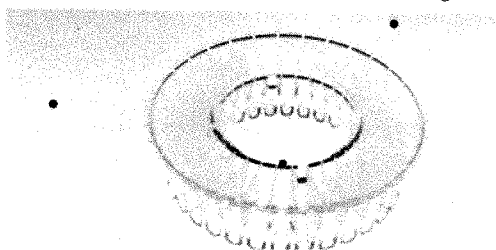
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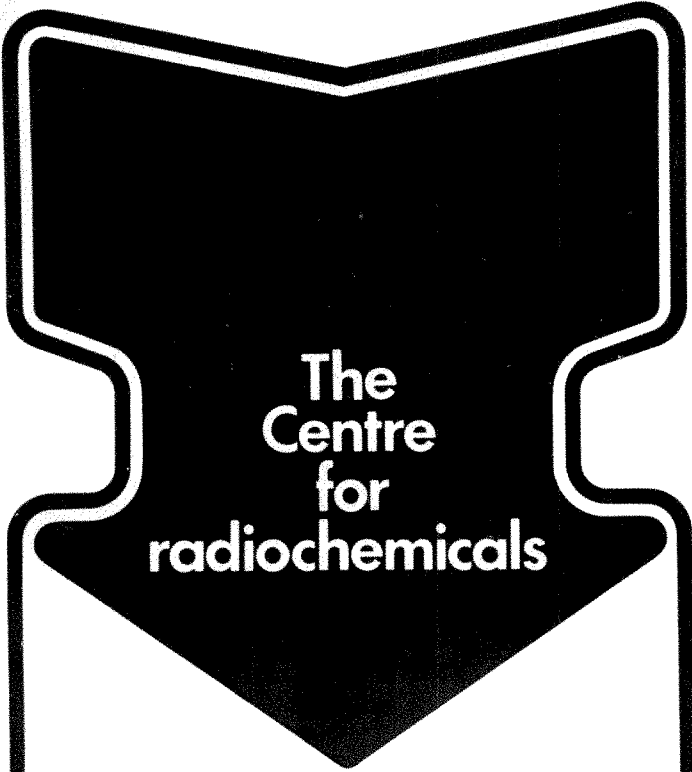
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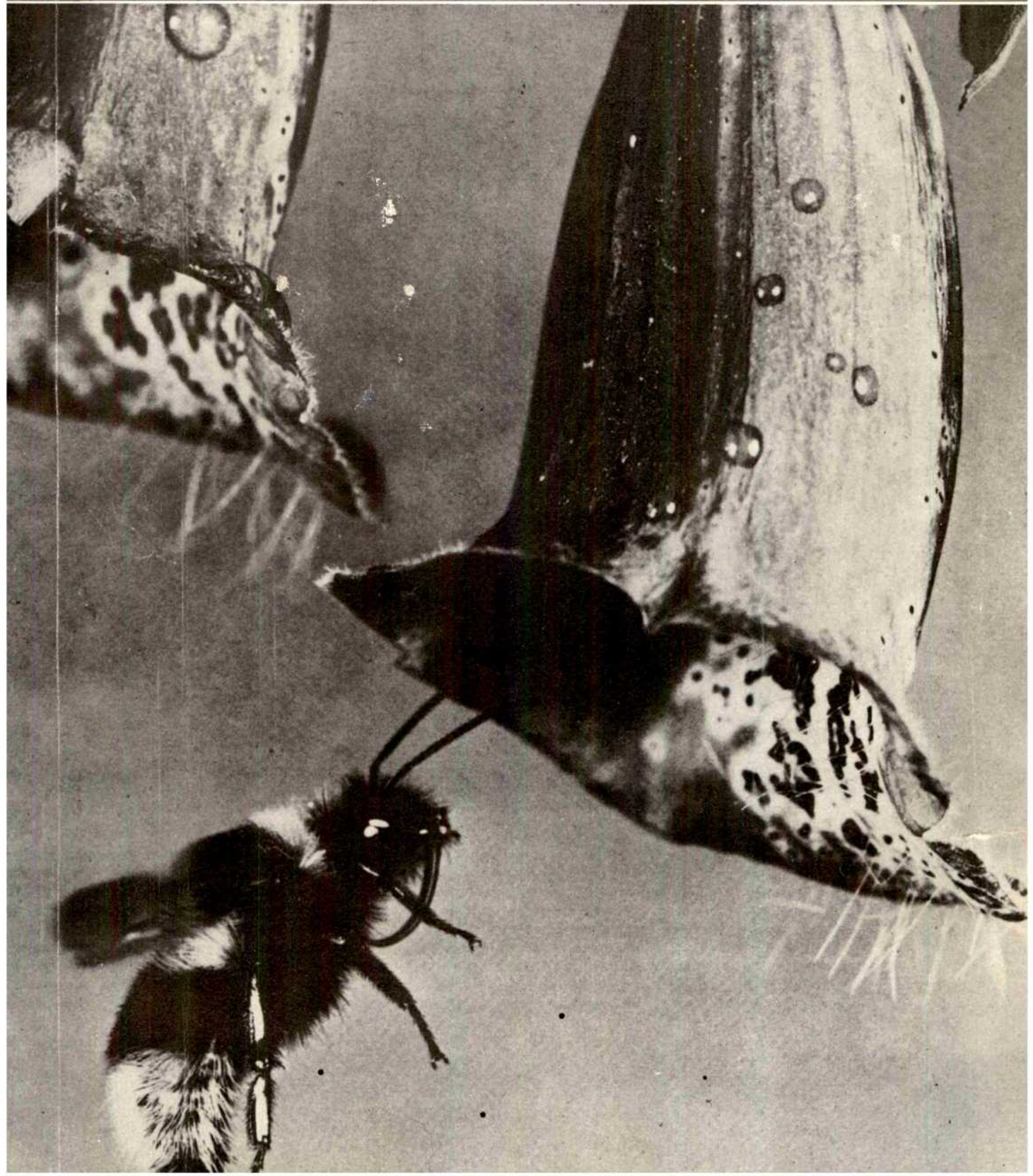
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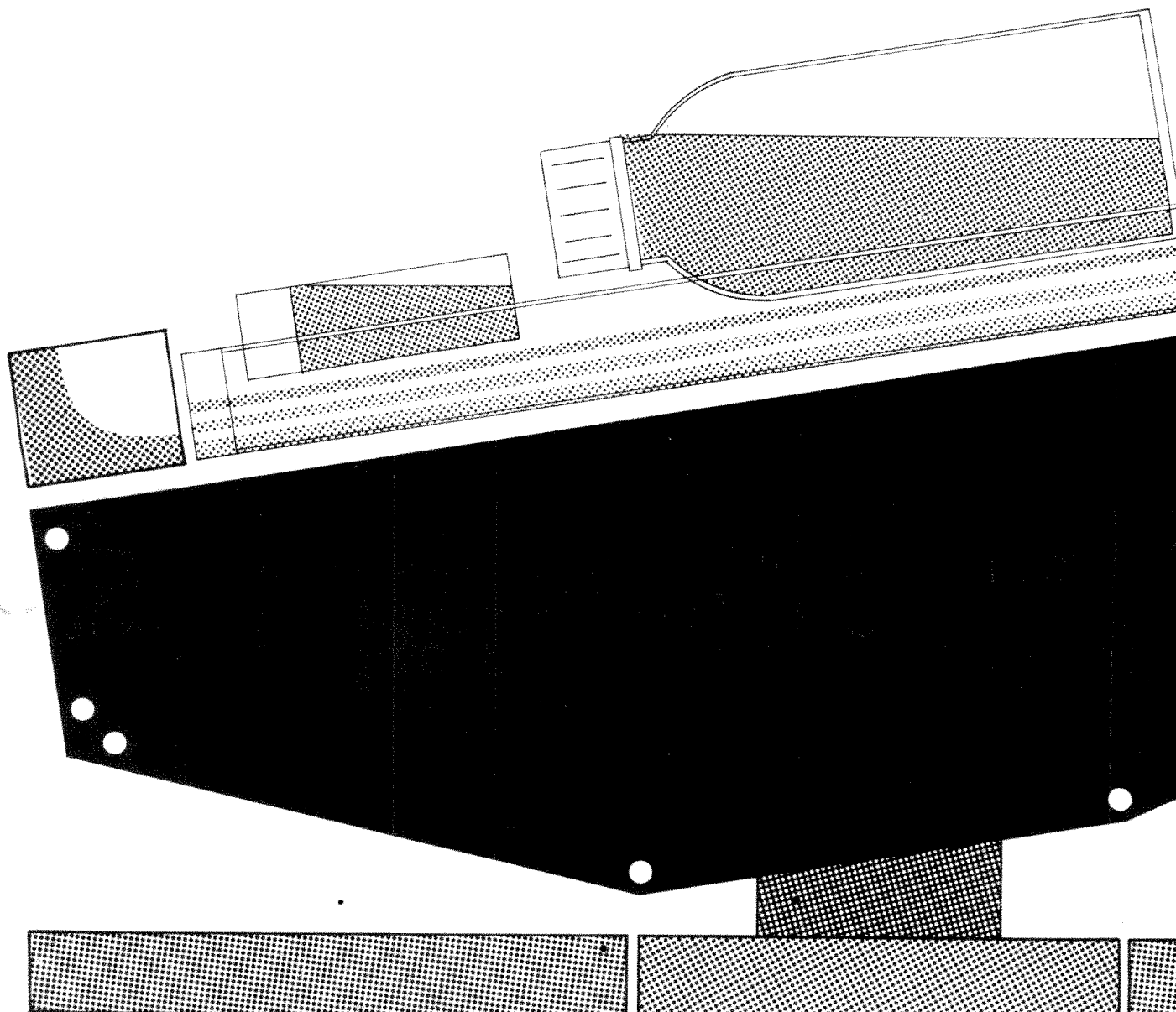
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Cover picture

Bumble-bee flying towards the bell of
a foxglove, from *Insects in Flight* by
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Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. $\exp(a)$ is preferred to e^a if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

(i) If it is necessary to refer to several references by the same author at once, only one reference number need be given.

(ii) The last page as well as the first of any reference should be cited.

Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

A fuller guide appeared in *Nature* (246, 238; 1973).

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The time-bomb ticks on

IN 1969 the Institute of Race Relations in London produced a massive report on race in British society, and in particular the problem of the coloured immigrant. *Colour and Citizenship* was a mine of information, not all of it very palatable. It also had some strong recommendations to make on the subjects of education and employment.

Broadly, the report recommended that the Race Relations Board "should make full use of its powers to initiate investigations". It continued, amongst other things, to say, "it is essential for a non-discriminatory employment policy that records be kept which distinguish employees by ethnic origin." This would be seen by some as discriminatory in itself, but there was felt to be no other way to determine whether minorities were afforded equal opportunities.

Five years later, the House of Commons Select Committee on Race Relations (in a report issued last week) is still loath to grant the Race Relations Board legal powers to initiate investigations, being dissatisfied with the preventive work it has done so far.

But how does all this affect the scientist; surely this is a matter with which he may have some general liberal sympathy but is it not really an affair for the industrial shop-floor?

About 2% of the population of Britain is coloured immigrant or of immigrant descent. West Indians comprise half this figure, Indians and Pakistanis another 40% of it. By 1986 the 2% will be up to 3% or maybe 4%, depending on immigration policies and fertility. It is erroneous, even now, to assume that the distribution by age of this section of the population is such that the children of coloured immigrants are not yet at an age at which others seriously consider further education, say 16 to 21. The percentage of immigrants in this age bracket is close to the percentage of the general population in the same bracket. Two percent of those eligible, in terms of age, for a university education are immigrants or of immigrant descent.

The next question is, obviously, how many are getting a university education, and it is at this stage that problems arise. Every university campus is, of course, a very multiracial affair but many of those contributing to this are temporary immigrants, in Britain for educational purposes. It is impossible to get statistics about permanent immigrants at university; these figures do not exist, indeed some university registrars expressed surprise that we should wish to know the numbers, as in order to be scrupulously fair they could not possibly ask of candidates for admission anything more probing than their place of birth.

This seems a wrong if well intentioned policy, and the sooner it is rectified in universities the better; the select committee has come to a similar conclusion in the more general fields of employment (duplicating the 1969

proposals of the Institute of Race Relations).

Those who do not learn from history are forced to relive it, and there are the clearest lessons from recent American history. American experience—with a much larger minority group population—is that passivity in matters of race relations leads to greater alienation. It was simply not good enough to bemoan the lack of suitably qualified applicants and place the blame elsewhere—in the schools, in the social system or whatever. Accordingly the whole process of acquiring staff as well as students in many American universities, and particularly those with government contracts, is a complex business of satisfying law enforcement agencies that positive steps have been made to recruit minorities. This is a tedious process in many ways, and is both irritating to the academic community and somewhat humiliating to the minorities involved. On the other hand, blunt as it is, it is the only tool available to ensure that at least those who have some aspirations towards higher education do get reached.

It would probably be wrong at present to go for measures as drastic as this in Britain if there is any hope that lesser measures can succeed, and there may be a few years left before alienation is so total amongst some immigrants that the thought of a university education is anathema. But the next few years will require some positive action—one idea widely supported is that since many immigrants only discover in their twenties that they have unrealised potential for which higher education would have been beneficial, universities should come to terms with a fairly regular intake of more mature immigrant students. Another need expressed by workers in areas of high immigrant numbers is for more people from places of higher education to visit their community, not to announce that they are lowering standards for immigrants, but to give potential students the challenge of raising their own standards.

100 years ago



LADY BARKER'S "LESSONS ON COOKING"

First Lessons in the Principles of Cooking. By Lady Barker (London: Macmillan and Co., 1874).

IN this little volume the authoress has proved beyond all manner of doubt how completely she is the right woman in the right place. Surely nowhere could the Committee for the National Training School for Cooking have found a lady superintendent better fitted than Lady Barker to put life and spirit into the scheme which they advocate, or one more thoroughly qualified to train and marshal the feminine bands that are now being drilled under her supervision in the South Kensington Schools of Cookery to invade and revolutionise the kitchens of the future in every part of the empire.

From *Nature*, 10, 283, August 13, 1874.

The unendangered whale

Dr Ray Gambell, of the Whale Research Unit at the British Museum (Natural History), discusses the new rules for whaling adopted by the International Whaling Commission this year and argues against previous calls for a complete moratorium.

EVER since the 1972 Stockholm conference on the human environment there has been a persistent call to impose a 10-year ban on commercial whaling. This year's annual meeting of the International Whaling Commission (IWC), held in London in June, again considered this proposed moratorium. The IWC's Scientific Committee advised that because the concept of individual species management is now operative, there is no biological requirement for a blanket ban on all catching, and indeed, catching of some species may be necessary to promote the maximum rebuilding of other depleted stocks because of interspecific competition.

The IWC, which regulates about 90% of the world's whaling, therefore adopted an amended moratorium proposal which sets out the criteria by which the various whale stocks will be harvested in future. Briefly, these divide whale stocks into three categories; 'protection stocks', which will not be hunted at all; 'sustained management stocks', which can be caught at carefully controlled levels; and 'initial management stocks', which are very abundant and can also be taken in suitably regulated numbers to prevent any risk of over-exploitation.

By adopting this regime for future commercial whaling activity, the IWC has effectively placed the regulation of whaling in the hands of its Scientific Committee. This committee is charged with the responsibility of allocating the various whale stocks into the three categories defined above, and then deciding the level of catch which each can sustain if it is available for exploitation. The original Convention for the Regulation of Whaling signed in 1946 was formulated to provide for the conservation, development and optimum utilisation of the whaling resources. This was to be based on scientific findings, but could also take into account the interests of the consumers and the whaling industry. In the past the

economic arguments of the latter two groups often outweighed the advice given by the scientists, with the consequent decline of some of the major whale stocks. Now the emphasis has swung very much towards the scientific side. It remains to be seen just how well the scientists can resist the political and economic pressures which up till now have been exerted mainly in the full commission meeting rather than in the Scientific Committee.

The present scheme for the management of the whale resources can be seen as part of the general development of the policy adopted by the IWC in recent years. This is based on the concept of sustainable yield. In a stable, unexploited population the number of new recruits exactly balances the deaths due to natural mortality. When the population size is reduced by whaling, the recruitment rate increases through a lowering in the age at which the animals become sexually mature, and also by an increase in the pregnancy rate. The natural mortality rate also falls as whales are caught before they have a chance to die naturally.

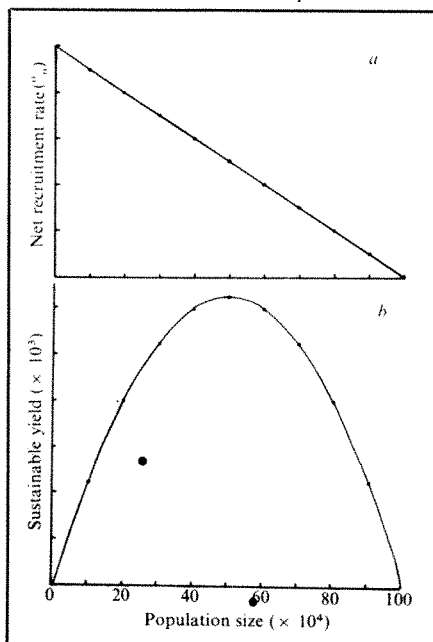
The exact forms of the relationships between the changes in recruitment rate and natural mortality rate with decreasing population size are not known for

certain in the whales, although there is some evidence from blue, fin and sei whales that the recruitment rate increases linearly. In Fig. 1a such a linear form for the variation in a theoretical net recruitment rate with population size is shown. This represents the gross recruitment by reproduction less the natural mortality. Multiplying every population size by the appropriate net recruitment rate gives a number, plotted in Fig. 1b, which is the surplus of recruitment over natural deaths. This surplus is a yield which can be harvested indefinitely without changing the overall population size. As can be seen from Fig. 1b, the yield is small in populations which have been much depleted in size. It is also small in those populations which have been but little reduced. The yield reaches a peak, the maximum sustainable yield (MSY), at some intermediate population size which is generally around half the original population number. Catching the sustainable yield will hold a population at its present level. Catching less than the sustainable yield will mean an addition to the total population size, which will therefore increase in numbers. For a population which is smaller than the level giving the MSY, this will allow some rebuilding towards that level; a population above that level will increase still further. Conversely, catching more than the sustainable yield will deplete the population, and for a population already below the MSY level will make it still less productive.

It is relevant now to consider where the various whale populations stand in relation to their MSY positions. In both major whaling areas, the Southern Hemisphere and the North Pacific, the same species are in very similar states. Blue, humpback and right whales are very seriously depleted and far to the left in the yield diagram (Fig. 1b). This is why they are totally protected from catching by the IWC. Grey whales in the North Pacific are also protected, although they have recovered from a very low level and now seem to be stabilised near their former original number.

Sei whales are close their MSY levels in both regions, although there are some variations between the component stocks in each case. Male sperm whales are also close to their MSY levels, but the females are relatively unexploited and far to the right of the yield diagram. Antarctic minke whales are also very

Fig. 1 *a*, A theoretical relationship between the net recruitment rate and a whale population size. *b*, Sustainable yield at each whale population size derived from the theoretical recruitment relationship.



The end of the line.

Copyright: National Institute of Oceanography.

abundant, as they have only been caught in large numbers in the past two seasons.

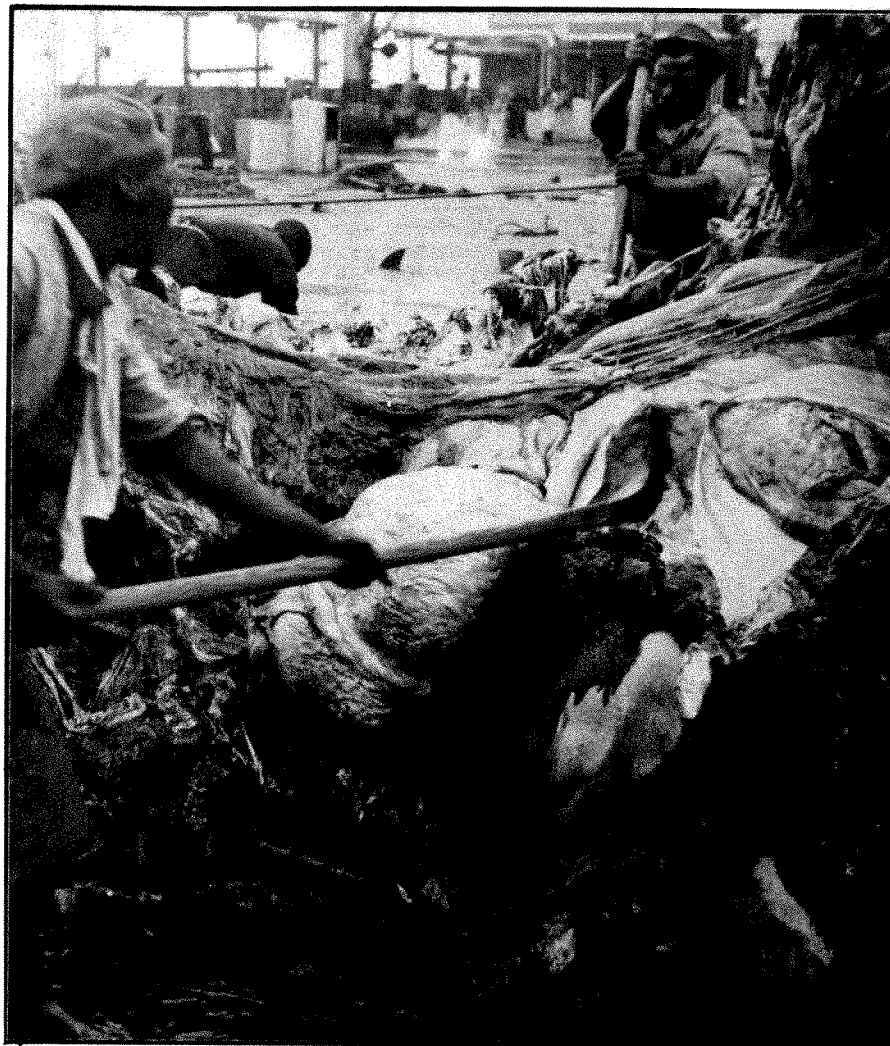
Fin whales, although a major component of the whaling industry's economy, are only at a third or a half of their MSY levels, and so need to rebuild in both regions.

Since 1965 the IWC has had the stated intent of setting the catch limits below the sustainable yields as calculated by continuing assessments. At that time the catch limits for the Antarctic baleen whale were set in terms of Blue Whale Units (BWU). One BWU equalled 1 blue whale, 2 fin whales, $2\frac{1}{2}$ humpbacks or 6 sei whales. These values were based on the relative oil yields of the different species, but the system was unfortunate because it took no account of the fact that each species may need a different degree of protection. The same criticism can also be levelled at the idea of a total moratorium on whaling, for not all whale stocks require such protection.

Catches of whales in the North Pacific have always been limited on a species basis, and from 1972 the Antarctic catch limits have also been set species by species. An even finer degree of control would be achieved by regulating the breeding stocks independently, but these cannot yet be fully identified. As a move in this direction, though, the IWC has now subdivided the Antarctic into three areas, and imposed maximum catches for each species in each of these areas.

Sperm whales, the largest of the toothed whales, did not come into the BWU system. In 1972 the Scientific Committee recommended that the catches of this species should be limited by each sex separately. This is practicable because the male sperm whales grow considerably larger than the females. The females had been protected from much catching by a minimum size limit. The Scientific Committee therefore also recommended that this size limit should be reduced, so that more females could be caught to bring the sexes into better balance. This is reasonable with the total catch limits imposed, which include a subdivision of the Southern Hemisphere into three areas with separate limits for the sexes in each.

The overall catch limits set this year are summarised in the Table, together with the estimates of sustainable yields. It is apparent from all this evidence that



the catches will not cause any species to be in danger of becoming extinct. Even the fin whale, which is the most reduced of the major species still hunted, should be rebuilding slowly. In addition, the IWC is committed to stop the capture of all fin whales in these two regions at least by 1976, so that the populations will then increase at the maximum rate possible.

The Scientific Committee will hold a special meeting to consider the assessment and status of all whale stocks in time to advise the commission next year on these matters. In addition, it will put forward detailed plans for extensive monitoring and research on the stocks. It is hoped that the costs of these projects, running into several millions of pounds, will be met by the United Nations (UN) Environment Programme, the UN Food and Agri-

cultural Organisation and other similar agencies.

There is much research needed. Up to the present, each whale species has been considered in isolation, without regard to the other whale species or the rest of the environment. Evidence of intelligent actions is now becoming available. In addition, concepts involving the biology of whales rather than their numbers will require study before they can be incorporated into the sustainable models already employed.

At all events, the future for whales looks bright. There is no danger of any of them disappearing through overexploitation under the present controls, and there is the prospect of management being grounded on rational policies designed to provide the greatest long term benefit from this considerable natural resource of the oceans.

Overall catch limits set by the IWC and the estimated present sustainable yields of the whale populations

	Antarctic		North Pacific	
	Quota	SY	Quota	SY
Fin	1,000	3,200	300	750-900
Sei	4,000	5,200	2,000	2,500
Minke	7,000	7-12,000	—	—
Sperm* ♂	8,000	8,500	6,000	6,200
♀	5,000	4,500	4,000	1,700

*Whole Southern hemisphere.

international news

Two years ago a massive research programme on cardiovascular disease was launched in the United States when Congress passed a bill inelegantly entitled the National Heart, Blood Vessel, Lung and Blood Act of 1972. Modelled on the rhetorically overburdened campaign for the conquest of cancer, the research programme has received enthusiastic endorsement by President Nixon, who has repeatedly reaffirmed the administration's commitment to find ways of reducing the death toll from heart disease—"the nation's number one killer". But an expert committee, which is supposed to keep an eye on this frenzied research programme has charged that the effort so far has been grossly underfunded and badly misdirected. In short, the Administration's commitment has been long on rhetoric but short on dollars.

The criticisms were made in a report to Congress by the National Heart and Lung Advisory Council which was actually written in December last year but which has spent the past seven months being reviewed by the Office of Management and Budget and the Department of Health, Education and Welfare. President Nixon finally passed the document on to Congress last week with the message that it "merits serious consideration", although it contains recommendations which are "at variance with the Administration's views".

Chief among those recommendations is that the National Heart and Lung Institute (NHLI) should be given a budget this year of \$520 million, instead of the \$309 million proposed by the Administration. The council charged that the amount of money being spent on cardiovascular research was actually

Heart research short on cash

by Colin Norman, Washington

less last year than in 1968 if inflation is taken into account, and it reported that "the resources for this accelerated attack [on heart disease] have not been forthcoming".

Such complaints have come as no surprise to the Administration, or to anybody else for that matter, since the council is composed chiefly of scientists directly or indirectly engaged in research on cardiovascular disease, and the report thus has all the appearances of special pleading by a section of the biomedical community. Although the Administration has so far made no formal public response to the council's recommendations, officials point out that the National Heart and Lung Institute (NHLI) has in fact fared relatively well. Since this year the Administration proposed a hefty increase in its budget (\$25 million) while all the other institutes at the National Institutes of Health with the exception of the National Cancer Institute either had their budgets cut or kept static.

Nevertheless, it is difficult to deny that much of the proclaimed attack on heart disease has gone off at half cock, at least when it is measured against the intentions of the 1972 Act. The Bill suggested that \$460 million should be spent by NHLI in the 1974 fiscal year—which ended on July 1 this year—and that \$520 million should be spent in the 1975 fiscal year. Only \$280 million was

spent last year, however, and the Administration has proposed a budget of \$309 million for 1975.

Aside from the question of funding, the council also takes the Administration to task for a variety of other sins, chief of which is the move early last year to abandon the policy of providing fellowships and traineeships for biomedical scientists. Although the council neglects to mention that in July last year the Secretary of Health, Education and Welfare, Caspar Weinberger, announced that the Administration would not scrap the training programmes after all, it states that the unavailability of funds for biomedical training "has reduced the research manpower and the productivity of many of the very best research groups supported by the National Heart and Lung Institute." (Last month, Congress finally approved a bill which will more than double the Administration's proposed expenditure on biomedical research training. Administration officials said last week that they are looking at ways to implement the bill, and presumably that will still the criticisms of the council.)

The council also comes up with a clutch of recommendations for endowing chairs at universities for professors engaged in cardiovascular research, for maintaining a good balance between grants and contracts, and for establishing special research and demonstration centres around the country. Another prominent suggestion is that Congress should establish minimum qualifications for members of the council itself; this is believed to be a reference to the fact that the singer Frank Sinatra was appointed to the council last year, but failed to attend any of its meetings.

A SOUTH AFRICAN gynaecologist is pioneering a technique for fallopian tube transplant operations which may offer a better answer than *in vitro* fertilisation for childless women.

For *in vitro* fertilisation to take place the egg must be caught at the precise moment of ovulation, and since ovulation can occur at any time, doctors must be on constant alert and time-consuming observations of the woman is needed. By contrast, the suggested transplant technique is much simpler and cheaper, because after a short operation nature can take its course.

According to Dr Abraham Rubin, he and his colleagues at the University

Transplant babies?

from Ian Ridpath

of the Witwatersrand Medical School in Johannesburg are hoping to start soon an experimental programme that will lead to the first operation of this kind in women.

Fallopian tubes for transplant are readily available from women who have had a hysterectomy. Substituting them for diseased tubes is a relatively simple operation that is little more than an extension of existing surgical techniques regularly used by gynaecologists. Successful fallopian tube

transplants have already been performed on sheep by Dr M. Katz at the University of Cape Town, and Dr Rubin thinks that the same technique can be extended to humans.

It would be particularly valuable where infection of the fallopian tube is common, says Dr Rubin. Unlike other forms of transplant there are no worries about rejection, since the technique is to use the tube for one cycle and then it can be removed. Rejection only becomes a problem after three to four weeks. Donors do not therefore have to be specially selected, which helps to make the operation more widely available, and Dr Rubin envisages it being performed regularly.

Geologists as professionals

from Peter J. Smith

PHYSICISTS, chemists, biologists and mathematicians in Britain have their respective professional institutes, and there are no less than 15 chartered institutions catering for the needs of the various types of engineer. British geologists, by contrast, have no professional body, although members of certain subdisciplines within the earth sciences are eligible to join appropriate existing organisations (some geophysicists, for example, are members of the Institute of Physics). So is there a case for the formation of a professional Institute of Geology? A working group sponsored by the Geological Society believes there is, and in its published report (*Report of the Working Party on Professional Recognition*, Geological Society of London; 1974) recommends that the degree of support for a professional organisation in geology be widely tested.

The Geological Society itself is a learned society, but has lately been finding it difficult to reconcile its traditional role with the need to represent British geology to the outside world. As an example of this need, the report cites the setting up in 1973 of the Council of Environmental Science and Engineering by the two councils representing, respectively, the professional science institutes and the chartered engineering institutions. Because there is no professional body of geologists affiliated to the two sponsoring councils, geologists have no formal participation in an organisation concerned with environmental problems.

Because earth science is one of the two branches of science most directly concerned with the environment, the need for a 'voice of British geology' is clearly much more than a parochial academic matter. The Geological Society working group has therefore examined in some detail the case for a professional body which "would be expected to provide representation of geological interests at different levels". But external representation is not the only, nor perhaps even the principal function of a professional institute; and it is envisaged that the proposed Institute of Geology would also operate a code of ethics and professional conduct, set professional standards for the various categories of membership, provide advice on career structures and personal security, and disseminate information on general and professional matters.

But if the idea of an Institute of Geology (by this or any other name) comes to be widely accepted, how could it be put into practice? The re-

port considers five "alternative [sic] pathways" (in addition to maintenance of the status quo) but in the end rejects four of them as unlikely to succeed. The first—the creation of a professional class of Fellowship within the Geological Society—was rejected at an early stage in the discussions because although it would strengthen the society financially and allow it to speak for professional geology nationally, it would probably lead to a dissension among the ranks of the non-professional geologists (the "second class Fellowship"). Moreover, there is some doubt as to just what professional services the society would be allowed to offer under the terms of its Charter; the lack of significant additional services recently led to the failure of a similar scheme in South Africa.

Indeed, the Charter seems to be a major stumbling block, for both it and the society's grace-and-favour apartments might also be put at risk by two other possible schemes — a more thoroughgoing conversion of the society into a professional-cum-learned society (a solution adopted by the physicists) or the creation of a new body under the society's direct sponsorship. This then leaves just two other possibilities—the creation of a new body by independent sponsorship, either with no society participation or with the society's general support. The first of these is rejected because of the high costs arising from the need for administration and accommodation separate from the society and because of the possibility of conflict with the society. In the end, therefore, the working group recommends independent sponsorship with society support.

The working group is now soliciting views from as many geologists as possible, both on the idea of a professional body as such and on the proposed structure as set out in an appendix to the report. Those who respond should pay particular attention to the latter, for experience suggests that detailed organisational points which appear unimportant can, in fact, have profound consequences. For example, the working group suggests, without discussion, that the new institute should "be established as a charitable body"—an apparently innocuous enough statement. But under the present, admittedly chaotic, laws governing charitable bodies in Britain this would prevent the institute from taking part in any activity remotely political. Registration as a charity would therefore place constraints on the institute which are in many ways comparable to those now acting on the Geological Society itself. Would this be in the best interests of the geology profession?

Finally, there is at least one surprising omission from the report. The

working group makes the contentious point that "the science of geology embraces many diverse fields including . . . geophysics . . .", but fails to consider, or even mention, the anomalous position of British geophysicists within the Royal Astronomical Society. Although the working group does not regard itself "in any sense as representative", it would surely have been sensible to have had a geophysical representative of that society on the group right from the start.

Ispra's 'factor up' energy programme

THE energy crisis has done a service to both the solar and hydrogen energy projects at the largest of the European Community's Joint Research Centres, that at Ispra on Lake Maggiore, originally an exclusively Euratom establishment. The aim of the solar work, which was only initiated in 1973, is to probe promising areas not well covered either by industry or national research establishments in member countries, and a substantial increase in funding is expected this year.

The approach may be described as a 'factor-up' programme. Fundamental to work at Ispra is that solar energy—although abundant and nonpolluting—is both diffuse and intermittent. Accepting that it is already possible to heat a house by solar energy—it is being done in the French Pyrenees through air convection generated in hollow blackened walls—Ispra is investigating higher efficiency systems. For domestic heating it is pursuing a plug-in, fixed-angle collector unit (not a house) capable of generating 100° C where preser collectors do not surpass 65° C, large because of heat loss from the surface of the collector. Small scale tests of a method are in progress and these involve adaptation of the 'anti-radiation' cells developed by Francia.

A temperature of 100° C is a threshold value for another small 'domestic' application of special interest in underdeveloped arid regions. "To use the Sun to pump water out of the desert" as the brisk energy director at Ispra, Dr Joachim Gretz puts it. A heat source of this order could be coupled to a 1-kW power set able to pump up water for 8 hours a day (during sunlight) for human and animal subsistence. Dr Gretz points out that in many areas primitive concepts of the natural world mean that communities are dying of thirst while there is pumpable water only 16–60 m down.

Looking further ahead Ispra sees real promise in quantum devices with which yields of 1 kW m⁻² would be possible through photoelectric or photochemical conversion. □

correspondence

Victimisation

SIR,—In your issue of July 12 you publish the appeal of Dr Peleska, the Czechoslovak scientist, to the World Federation of Scientific Workers.

Unfortunately, the WFSW has many allegations of victimisation against scientific workers brought to its attention, involving many different countries. For example, in the same letter as that in which I reported the receipt of Dr Peleska's letter to the vice-presidents of the federation, I also had to report two other allegations, in some respects even more serious. There was the case of Professor José Ferreira de Alencar, a Brazilian anthropologist and sociologist, who was said to have been imprisoned and ill-treated as a direct consequence of reports he has written on the social status of the people of North-East Brazil. There was also the case of Professor Horst Holzer, one of the leading sociologists in the Federal German Republic, who was dismissed in April 1974 by the Bavarian Ministry for Education and Culture because of his membership of the German Communist Party.

There is no doubt of the policy of the World Federation of Scientific Workers on the question of the right of scientists to work. It is, for example, dealt with in the Declaration on the Rights of Scientists, adopted at the Ninth General Assembly of the Federation in Paris in 1969. Thus:

"3.2. Scientific workers should have the right to work in accordance with their scientific capacities and Governments should endeavour to ensure this right.

"3.5. Scientific workers should have equal rights in their professions, regardless of sex, race, nationality, creed or political conviction."

We have affiliated organisations of scientific workers in 30 countries and they all accept these basic principles of the Federation. When a serious *prima facie* case of victimisation of a scientist is brought to our attention we refer it to our affiliated organisation if there is one in the country concerned, ask them to look into the matter and, where appropriate, either take action themselves or suggest possible action the Federation can take. In some cases in which we have intervened in this way appropriate remedial action has been taken. In other cases new circumstances have been revealed

which have put a different complexion on the case.

It is difficult to see what further action is open to us. The preamble to our Constitution states quite clearly: "The Federation will endeavour . . . to develop relations between scientists having regard to the autonomy of each organisation, to the equality of rights, to the avoidance of interference in the affairs of national organisations." It is difficult to see how an international body could be based on any other principle. Differences of practice between different countries are very great and appropriate allowance must be made for this. Very many useful initiatives have been taken by the Federation and the close contacts maintained between our affiliated organisations in different countries are beneficial to scientists and for science itself. Our aim must be to strengthen these contacts.

Yours faithfully,

E. H. S. BURHOP

World Federation of Scientific
Workers,
London, UK

Ill reactor?

SIR,—The United Kingdom is now not only a *de facto* but also a *de jure* partner in the Institut Laue Langevin, built around the high flux neutron beam reactor. Since we are equal partners in the running and use of the establishment, we might also claim an equal share in its name. Fortunately, the choice of a suitably alliterative British scientist is obvious. What could be better than to call it the Institut Laue Langevin Lonsdale. Such a choice may be justified on several grounds:

Kathleen Lonsdale was a most distinguished scientist, the first woman elected Fellow of the Royal Society. She was a crystallographer, hence her name has obvious associations with the high flux beam reactor.

Just as von Laue and Langevin, Kathleen Lonsdale was all her life a champion of dignity and freedom.

Changing ILL to ILLL would have the advantage that newspaper headlines could no longer be misunderstood, and there would be no danger of an announcement like "Ill Deputy Director Returning to England" causing anguish to Dr W. M. Lomer's friends.

Yours faithfully,

N. KURTI

Clarendon Laboratory,
Oxford

Anonymous refereeing

SIR,—It seems that the best way to obviate the misuse of the unilateral anonymity granted to reviewers is to extend anonymity to authors as well. When the reviewers get a paper from the editor but have no idea who the authors are or what their affiliation is, they would find less pleasure in making unnecessary and uncivilised remarks. In addition, the reviewers would be able to judge a paper more justly and without prejudice.

This bilateral anonymity programme is simple, and can work. And yet, after this is adopted I should like to go one step further in proposing that all papers be not only reviewed but also published anonymously.

The idea "publish or perish" did stimulate scientific research for a while. But it has now come to the stage where too much energy is wasted in unnecessary publications. I believe most scientists would agree that the total amount of papers published yearly could very well be cut 50% or more without showing any significant impediment in the progress of science. If this immense waste is allowed to continue, the advancement of science will actually be slowing down rather than moving faster. The main reason for this is that we place too much emphasis on the number of papers that a scientist has produced. One obvious result has been much unnecessary repetition.

If all papers were published anonymously, then the "status" of being a prolific writer would be diminished. The fight among authors about whose name should appear first would disappear. The scientific community could, furthermore, judge a paper solely on its merit.

My ultimate hope is to dissociate totally the name from the achievement. If Einstein were alive today, I believe he would not mind if people discussed and utilised the great theory of relativity without mentioning or even knowing his name. But I dare not advocate such a radical proposal at this time. Scientists are human, and as such must be selfish beings after all.

Yours faithfully,

TA-MING-FANG

Division of Engineering and Applied
Physics,
Harvard University,
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news and views

Older comets lose their sparkle

ON each successive approach to the Sun a comet becomes fainter. This phenomenon can be explained as follows. The nucleus of a comet can be considered as a conglomerate of ices and dust, and the regular heating of the nucleus each time the comet moves through the perihelion position of its orbit leads to sublimation of the ices, the copious emission of volatiles, a concomitant loss in mass and either a reduction in the size of the nucleus or the production of a relatively ice-free, porous dusty mantle around the nucleus. Thus, the emission of volatiles decreases each time that the comet goes past the Sun and the maximum brightness of the comet therefore diminishes. Since the 1920s, however, there has been a considerable controversy as to the rate of this secular decrease in brightness. The protagonists today are Sekanina (Smithsonian Astrophysical Observatory, Cambridge, Massachusetts), Vsekhsvyatskij (Astronomical Observatory, Kiev University), and Kresák (Astronomical Institute of the Slovak Academy of Sciences, Bratislava). A recent article by Kresák (*Bull. astr. Insts Csl.*, **25**, 87; 1974) adds fuel to the debate, the main point of which is not the fact that short period comets (those with periods less than, for instance, 20 yr and with a median period of 6.8 yr) are short lived objects in Solar System terms, but whether their decay process has actually been observed as a secular decrease in brightness within the age of accurate astronomical observation, that is, the last century and a half. Sekanina and Vsekhsvyatskij think that decay has been observed; Kresák disagrees.

A principal difficulty is that photometric observations of comets, collected by Vsekhsvyatskij, have been used by other researchers without due consideration of the great hazards involved in comparing observations from different epochs between which instruments and observing techniques have both developed enormously. Use has sometimes been made of visual magnitude data quoted to ± 0.1 mag without any consideration of systematic errors which may amount to many magnitudes (magnitude measures brightness on a logarithmic scale; one magnitude is equivalent to a 2.5 fold change in brightness).

The head of a comet consists of a diffuse coma which surrounds a central condensation or nucleus. Observations made with comet seekers of low focal ratio and moderate magnification, or with small cameras, allow the intrinsic magnitude of the diffuse comet head to be compared with the point images of reference stars, without the introduction of appreciable systematic error. If, however, the focal length and exposure time are increased, the apparent motion of the comet is accentuated and the image is trailed. Furthermore, in a telescope with a long focus a typical comet head of several minutes of arc in diameter may cover the whole visible field of view, adding to the sky background, and leaving only the nucleus to be measured. The luminosity of the nuclear condensation may be as low as 0.1–1% of the total luminosity of the comet. During the past two centuries magnitude estimates using small instruments or the naked eye have been replaced by the application of large telescopes and astro-

photography. That improves the detection limit of point sources but only partially affects the detection of large and diffuse comets.

Kresák illustrates this problem by considering published observations of the 1962 appearance of Comet Tuttle-Giacobini-Kresák, which had a very small nucleus and a large coma. The observations show systematic differences: there is an intensity ratio of 1000:1 between the most divergent observations.

Incompleteness of observations also introduces a difficult problem: for example, the absolute magnitude (the magnitude that the comet would seem to have if it was placed in a standard position 1 AU from the Earth and the Sun) is not the only decisive factor effecting the probability of detection; there are also important geometrical conditions of a seasonal nature. If there is a Gaussian random walk in the absolute magnitude at each return, then the comet will most probably be discovered when the random fluctuation makes it brightest (thus, the first observation is non-typical). Moreover, as observing conditions determine whether or not it will be observed on subsequent returns, the actual observations will be biased. As the limits of detection have decreased with time because of instrumental improvements, minimum values which are detected easily now would, in previous decades, have been missed. Both these effects bias the data towards a rate of absolute brightness decrease which is too steep.

Kresák, using observations of the total brightness of the whole comet head, concludes that the secular decrease in brightness of the periodic Comet Encke is only 1 mag per century and not 3 mag per century, as was found by Sekanina (*Astr. astrofiz.*, USSR, **4**, 54; 1969). And the rate of decrease is not accelerating. Kresák checked his absolute magnitude results by using the maximum visual magnitude at each apparition and obtained a similar decrease.

A rapid secular decrease in the brightness of short period comets would imply that the objects observed today were considerably brighter in preceding centuries, assuming, that is, that they have not in the meantime been captured from orbits of large perihelion distance. Unfortunately, short period comets are mostly too faint to be recorded as naked eye observations in mediaeval ancient records, and Jovian perturbations drastically alter their orbits, which makes backward extrapolation from today's observations exceedingly difficult. One exception is Comet Encke, which has a relatively stable orbit well inside that of Jupiter.

Ho Peng Yoke (*Vistas Astr.*, **5**, 127; 1962) produced a catalogue of ancient and mediaeval Chinese observations. A backward extrapolation of the secular brightness of Comet Encke would imply an easy identification of Encke in Ho Peng Yoke's catalogue; for example, extrapolation using Sekanina's value would make the comet frequently brighter than Venus before 1700. Kresák, however, found no evidence in the catalogue of periodic appearances of Comet Encke, and thus concluded that Sekanina's result is too large.

The high decay rates found by Sekanina and Vsekhsvyatskij have been applied by many authors to predict death dates for individual comets. Kresák shows not only that these projections would remove most of the well observed short period comets from our view within the

next decade or so, but also that many comets are still visible long after their predicted demise, two more facts in favour of a slow decay rate. Vsekhsvyatskij's decay figures of 0.3–0.4 mag per revolution gives short period comets a lifetime of two centuries, and proposed accelerations of this rate reduces this life to a few decades. Kresák finds that during the period 1930–1970 the discovery of short period comets has stabilised at a constant level of seven per decade. This perturbational capture rate is, in fact, no adequate substitute for the rapid loss predicted by Sekanina. If the short period comet population is in equilibrium, Kresák's low values of decay rate must be applicable.

Kresák concludes that the process of ageing of comets, and its effect on the variation in absolute brightness, cannot be satisfactorily explained by a simplified model of continuous mass loss which induces a continuous secular variation in absolute magnitude. Rather, observational evidence suggests strongly that the evolution proceeds stepwise, with short periods of abrupt evolution interspersed with long, relatively latent periods during which the brightness undergoes some fluctuation but in general remains essentially unchanged.

DAVID W. HUGHES

Dirac completes his theory of large numbers

It is not often that a man begins a new physical theory in his thirties, and then returns to finish it off forty years later. In a letter to *Nature* in 1937 (139, 323) Dirac added to his store of brilliant ideas a fundamental hypothesis concerning the values of various naturally occurring dimensionless numbers. In the past two years he has returned to this hypothesis to explore its consequences in detail. His latest article (*Proc. Roy. Soc.*, A338, 446; 1974), deals with certain astronomical and cosmological topics.

Dirac's theory begins with the question: what is the age of the Universe expressed in some naturally occurring units? A fundamental unit of time is the interval required for light to cross a characteristic atomic dimension, say 10^{-13} cm. Then the age of the Universe comes out at about 10^{39} in these units. The significance of this number is that it turns out to be simply related to other large dimensionless numbers formed from astronomical and atomic data; for example, the total number of particles in the observable Universe is around 10^{78} , that is $(10^{39})^2$, and the ratio of the electric to gravitational attraction between an electron and proton, e^2/Gm_em_p , is also about 10^{38} . In view of the fact that the first number obviously increases with time, the coincidences involved by these simple relationships seems great. Dirac's explanation of this curiosity is that all such large quantities are connected, so that as the first increases with time so do the others. This is already a radical departure from conventional physics, which in addition imposes severe restrictions on cosmology. Dirac calls this connection the large numbers hypothesis, in which he expresses "great confidence".

In his 1937 article, Dirac was led by this hypothesis to a model universe which expands with time t like $t^{1/3}$, making it about half as old as the more conventional big-bang models. In his recent work, however, the bald hypothesis is accompanied by a detailed gravitational theory which bears some similarity to the work of Hermann Weyl and E. A. Milne. A central feature of this work is the proposal

that there are really two space-time metrics; one, which is unmeasurable directly, enters into the Einstein equations (which remain valid) and the other is what is actually measured in laboratory experiments involving atomic apparatus. A connection between these two metrics is provided by a consideration of the motion of the Earth around the Sun, which in Newtonian approximation is essentially determined through the relation $GM = v^2 r$ connecting the Newtonian constant of gravitation G with the solar mass M , velocity of the Earth v and radius of the Earth's orbit r .

There then follows an argument which is typical of that used throughout Dirac's work. In terms of Einstein units, the quantities in this equation are constant. But in atomic units one must satisfy the large numbers hypothesis, so that G must decrease as t^{-1} in order that e^2/Gm_em_p increases proportionally to t . Theories involving a changing constant of gravitation are not new: for example, Brans-Dicke theory predicts a similar effect. The change is, however, very small and barely detectable with current technology.

Once again, the number of particles in the Universe $(10^{39})^2$ at present, is required by the large numbers hypothesis to increase as t^2 , which is interpreted by Dirac as a form of continual creation along the lines of the steady state theory.

Two possible creation mechanisms are proposed, with the new matter either appearing concentrated in existing masses (multiplicative creation) or spread out in the intergalactic spaces (additive creation). In the latter case the mass M in the above equation is constant, so that r in atomic units varies like t^{-1} . In the former case $M \propto t^2$ and $r \propto t$. It follows that the ratio of the two metrics can be t^{-1} or t , and that the Solar System must be either expanding or contracting with time.

In terms of Einstein units, if multiplicative creation is adding continuously to the mass M of the Sun, conservation of energy requires that the nucleons in the Sun decrease in mass as t^{-2} to compensate. Through the relation $e^2/Gm^2 \propto t$ this further requires $e \propto t^{-3/2}$ and \hbar (Planck's constant) $\propto t^{-3}$. A number of interesting cosmological consequences follow. First Dirac argues that any universe consistent with the large numbers hypotheses must be static, to avoid the introduction of a characteristic cosmological epoch (that is a constant large number). The red shift of distant galaxies, normally interpreted as a recessional effect, is instead accounted for by the decrease in the unit of atomic time interval. The Universe is prevented from gravitational collapse by the introduction in Einstein's equations of the cosmological constant which may be non-zero for multiplicative creation because of the correct time dependence of the metric ratio in this case. Dirac thus arrives at a version of Einstein's original static model universe.

In the case of additive creation Dirac proposes to conserve energy by the introduction of an unobservable negative energy field spread throughout the Universe, along the lines of the C-field of Hoyle and Narlikar. The total energy density of the Universe thus vanishes, so that the global geometry is just the Minkowski space of special relativity. In comparing the two models, Dirac inclines to the latter, on the basis that the creation of new atoms in existing material would lead to insuperable difficulties concerning the crystalline structure of very old rocks.

Dirac's article is written in his usual lucid and direct style. The ideas it contains, a mixture of old and new, are probably unpalatable for most modern cosmologists. Yet they are the product of a lively imagination, challenging the fundamental principles on which modern theories of astronomy, cosmology and physics itself are founded. Coming from a physicist of Dirac's stature, that is at the very least thought provoking.

P. C. W. DAVIES

Passive transport of insect urine

from our *Insect Physiology Correspondent*

It has long been realised that the Malpighian tubules of insect constitute a filtration-reabsorption system comparable with that of vertebrates. But in the absence of a glomerulus the flow of water is sustained by secretion in the upper region of the tubule, as in the aglomerular kidneys of some fishes, and not by pressure of the blood. Reabsorption of water can occur in the lower segment of the tubules, and in the hindgut and rectum—as in the kidney tubules and cloaca of many vertebrates. As was first shown by Ramsay, the osmotic driving force for the flow of water is produced by the active secretion of ions, particularly potassium; although sodium and chloride can play a part in some insects. The wall of the tubules was believed to be readily permeable to small organic molecules, so that sugars, amino acids and the like entered along with the water; the valuable components were reabsorbed, while toxic materials and waste products were discarded.

This problem of the passive permeability of the Malpighian tubules to organic solutes has now been re-investigated by Maddrell and Gardiner (*J. exp. Biol.*, **60**, 641; 1974) in the blowfly *Calliphora*, the locust *Schistocerca*, the hawk-moth *Manduca* and the blood-sucking bugs *Triatoma* and *Rhodnius*. Some eighteen compounds, labelled with carbon-14 or tritium were used as test substances; mostly amino acids and sugars, but including urea, urea acid

and inulin. Most of the experiments were done on Malpighian tubules isolated *in vitro* in appropriate saline solutions; but the results were confirmed by *in vivo* observations. The concentrations of the test substance in the bathing medium (*M*) and in the secretory product (*S*) were measured, along with the rate of fluid secretion, which could be varied experimentally by appropriate stimulation. It was found that there was always a linear relation between the *M/S* ratio and the rate of fluid secretion—as was to be expected if the transport was purely passive, the slope of the line being determined by the permeability of the tubule to the substance in question. There was thus, for example, a very gentle slope for the small molecules of xylose, as compared with the steep slope for the large molecules of inulin. Highly charged molecules penetrate more slowly than those more nearly neutral electrically: thus L-valine accumulates at one fifth the rate of D-xylose. The permeability of the tubules to such materials as inulin (molecular weight 5,200) is surprising. In some experiments, where the tubules are secreting very slowly, inulin may reach a concentration in the secreted fluid nearly 50% of that in the bathing medium. The authors suggest that much of this passive permeability lies in the intercellular spaces (see figure).

These experiments seem to have established the fact that the content of small organic molecules in the Malpighian fluid is determined by a passive non-discriminatory process. This means that the onus for conservation and discrimination resides in the re-

absorptive system. Here one is less well informed. The reabsorption of ions by the rectal epithelium has been well studied; and some absorption of amino acids and sugars occurs here. But a large part of the reabsorption probably takes place in the Malpighian tubules themselves, notably in the lower segments, and perhaps in the ileum where this exists. Furthermore, not all 'secretion' by the tubules is passive: some materials such as biliverdin, are transported by a process of endocytosis and exocytosis; the excretion of indigo carmine, which was so widely studied in the last century, presents some curious features that are not fully explained.

Modified nucleotides in messenger RNA?

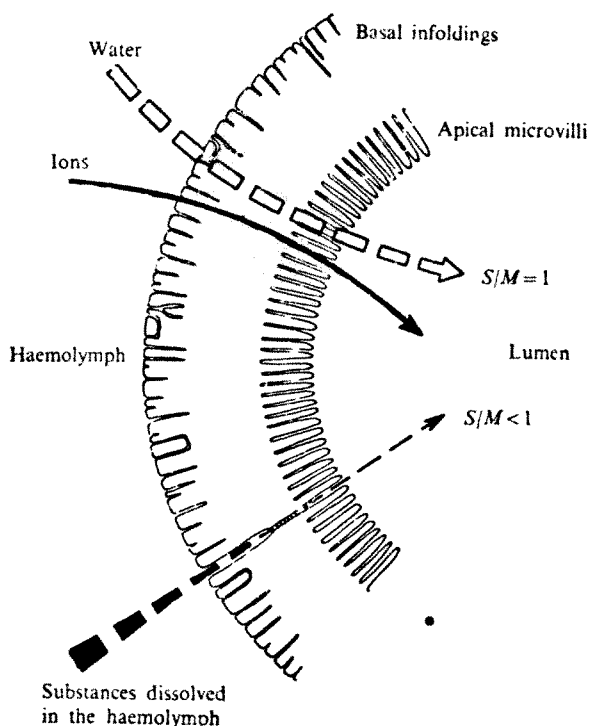
from Alan E. Smith

UNTIL recently it was thought that minor bases were present only in transfer RNA (tRNA) and ribosomal RNA (rRNA), but the difficulties in obtaining sufficient pure messenger RNA (mRNA) prevented definitive analysis of the latter. The presence of poly (A) in mRNA and heterogeneous nuclear RNA (hnRNA) can now be utilised to obtain purified fractions of these species and they can be examined for nucleotide composition without risk of contamination by rRNA and tRNA. In the first issue of *Cell* (**1**, 37–42; 1974), Perry and Kelly reported the results of such an analysis of mouse L-cell mRNA and showed that on average there are about 2.2 methyl groups per 1,000 nucleotides. This compares with about 13 methyl groups per 1,000 in rRNA, and means that an average cellular messenger molecule containing, say, 3,000 nucleotides has about 6 or 7 methyl groups.

hnRNA, on the other hand, contains many fewer methyl groups and Perry and Kelly therefore suggested that in eukaryotic cells methylation like the addition of poly (A) constitutes a post-transcriptional modification of mRNA precursors. These authors, however, did not identify the minor bases present in a specific mRNA species nor did they position them within the molecule.

The first identification and precise location of a minor nucleotide in mRNA has now been reported using a somewhat exotic system. In an elegant paper (*Nucleic Acid Reports*, **1**, 809–822; 1974), Furuichi shows that the mRNA of silkworm cytoplasmic polyhedrosis virus (SCPV) contains a 2'-O-methyl adenylic acid in the 5' terminal position, and that transcription to give mRNA is intimately coupled to the methylation reaction.

SCPV, like reovirus, contains a frag-



Schematic section of the wall of a Malpighian tubule to show the routes of transport of ions and water and diffusion of organic substances through the cell wall. *S/M* is the ratio of concentrations in the secreted fluid and in the bathing medium or haemolymph.

mented double-stranded RNA genome which consists of ten different segments. The terminal nucleotides of the double stranded RNA have recently been reported (*J. molec. Biol.*, **85**, 31–48; 1974) and the 5' nucleotide sequence of one strand of each of the segments is A*GU, where A* is a methylated adenosine. SCPV contains a virion-associated transcriptase which transcribes all ten double stranded RNA fragments *in vitro* to give mRNA molecules each of which begins with a 5' terminal sequence ppAG (*J. molec. Biol.*, **85**, 21–30; 1974; see also *Nature*, **250**, 13–14; 1974).

Since the activity of the SCPV transcriptase is low compared with that of the enzyme from reovirus, and because the genomic RNA is methylated, Furuichi tried adding a donor of methyl groups to the *in vitro* reaction mixture, and observed a huge stimulation of mRNA synthesis on addition of S-adenosyl-methionine (SAM). The product formed *in vitro* was found to be virus-specified and consisted of complete copies of one strand of each of the genome segments containing, on average, one methyl group per RNA molecule. The methyl group incorporated into the viral mRNA was detected in 7-O-methyl adenylic acid which is also present at the 5' terminus of the genomic double stranded RNA.

Furuichi examined the RNA made during very short incubations and found that methylation occurs early in the transcription process when the nascent chains are less than ten residues long. Since mRNA synthesis is almost totally dependent on addition of the methyl donor, and methylation is such an early event, Furuichi suggests that methylation may be involved in the initiation of mRNA synthesis and that SCPV transcriptase utilises a methylation-coupled transcription system, which is so far unique.

It will be of interest to establish whether other viral transcription systems are similarly coupled. It is already known, for example, that the RNA tumour viruses contain virion-associated methylase enzymes, as well as reverse transcriptase. So far the effect of SAM *in vitro* has not been reported in this system.

Of even greater interest will be an understanding of the initiation of hnRNA synthesis in normal cells. Bajzar, Samarina and Georgiev (*Molecular Biology Reports* **1**, 305–310; 1974) have isolated the 5' terminal nucleotides of mouse cell hnRNA and identified them as pppAp and pppGp. So far there is no evidence for methylation in these positions. It is possible, however, that methylation occurs at internal positions in hnRNA and that such modifications act as markers for subsequent cleavage reactions. It may be significant that

the chromatographic mobility of some of the methylated nucleotides detected by Perry and Kelly in mouse mRNA suggested that more than one phosphate group is present. Perhaps some of the methyl groups are attached to nucleotides (pX*p) which originate from the 5' end of mature cellular mRNA.

Models of magnetic anomalies

from Peter J. Smith

A MAGNETOMETER towed at or near the ocean surface will record the well known linear magnetic anomalies which arise from alternating normal and reversed magnetic material within the oceanic crust. A magnetometer towed along very close to (say, within 100–200 m) of the sea floor, on the other hand, records anomalies which are much narrower and yet much greater in amplitude. But to what is this fine structure due? Do these small scale variations within magnetic polarity epochs and events reflect real physical variations of internal crustal properties or are they merely the result of topography or some other equally uninteresting phenomenon?

Conclusions on this point seem to differ. Atwater and Mudie (*J. geophys. Res.*, **78**, 8665; 1973) measured near-bottom anomalies on the flank of the Gorda Rise and found that almost all of them could be accounted for by a uniformly magnetised basement with topography. But some years ago, Luyendyk (*J. geophys. Res.*, **74**, 4869; 1969) found such an explanation unsatisfactory in connection with the fine structure in an area on anomaly 10 west of southern California. Instead, he invoked variations in magnetisation which arise either from ancient magnetic field variations or changes in petrology with distance from the ridge crest. Similarly, Larson and Spiess (*Science*, **163**, 68; 1969) concluded from a profile across the East Pacific Rise crest that small scale anomalies within the Brunhes epoch are unrelated to basement topography, and again appealed to fluctuation in palaeointensity.

Insofar as these conclusions refer to difficult areas they are not necessarily inconsistent, for it is quite conceivable that apparently similar phenomena in different regions could be explained in different ways. On the other hand, it is clearly important to know whether a common observation may be dismissed as an effect of little significance or whether it is necessary to look for deep-seated physical causes. So to investigate this matter further, Larson *et al.* (*J. geophys. Res.*, **79**, 2686; 1974) have constructed and analysed magnetic

block models based on the volcanic basement profile actually obtained across the East Pacific Rise by Larson and Spiess—a profile carefully obtained by continuously and simultaneously measuring the depth of the magnetometer with an up-looking sonar, the height of the instrument above the sea floor with a down-looking sonar, and the thickness of sediment with another down-looking sonar.

The first model was given a constant magnetisation of 0.011 e.m.u. cm⁻³ to see if such uniformity could reproduce the observed magnetic anomalies. As far as general widths and amplitudes were concerned, agreement between model and observation was quite good; and some specific anomalies were particularly well matched. In such cases it follows that the relevant near-bottom anomalies may be accounted for solely in terms of the shape of the basement profile and variations in the magnetometer depth. But in many cases specific anomalies were reproduced poorly by the uniform model, notwithstanding its topography. For such anomalies good agreement between model and observation could only be obtained by allowing magnetisation to vary along the profile between extremes of 0.0048 and 0.0257 e.m.u. cm⁻³.

In summary, then, it would seem that however valid were the conflicting conclusions of Luyendyk and Atwater and Mudie in their respective geographic contexts, neither party is completely correct in general. Moreover, it is now clear that Larson and Spiess were not entirely correct in the specific case of the East Pacific Rise in attributing small scale magnetic anomalies solely to lateral variations of magnetisation. Near-bottom anomalies apparently have no unique cause; in any given instance it may be necessary to invoke both topographic and magnetic effects.

Antitumour immunity

from A. J. S. Davies

THE antagonists of the notion of an immunological antitumour defence mechanism very properly ask why the process involved seems so often to fail. The explanations are increasingly ingenious. Initially, antibody was thought to block the response of hostile lymphocytes to the antigenic tumour; then antigen-antibody complexes and finally antigen have become popular as the blocking factor. It is not too difficult to envisage that antibody would mask those sites on the tumour cell which would otherwise act as recognition foci for lymphocytes. The antibody component of an antigen-antibody complex could do the same as long as it has some free binding sites (that is, the

complexes are in the presence of an excess of antibody). Antigen either free or complexes in antigen excess could presumably combine with any lymphocytes capable of reacting against the malignant cells, thus frustrating their attack.

Overall there is the possibility that some central paralysis of the immune response arises which does not operate by one of the mechanisms already considered. This last possibility is not deemed likely as in many of the instances in which a tumour is growing it is possible to detect by *in vitro* methods the existence of cytotoxic cells and antibodies which have some degree of specificity for the relevant tumour. There is a variety of other explanations which involve non-specific suppression of immunological responses particularly in terminal cases as a consequence of the poor physical condition of the tumour-bearing host.

Hattler and Soehnlen have illustrated the operation of one of these mechanisms (*Science*, **184**, 1374; 1974). These investigators cultured the blood lymphocytes of cancer (usually squamous cell carcinoma of the lung) patients together with their irradiated tumour cells and measured the response of the lymphocyte populations by their capacity to incorporate tritiated thymidine some days later. The response was in all instances much improved by washing the lymphocytes five times before they were allowed to make contact with the tumour target cells. This result is reminiscent of those of Curry and Basham (*Br. J. Cancer*, **26**, 427; 1972) who found that in melanoma patients it was difficult to detect the cytotoxicity of lymphocytes against their specific target tumours unless the lymphocytes were washed six times before the *in vitro* test.

The cell wash eluates of Hattler and Soehnlen effectively inhibited the performance of the cells washed five times but failed to influence the (poor) response of cells washed only once. Fractionation of the eluates yielded a low and a high molecular weight entity neither of which was a particularly effective inhibitor but which in combination did inhibit the cells washed five times. The authors suggest that their results are compatible with the notion that the lymphocytes of the cancer patients may be blocked by antigen-antibody complexes. *In vitro* these complexes can be eluted from the lymphocytes permitting the demonstration of specific antitumour responsiveness. The further exercise of fractionation and the demonstration of two component inhibitors certainly seem to support this idea.

The work of Curry (*Br. J. Cancer*, **28**, 153; 1973) again amplifies the concept. In extension of his earlier studies,

Curry found that the serum of patients with extensive malignant disease contained a blocking factor of low molecular weight which had affinity for lymphocytes but not for tumour target cells. In the serum of patients with less extensive tumours the blocking factor was of large molecular weight and had affinity for both lymphocytes and tumour cells. Curry supposed that this smaller blocking material was antigen and the larger antigen-antibody complexes.

These kinds of studies are extremely valuable in pointing to the complexity of antitumour immunity. A complexity which, without considerable luck, must be mastered before effective and rational immunotherapeutic procedures can be adopted.

Alpha A and beta S

from a Correspondent

INTEREST in the detailed mechanism of the aggregation of deoxy sickle-cell haemoglobin (Hb-S) continues to stimulate the application of new experimental approaches. Wilson, Luzzana, Penniston and Johnson (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 1260; 1974) have used the quasi-elastic light scattering method to study the pregelation aggregation of deoxy Hb-S, and obtain results consistent with a linear association between aggregates. This scheme implies that deoxy Hb-S molecules are bifunctional, with respect to aggregation, and interact in a non cooperative manner, rather than by a concerted scheme in which monomers rather than aggregates are in equilibrium with the gel. Both these alternatives have been considered in previous discussions of the sickling process.

Benesch, Benesch and Yung (*ibid.*, 1504) have exploited their earlier success in the specific pyridoxylation of either the α or the β chains of normal adult haemoglobin (Hb-A) to show that substitution of the N-terminal groups of Hb-S inhibits gelation and increases the solubility of the deoxy form. Modification of the α chains has much more effect than that of the β chains, to an extent equal to that produced by dilution of Hb-S with an equal amount of Hb-A. If this effect could be achieved in the intact red cell, it would be equivalent to reducing the sickling tendency from the degree found in homozygous sickle-cell anaemia to the lesser extent experienced in the heterozygous trait condition. Surprisingly the α -chain modified form of Hb-S obtained by using pyridoxal 5'-phosphate, viz. $(\alpha^{PLS})_2\beta_2^S$ scarcely differs from Hb-S itself in its oxygenation parameters, and the decreased gelling tendency must, therefore, be caused

by changes in the structure of the deoxy form *per se*.

The same strategy of selective irreversible pyridoxylation of α or β chains has been used by Suzuki, Benesch and Benesch (*Biochem. biophys. Acta*, **351**, 442; 1974) to demonstrate that the Bohr effect in Hb-A is reduced in both α -chain and β -chain pyridoxylated Hb-A, but to a greater extent in the species with modified α chains. It is known from previous work that about one-half of the oxygenation-linked proton exchange of the Bohr effect is associated with the C-terminal β -146 histidines, and about one-quarter with the N-terminal amino groups of the α chains. Elimination of the ionisable protons of these α -chain N-terminal groups by pyridoxylation can account for the observed diminution of the Bohr effect. For the β -chain modified species there is no elimination of an existing Bohr proton binding site, so the change is probably caused by the introduction of a new ionising group whose acid strength decreases with oxygenation, possibly the 5'-phosphate of the attached pyridoxal phosphate reagent used to prepare the β -chain modified species, and which is presumed to form a salt bridge with β -82 lysine in the deoxy but not in the oxy conformation. On oxygenation this residue would therefore bind protons, accounting for the observed decrease in the Bohr effect. These two papers provide an excellent example of the way in which specifically modified but still functional haemoglobin species provide powerful tools for elucidating details of structure-function relationships.

Finally a paper concerned with the red cell itself, and of particular interest in that it deals with the study of individual red cells, always an approach that appeals to biologists and haematologists, who are concerned with red cell populations rather than haemolysates prepared from millions of cells. Brunori, Giardina and Antonini (*J. molec. Biol.*, **86**, 165; 1974) have studied this distribution of the three major haemoglobin parts of trout (*Salmo gairdneri*) blood by single-cell spectroscopy. They find that the erythrocytes contain the component (Hb-IV) which is characterised by the Root effect in the same proportion as in a haemolysate, and which is therefore uniformly distributed. The Root effect (*Biol. Bull.*, **61**, 427; 1931) refers to a very marked dependence of the oxygen binding curve on pH, such that below pH 7 the haemoglobin is only partially saturated in air. It is characteristic of teleost fish haemoglobins, and related in some way to the mechanism of gas secretion into the swim bladder. Brunori *et al.* speculate that the synthesis of several different normal

haemoglobin components in definite proportions in all erythrocytes may be required to maintain a functional situation dependent on a balance of properties of the various components.

J-dependence in nuclear reactions

from P. E. Hodgson

THE general character of the differential cross sections of direct one-nucleon transfer reactions is dominated by the orbital angular momentum L of the transferred particle. Except at low energies when the angular distributions are backward-peaked and similar for all L values, the angular distributions are peaked at an angle in the forward hemisphere that increases with the value of L . In most cases a comparison between the experimental data and distorted wave calculations enables the value of L to be determined.

Since the transferred nucleon also has a spin of $\frac{1}{2}$, the total transferred angular momentum J is the vector sum of L and $\frac{1}{2}$, and since these can be added or subtracted, $J=L\pm\frac{1}{2}$. If the target nucleus has zero spin, this J is just the spin of the final state of the residual nucleus.

The angular distributions of reactions of the same L but different J are usually very similar, but in some cases small but systematic differences have been found. These are called the J -dependent effects, and have proved quite useful in assigning spins to nuclear states simply by the shape of the angular distribution, without any detailed calculations.

These J -dependent effects depend on the transferred orbital angular momentum L and on the incident energy, and are more marked in some cases than in others, so it is clearly important to understand how they arise, both for their intrinsic interest and in order to enhance their utility in nuclear spectroscopy.

The most marked J -dependent effects are found for $L=1$ transitions, and Robson has shown that these can be accounted for very well by distorted wave calculations with a spin-orbit interaction in the incoming and outgoing channels, provided the optical potentials are chosen to fit both the differential cross section and the polarisation of the corresponding elastic scattering.

For $L=2$ and 3 the J -dependent effects cannot be accounted for in this way and several attempts have been made to develop the distorted wave theory, in particular by including the deuteron D-state. This is a complicated calculation, and some of the results show J -dependent effects that are similar to those observed. The difficulty of the calculations has however deterred

an extensive study, so most of the J -dependent effects remain unexplained.

Quite recently a new explanation of J -dependent effects has been proposed: they are attributed to the contributions of two-step processes to the reaction amplitude. The importance of two-step processes is now widely known, especially when the nuclei are deformed and when the direct one-step transition is forbidden or inhibited by some selection rule. A substantial proportion of the reaction can proceed by the incident particle first exciting the target nucleus to a low-lying collective state by inelastic scattering, followed by the particle transfer to the final state. Similarly, the particle transfer can take place first to the ground or to an excited state of the residual nucleus, which can then be excited to the final state by an inelastic process as the outgoing particle leaves. All possible processes of this type combine to give the observed cross section.

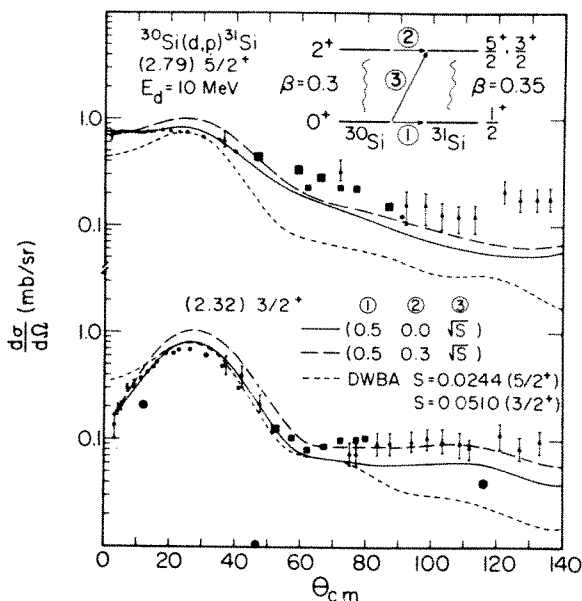
In recent years the formalism for calculating such processes has been extensively developed, and many studies have shown the importance of including two-step processes. It has now been applied by Hoffmann, Udagawa, Coker, McIntyre and Mahlab (*Phys. Lett.*, **50B**, 249; 1974) to study the J -dependent effects in the reaction $^{30}\text{Si}(\text{d},\text{p})^{31}\text{Si}$ at 10 MeV to the 2.32 MeV ($3/2^+$) and 2.79 MeV ($5/2^+$) states of the residual nucleus. As the figure shows, the cross sections of these two reactions differ markedly in the forward direction, although they are both $L=1$ and very similar in energy. The short-dashed curves are standard DWBA calculation, which gives very similar angular distributions for the two reactions, and does not account at all for the difference between them. The calculation including the two-step process via the

$1/2^+$ state of ^{31}Si at 0.75 MeV gives the full curves, which are in excellent accord with the data in the forward direction. The small differences in the backward hemisphere are probably attributable to compound nucleus contributions. A final calculation including those two-step processes through the lowest 2^+ state of ^{30}Si gave the long-dashed curves which do not agree with the data, which is expected since the excited states of the two states of ^{31}Si have a small parentage in terms of the 2^+ state in ^{30}Si , so that this process does not contribute significantly to the reaction.

Inclusion of the two-step contribution to this reaction is thus able to give a very good account of all the J -dependence of the cross sections to the $3/2^+$ and $5/2^+$ states of ^{31}Si . Other effects, such as those caused by the deuteron D-state, may contribute, but in this case at least they are small.

Further calculations by Coker, Udagawa and Hoffmann have shown that the two-step process is also able to account for the $L=2$ J -dependent effects in the $^{28}\text{Si}(\text{d},\text{p})^{29}\text{Si}$ reaction at 10, 13 and 18 MeV to several final states.

This work shows that two-step processes via low-lying collective excitations account for some of the J -dependent effects in one-nucleon transfer reactions in deformed nuclei. The J -dependent effects, however, are also found for nuclei to which this explanation cannot apply. It is therefore necessary to carry out a series of analyses for a range of nuclei and incident energies for different L -values with consistent parameters for the distorting potentials. This will make it possible to establish in a systematic way the contribution of two-step processes to J -dependent effects in nuclear reactions.



Differential cross sections for the one-nucleon transfer reaction $^{30}\text{Si}(\text{d},\text{p})^{31}\text{Si}$ at 10 MeV to the 2.32 MeV $3/2^+$ and 2.79 MeV $5/2^+$ states of ^{31}Si compared with distorted wave calculations. The short-dashed curves are the simple one-step calculation and the full curves show the effect of including the two-step process (1). The long-dashed curves show the effect of including the coupling (2) between the excited states, which does not contribute significantly. The figures in the brackets are the spectroscopic factors for the various transitions.

Deterioration of high school students' attitudes to physics

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Australian students taking a physics course based on PSSC displayed a sharp decline in enjoyment of physics. Pupil personality and teacher behaviour variables affected the extent of this decline.

SCIENCE teachers are still concerned about the attractiveness of physics courses. In 1971, in Victoria, Australia, high school physics enrolments showed an absolute decline for the first time in recent history, and a further decline occurred in 1972. In Britain, 2,400 university science places were unfilled in 1973-74, and a complete halt in all university science building programmes for a decade has been predicted¹. Hopes for large increases in the proportion of girls taking physical sciences² have not materialised. There have been attempts to explain this in terms of the poor 'image' of physics and physicists³, a modern distaste for science and technology⁴, the failure of physics curricula to include social aspects of science which are of interest to adolescents, particularly girls³, and the operation of economic forces outside the control of the science teacher⁵.

The classroom and the people within it, however, probably remain the central arena of interest for the science education researcher seeking to explain attitudes to science and enrolments in science subjects. There have been many searches for relationships between attitudes or enrolments and other pupil and teacher variables. In most of these studies pupil or teacher variables (but not both simultaneously) have been correlated with pupil outcomes. Although such studies are often interesting they do not permit investigations of the complex interactions which may exist between pupil and teacher variables.

Physics questionnaire research study

The physics questionnaire research study (PQRS) project was set up to investigate the relationships between pupil personality, teacher behaviour and pupils' attitudes to physics. The study was done in Victoria, Australia on Grade 11 pupils taking the first year of a 4 yr course based on the United States PSSC materials. To exert a moderate amount of control over a number of extraneous but potentially influential variables, such as school facilities and home background, the sample was restricted to pupils in coeducational State high schools in the more affluent areas of Melbourne.

Three instruments were used in the project: the physics attitude index (PAI) was employed as a pretest and 8 months later as a post-test; the personal preference index (PPI) and the physics classroom index (PCI) were given as mid-tests. A total of 1,014 students (798 boys, 216 girls) in 58 classes in 34 schools were studied.

The PAI is a 40-item Likert-type scale, yielding scores on four attitudes: first, towards unauthoritarian modes of learning; second, towards physics as an open, flexible, dynamic discipline; third, towards scientists, and fourth, per-

sonal enjoyment of physics. The pretest and post-test means⁶ reveal a favourable initial attitude towards active, participatory modes of learning, an attitude which was maintained during the year. The students also entered the course with the view that physics is an open discipline; at the end of the Grade 11 year, their attitude was less open, the difference being statistically significant, although rather small. The students also held a favourable view of scientists as fairly normal people; again, there was a significant although small decline during the year. The most striking finding, however, was on the fourth scale: although on entry to the course, students expressed a high level of enjoyment in learning physics, eight months later their attitude had declined sharply. Since most science educators would want students to increase, or at least maintain, their interest in a subject, this decline in enjoyment is disturbing.

From the PCI and PPI data some inferences may be drawn about pupil and teacher variables which affect this decline. The theoretical framework underlying the two instruments is the needs-press model of Murray⁷ and Stern⁸. The model indicates that human behaviour may be understood in terms of an interaction between aspects of personality ('needs') and relevant aspects of the social environment ('press'). The PPI contains eight needs scales, taken from Stern's Activities Index: achievement; conjunctivity; deference; play; understanding; order; nurturance and energy. The PCI was devised especially for the PQRS project, and contains eight press scales which correspond to these needs: competitiveness; organisation; compliance; pleasure; intellectualisation; compulsiveness; warmth and stimulation.

A 4×4 analysis of covariance design, with an unweighted means adjustment for unequal cell frequencies, was devised to analyse the data. The analysis of the effects of competitiveness and achievement on enjoyment illustrate the design. Class mean scores on the PCI scale were used to divide the 58 classes into four approximately equal quartile groups, each containing either 14 or 15 classes: classes with teachers regarded as very high, high, low, or very low on competitiveness. Individual scores on the corresponding PPI scale were then used to divide the 1,014 students into four approximately equal quartile groups: very high, high, low, or very low on achievement. The covariance design permitted inferences to be drawn about the effects of these pupil and teacher variables on pupils' post-test attitudes, over and above any effects which could be ascribed to attitudes already present at the start of the course. This type of analysis was carried out 32 times to study the effects of eight needs-press combinations on each of the four attitude variables.

Findings

Of the 64 possible main effects, 25 were significant beyond the $P=0.05$ level, and of these 18 were significant beyond the $P=0.01$ level. Some further details of the findings may be found elsewhere⁹. The teachers' behaviour was found to have little effect on pupils' attitudes to nonauthoritarian

modes of learning. There was a weak inverted U-shaped relationship between attitude scores and teacher compulsiveness (that is, the tendency to organise meticulously the physical environment of the classroom.) Three personality variables were found to be significant: students who are motivated by achievement, serious (low on play) and intellectual (high on understanding) display more favourable attitudes. Factor analysis reveals that these personality variables all lie on one factor. Warm and outgoing students (high on nurturance) also display a more favourable attitude: 'discovery' learning, as it is organised in typical schools, generally requires cooperation with other people, and it is perhaps not surprising that students who are personally more outgoing should enjoy it more than students who are less interested in other people.

Students' views concerning the openness of physics were significantly related to only a small number of the predictor variables. Pupils motivated by achievement tend to hold a more open view, but achievement-pressing teachers (high on competitiveness) tend to promote a more closed view. Apparently, teachers who place heavy stress on achievement, success and examination performance are less likely to maintain the highly open attitudes that most of their students have on entering the course. The only other highly significant finding was the positive relationship between nurturance and openness: apparently, warmth towards other people and receptivity to new ideas are related qualities.

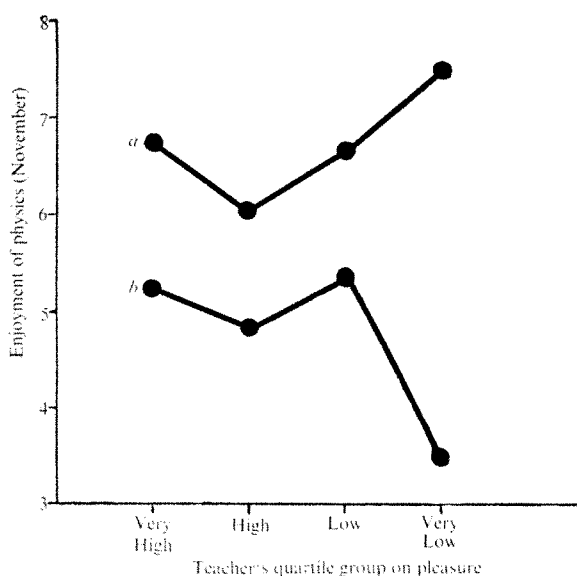


Fig. 1. Enjoyment of physics at end of year by serious and playful pupils under four levels of teacher behaviour. *a*, 'Serious' pupils (below median on play); *b*, 'playful' pupils (above median on play).

Students' attitudes towards scientists were strongly related to only two of the predictor variables: deference and nurturance (factor analysis indicates that these two personality variables lie on a single factor). Thus, pupils who are warm and friendly, and who are more likely to be submissive and conformist, are more likely to regard scientists with affection and tolerance. The relationship is sex-linked: girls significantly outscore boys on the two personality variables and on the attitude scale.

The enjoyment of physics scale displays the most significant findings, and nearly all the predictor variables are involved. In general, intellectually intense pupils—those who are serious, intellectual and motivated by achievement—and pupils who are warm and deferent tend to enjoy

physics more. Intellectually stimulating teachers—those who are intellectual, cognitively well-organised (high on conjunctivity), stimulating and achievement-pressing, and whose classrooms are physically well-organised—tend to be associated with greater enjoyment. Analysis shows that students who are intellectually intense with teachers who are intellectually stimulating display a highly favourable initial attitude, and this is maintained during the year. This is a rather small group: about 6% of the sample (the top quartile group of students in the top quartile group of teachers). Other students with other teachers display a greater or lesser decline. If maintenance of enjoyment is the criterion, the course is effective for a very small group of pupils.

The effects of teacher competitiveness are interesting for two reasons: first, because here is a teacher characteristic which tends to promote one desirable objective (enjoyment) while at the same time hindering slightly the attainment of another (openness); second, because there is an intriguing interaction effect with the corresponding pupil personality variable. Details are given elsewhere¹⁰; briefly, highly achievement-pressing teachers exert a beneficial influence on the enjoyment of highly achievement-motivated pupils, but a relatively deleterious effect on the enjoyment of pupils who are very low in achievement motivation.

Teacher pleasure and pupil play also exert interactive effects on enjoyment. As can be seen from Fig. 1, 'serious' pupils (those below the median on play) enjoy physics more than 'playful' pupils. Teachers in the top three quartiles on pleasure have no influence on enjoyment, but teachers in the lowest quartile—very serious teachers—exert diametrically opposing effects upon the enjoyment of serious and playful pupils. Had only class means been considered in this study, no relationship between teacher pleasure and enjoyment would have been found: the correlation between the class means was near zero.

Implications

The PAI has been used in earlier research to compare the outcome of different curricula in other Australian states¹¹. The magnitudes of the teacher behaviour and pupil personality effects found in the PQRS project are much larger than the effects associated with different curricula. Although teacher behaviour is probably more difficult to change than instructional material, the PQRS findings suggest that science educators who wish to bring about improvements in pupil's enjoyment of science subjects ought to concentrate more heavily upon teacher education. The current decline in support for nationally funded science curricula, and the concomitant growth of interest in the pre-service and in-service education of teachers reflect shifts of emphasis which seem to be appropriate.

¹ *Daily Telegraph*, 5 (November 10, 1973).

² *Physics in your high school* (American Institute of Physics, McGraw-Hill, New York, 1960).

³ Ahlgren, A., and Walberg, H. J., *Nature*, **245**, 187–190 (1973).

⁴ Roszak, T., *The making of a counterculture* (Doubleday, New York, 1969).

⁵ Reitz, J., *Science Educ.*, **57**, 121–134 (1973).

⁶ Gardner, P. L., *Aust. Sci. Teach. J.*, **19** (1), 71–78 (1973).

⁷ Murray, H. A., *Explorations in personality* (Oxford University Press, New York, 1938).

⁸ Stern, G. C., *People in context* (Wiley, New York, 1970).

⁹ Gardner, P. L., in *Contemporary studies in the curriculum* (edit. by Musgrave, P. W.) (Angus and Robertson, Sydney, in the press).

¹⁰ Gardner, P. L., *Research on teacher effects: critique of a traditional paradigm*, *Br. J. educ. Psychol.* (in the press).

¹¹ Mackay, L. D., in *Research 1971*, 109–118 (Report of the Australian Science Educational Research Association Conference).

Palindromic base sequences and replication of eukaryote chromosome ends

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A new theory is proposed to explain the synthesis of the 5' ends of linear DNA molecules. It suggests that chromosome ends consist of palindromic base sequences. These can form self-complementary hairpin loops, which can be converted by DNA ligase, a specific endonuclease, and DNA polymerase, into completely replicated ends.

DURING DNA replication, new DNA is made by DNA polymerases which add new nucleotides to the 3'OH end of growing polynucleotide chains. All known DNA polymerases are able only to extend existing polynucleotide chains and cannot start new ones. For this reason, the mechanism of initiation of new polynucleotide chains has for many years been a fundamental problem in the study of DNA replication¹. There is now good evidence that prokaryotes, eukaryotes and viruses all use a short RNA molecule as a primer for the initiation of new DNA molecules^{2,3}; this RNA primer is probably subsequently removed by nucleases³ before the completion of the daughter molecule by DNA polymerases and DNA ligase. The discovery that the transiently-formed, short pieces of DNA known as 'Okazaki pieces' have a short stretch of RNA⁴ at their 5' phosphate ends, strongly supports Okazaki's model⁵ for the discontinuous synthesis of DNA. This model, in which 'Okazaki pieces' are subsequently joined by DNA ligase to form a complete daughter strand, can now (in conjunction with the idea of RNA primers) provide a satisfactory explanation for the mechanism of replication of circular DNA molecules, though details, especially of the control mechanisms, remain to be filled in.

For linear DNA molecules, however, a fundamental problem remains. Watson⁶ has pointed out that when a linear virus chromosome replicates, the excision of the RNA primers from the 5' ends of the two daughter strands will leave a gap which cannot be filled in by DNA polymerase because there is no adjacent 3'OH end to serve as a template (Fig. 1a). Clearly this problem does not arise in the case of a circular chromosome which has no ends. This may be the reason why bacterial and viral chromosomes are often circular: linear chromosomes require a special mechanism for the replication of their ends. Watson suggested two such mechanisms. Circularisation as occurs in λ phage of *Escherichia coli*, and concatemericisation as in phage T7. Both mechanisms require identical sequences at the two ends of the molecules (terminal redundancy). Watson postulated that all linear DNA molecules must be terminally redundant and must replicate either by circular or concatemeric replicative intermediates.

Here I propose a third mechanism for the replication of the ends of linear DNA molecules which allows them to replicate as linear molecules and does not necessarily require terminal redundancy, circular intermediates, or concatemers. I argue that this third mechanism is more likely to apply to eukaryote chromosomes, which are probably essentially single DNA molecules, than either of

Watson's mechanisms. My theory has the added advantage that it can explain several long known, but hitherto unexplained, properties of the ends (telomeres) of eukaryote chromosomes. As it does not necessarily require terminal redundancy and circular or concatemeric intermediates, it may also be applicable to virus chromosomes, such as chick embryo lethal orphan virus⁷, which seem to lack these properties.

Replication of eukaryote chromosome ends

There is increasing evidence that each eukaryote chromosome consists of a single DNA molecule⁸⁻¹¹. Each chromosome contains many tandemly arranged units of replication (replicons)¹², and each replicon is comparable to the whole chromosome of a bacterium or a virus in that its replication is usually bidirectional. The simplest assumption compatible with the genetic evidence for linear linkage groups is that each chromosome is a linear, rather than a circular, DNA molecule. This means that exactly the same problem of completing the 5' ends of daughter strands will arise as in linear virus chromosomes. The basic problem in applying either of Watson's two suggestions for viruses directly to the replication of eukaryote chromosomes is that, if eukaryote chromosomes consist of single DNA molecules, these will often be many thousands of times longer than those of viruses. Because of the consequent considerable reduction in the effective 'concentration' of free ends, the chances of their ever meeting and pairing, whether to form circles or concatemers will be drastically reduced. The mechanism proposed here overcomes this problem.

The central assumption of my theory is that the nucleotide sequences at the ends of eukaryote linear DNA molecules are palindromic, that is if one always reads with the same polarity, the sequence of bases in one strand is

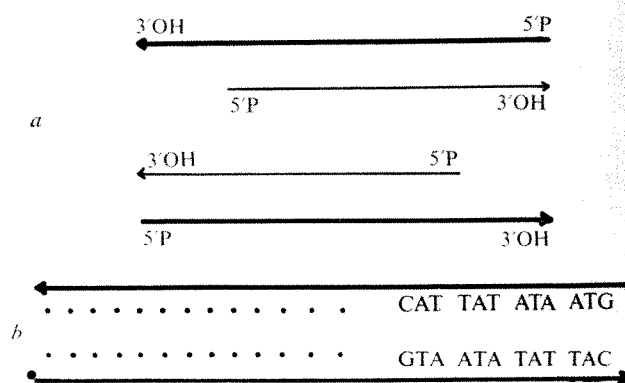


Fig. 1 *a*, The two daughter molecules which result from the replication of a linear DNA molecule, showing the gaps at the 5' end of the newly synthesised strands left by the removal of the RNA primers (the 3' end of a polynucleotide is shown by an arrow, newly synthesised DNA by a thin line). *b*, A terminal palindromic sequence. The sequence on each strand is the same when read with the same polarity.

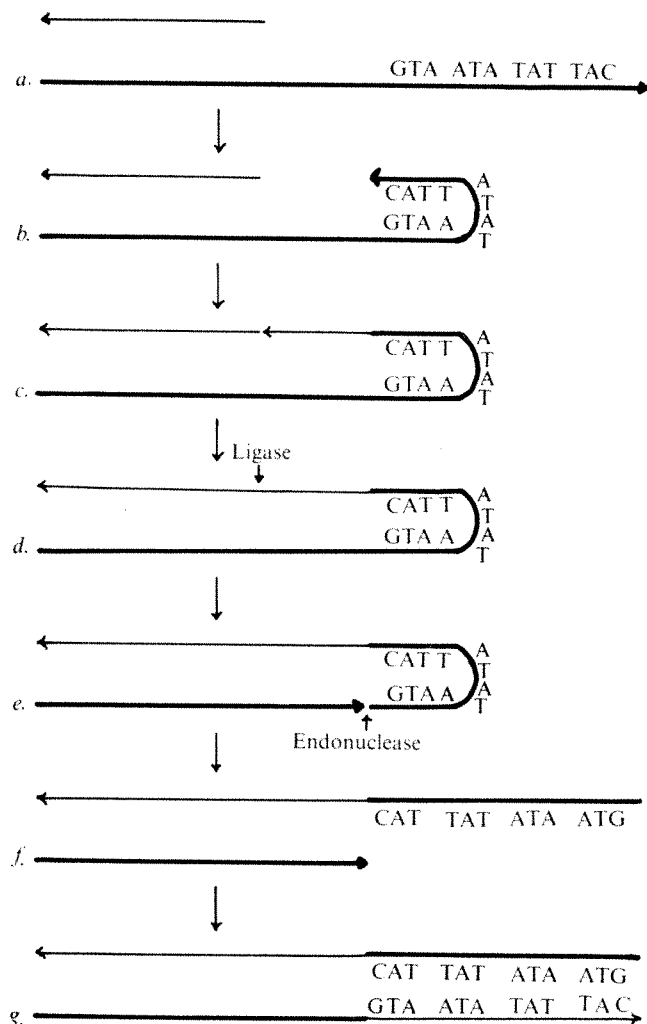


Fig. 2 Model for the synthesis of 5' ends of a daughter DNA molecule (the 3' end of a polynucleotide shown by an arrow, newly synthesised DNA by a thin line). *a*, Gap at 5' end of new DNA; *b*, The self complementary 3' terminal strand of the palindromic sequence base pairs to form a hairpin loop; *c*, 3' OH end serves as a primer for a DNA polymerase to fill in the gap; *d*, DNA ligase seals the remaining nick; *e*, A sequence specific endonuclease nicks the old strand; *f*, The loop unfolds; *g*, DNA polymerase completes the old strand with new DNA. Note this effectively results in the exchange of new and old DNA at one end of every chromosome.

the same as that on the other (Fig. 1*b*), so that the terminal region as a whole has twofold rotational symmetry.

Because of this symmetry the first base in the sequence on one strand is complementary to the last base on the same strand, the second base is complementary to the last but one and so on. This means that the unreplicated single stranded 3'OH end of a daughter chromosome can fold back on itself to form a terminal base-paired loop (Fig. 2*b*).

I suggest that this is the first stage in the replication of the end of the molecule. Subsequent steps are: the closing of any remaining gap by DNA polymerase (Fig. 2*c*); the sealing of the remaining nick by DNA ligase (Fig. 2*d*); the cleavage of the opposite strand by a sequence specific endonuclease (Fig. 2*e*) (this provides a new 3'OH end which serves as a primer for the final step); the unpairing of the loop to provide a template for the final step (Fig. 2*f*); and finally, the completion of the remaining strand by DNA polymerase (Fig. 2*g*).

The mechanism has only two special requirements: a palindromic terminal sequence, and an endonuclease which can recognise one end of this sequence and cleave the correct strand. Both assumptions should eventually be able

to be tested. Palindromic sequences and endonucleases which recognise them are well established in the restriction mechanisms of bacteria¹³. Perhaps they were ancestral to the mechanism postulated here. The palindrome need not be perfect since there must be at least two bases at the end of the loop which cannot pair and therefore need not be complementary.

Telomeres of eukaryote chromosomes

Although eukaryote chromosomes probably consist of a single DNA molecule, very little is known of the way this is folded in mitotic and meiotic chromosomes. In particular, it is not known whether the two ends of the molecule lie at the ends (telomeres) of the chromosome. In the case of meiotic chromosomes, however, the correlation in maize between the linear sequence of chromomeres and the linear sequence of genes in a linkage group makes it reasonable to assume that this is the case. If, therefore, one assumes that, as a general rule, the ends of the DNA molecule do in fact form part of the telomeres then the existence of terminal palindromic sequences could explain several intriguing properties of the telomeres.

First, there is evidence that broken ends of chromosomes behave differently from the natural ends in that they are 'stickier' and have a strong tendency to rejoin or to become permanently joined to broken ends of other chromosomes¹⁴⁻¹⁶. This is to be expected because broken ends are probably often single stranded, and if they contain partially complementary sequences (for example, one of the widely dispersed repetitive sequences¹⁷) or precisely complementary sequences (as in rejoining) can base pair and be irreversibly joined by polymerase and ligase action. By contrast, telomeres could not become permanently joined because of the sequence-specific endonuclease which would split them apart, if they did happen to pair and become joined when in the single stranded state.

A second property of telomeres is frequent temporary association of the telomeres of homologous chromosomes^{18,19}. Even in the absence of palindromic ends, the corresponding ends of homologous chromosomes could associate by 'side-to-side' base pairing if the appropriate strand of each is incomplete (whether because of exonuclease digestion or the delayed completion of replication, Fig. 3*a*). The existence of terminal palindromic sequences, however, would provide an alternative explanation. 'End-to-end' pairing to form a circular dimer would be possible if the 5' ends of the two homologous chromosomes were partially digested by exonuclease action (Fig. 3*c*). Indeed, this would provide an alternative pathway for the replication of 5' end if gaps in this dimer were repaired by DNA polymerases and ligase and then staggered nicks inserted (Fig. 3*d-e*), and the replication completed as before by DNA polymerase. This pathway, in contrast to the previous one, would be most efficient if the palindrome was perfect, since all nucleotides could take part in base pairing.

The occurrence of pairing between homologous telomeres in either of the two ways suggested here could explain cases of late separation of telomeres in mitosis²⁰.

In meiosis, the telomeres normally form strong end-to-end attachments at the beginning of diakinesis^{15,19,21}. Darlington's theory of the terminal chiasma²¹ postulated that this 'terminal affinity' was quite distinct from the normal lateral pairing of chromosomes during the earlier stages of meiotic prophase. I suggest that terminal affinity could be caused by the end-to-end association of palindromic ends as shown in Fig. 3*d*.

Nonhomologous chromosomes

Although the replication mechanism postulated does not require terminal redundancy, and would work perfectly

well even if the palindromic sequences at the two ends of a chromosome were different, there seems no obvious reason why they need be different. So it would be simplest to assume that the telomeres of all chromosomes in any one eukaryote are identical. The considerable evidence for

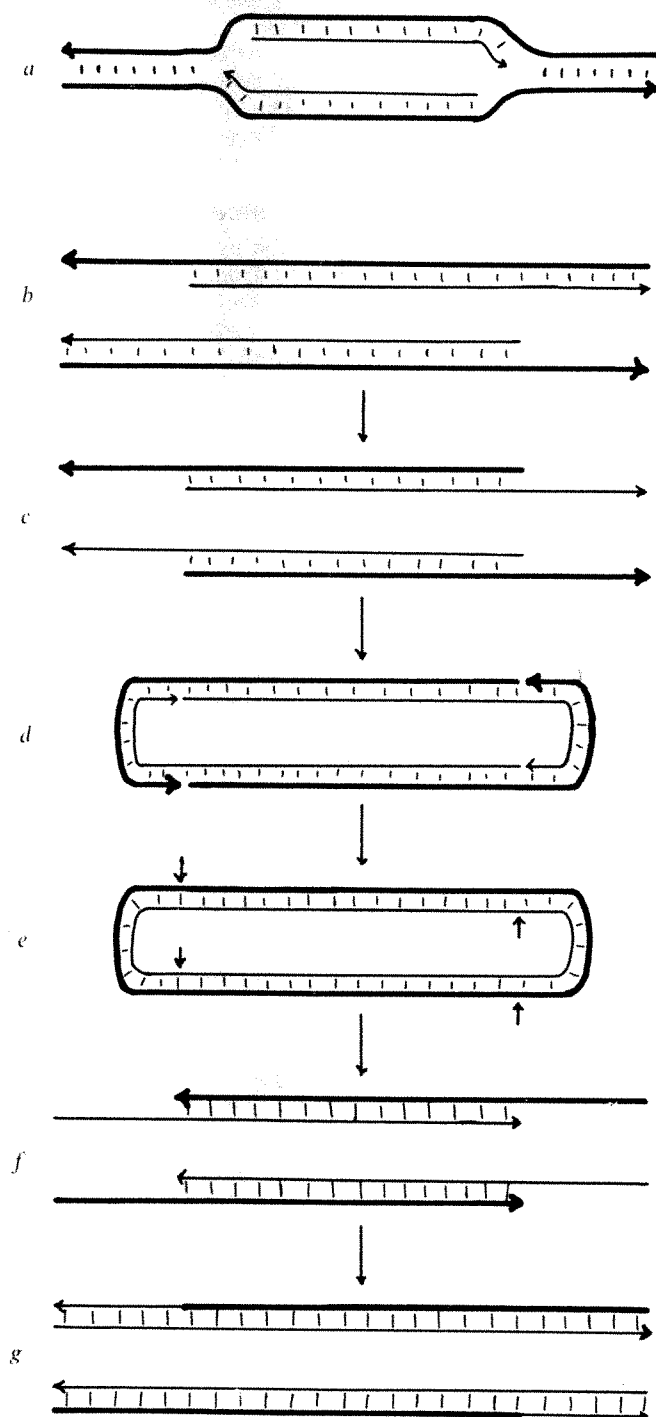


Fig. 3 *a*, Delayed replication provides a simple explanation for the late separation of telomeres at anaphase, which does not require palindromic sequences (the 3' end of a polynucleotide shown by arrow, newly synthesised DNA by a thin line). *b-g*, An alternative model for the replication of 5' ends and the delayed separation of telomeres. *b*, Gaps at 5' ends left by removal of RNA primer; *c*, an exonuclease digests the 5' ends of old DNA; *d*, base pairing between the single-stranded 3' ends of the two homologues; *e*, DNA ligase seals the nicks, and the sequence-specific endonuclease makes new nicks (arrows); *f*, unpairing; *g*, DNA polymerase completes the molecule using the newly exposed 3' ends as primers. Note that in contrast to the mechanism in Fig. 1, the new strand contains no old DNA. Instead an exchange of old DNA has occurred between the two daughters.

direct or indirect attachments between the telomeres of nonhomologous chromosomes¹⁸ lends some support to this idea.

At leptotene of meiotic prophase in many organisms, all the telomeres become gathered together and attached to a small area of the nuclear envelope, presumably so as to facilitate pairing during zygotene^{16,21,23}. This characteristic polarised 'bouquet stage' persists until pachytene when pairing is complete. This phenomenon strongly indicates that all telomeres possess some common structural feature, which might simply be a common palindromic sequence, which could serve not only for replication but for recognition by the apparatus that brings the ends together.

Dupraw¹⁸ has instanced several cases where many or all of the mitotic chromosomes are attached to each other by fine connectives. He even goes so far as to suggest that all the chromosomes of a eukaryote haploid set are simply parts of a single continuous circular DNA molecule, as in bacteria. If this were true it would make the replication mechanism postulated here totally unnecessary. However, Dupraw's model raises more problems than it solves. For example, he fails to discuss the difficult problem of how two haploid super chromosomes could be integrated to make a single diploid circle.

He does recognise, however, that this extreme model can only be compatible with the genetic evidence for the independent assortment of linkage groups if the postulated 'DNA connectives' between the nonhomologous chromosomes are unusually 'labile'. His explanation of this is that the connectives contain no functional genes and that the lability is caused by normal recombination. This would imply, however, that very large amounts of DNA contain no genes but simply serve to connect the chromosomes together. In maize and *Drosophila* such nonfunctional interchromosomal connectives would have to amount to 60% and 75% of the lengths of the functional part of the chromosomes, respectively, if they are to give a recombination frequency of 50%. The need to postulate such huge segments of nonfunctional DNA, whose sole function is to join nonhomologous chromosomes together in such a way as to allow independent assortment, can be totally avoided by supposing instead that the observed linkages are purely temporary and that their lability is not the result of normal genetic recombination.

Indeed, if as suggested here, the ends of all chromosomes have identical sequences, which are sometimes single-stranded, one might expect nonhomologous chromosomes on occasion to join together by base pairing between these cohesive ends. If this did occur, and if DNA ligase were to join the chromosomes covalently, the specific endonuclease would ensure that this joining was not permanent. In principle the chromosomes could associate reversibly into a single superchromosome as indicated in Fig. 4*a*. Though this kind of mechanism could explain some examples of chromosome association, others involve association between regions other than the telomere, and could be explained in this way only if it were assumed that the DNA ends are not at the telomere.

I am grateful to the referee of this paper for drawing my attention to a paper by White²², who suggested that the occurrence of multivalents of terminally associated chromosomes in the meiosis of F_1 hybrids (and other situations where normal pairing is reduced) could be caused by minute regions of homology at the tips of nonhomologous chromosomes, and not by reciprocal translocation as had hitherto been believed. This hypothesis of reduplicated telomere regions was supported by Callan, who in a footnote to White's paper said "all new telomeres are probably homologous". Despite criticisms¹⁹ it remains a reasonable interpretation of at least some of the data, which is fully consistent with my assumption that all telomeres in any one species are homologous.

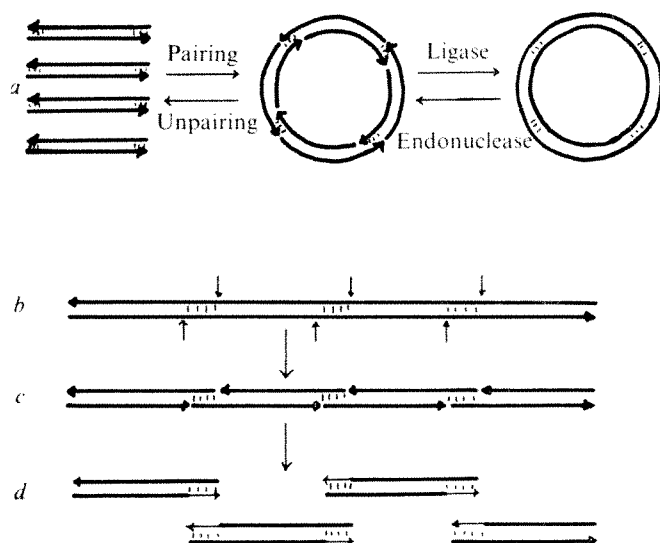


Fig. 4 *a*, The reversible formation of superchromosomes or, reading from right to left, an hypothesis for the origin of several linear eukaryote chromosomes from a single circular prokaryote chromosome (the 3' end of a polynucleotide shown by arrow, newly synthesised DNA by a thin line). *b-d*, Mechanism for the formation of minichromosomes during chromosome diminution or hypotrich ciliate macronuclear formation; *b*, the small arrows show the sites at which a specific endonuclease makes nicks at each end of special internal palindromic sequences; these sequences differ from the palindromic sequences at the original ends of the chromosomes. *c*, The nicks are made and, *d*, a DNA polymerase extends each newly formed 3'OH end, forming complete palindromic sequences at the new ends, and causing the new mini chromosomes to separate.

White pointed out that if many or most chromosomes have homologous telomeres, multivalent formation must normally be suppressed during meiosis. He suggests, however, that the homologies have no great evolutionary importance and originate quite fortuitously (though very commonly) by the reciprocal translocation of extremely minute segments, and the survival of resulting deficiency-duplication homozygotes. If the homologies are mere accidents, there is no obvious reason why the evolution of a general mechanism to suppress multivalents should have been selectively favoured over the simple alternative of eliminating the unnecessary duplications. By contrast, my suggestion that telomeres consist of identical palindromic sequences, with an indispensable role in replication, provides a strong selective advantage for the suppression of multivalent formation.

Chromatin diminution

In eukaryotes there are at least two instances where normal chromosomes are cleaved into smaller linear pieces or 'minichromosomes' which are subsequently replicated; these are chromatin diminution in the somatic cells of the worm *Ascaris* and other animals²³, and macronuclear formation in the ciliates *Stylonychia* and *Euplotes*^{24,25}. The replication of these 'new ends' will present exactly the same problems as that of the original ends of the normal chromosomes. I suggest, therefore, that these new ends must also consist of palindromic sequences, and that they are replicated in essentially the same way as the normal telomeres. Moreover, the site specific nuclease which functions in the replication of minichromosomes could be the same enzyme as the one which initially cuts the chromosome into minichromosomes, as both have to cut at the same site in the sequence. The only difference is that in replication only one strand is cut (Fig. 2e) whereas in making minichromosomes two staggered cuts are needed on opposite strands

(Fig. 4b). Two single-stranded ends will be produced and when the other strand is completed by DNA polymerase each minichromosome will have a complete palindromic end. To ensure that this cleavage does not occur in the germ line of *Ascaris* and in the micronuclei of the ciliate I suggest that the palindromic sequence and the endonuclease involved are different from those at the normal chromosome ends, and that the synthesis of the endonuclease is normally repressed and has to be derepressed to initiate chromatin elimination. This special endonuclease and palindromic sequence may have evolved from the normal ones involved in telomere replication.

Origin of eukaryote chromosomes

A necessary and crucial step in the evolution of a group of linear eukaryote chromosomes from a single circular bacterial chromosome must have been the evolution of some special mechanism for the replication of the chromosome ends. Unless this mechanism was present right at the outset, pieces would have been lost from the ends of the chromosome at each replication. Therefore I suggest not only that the palindromic telomere mechanism evolved from a bacterial restriction mechanism, but that this change was all that was needed to convert a circular chromosome into several linear ones. Thus Fig. 4a if read from right to left could be taken to represent the origin of separate eukaryote chromosomes.

It should be possible to test all the predictions of my model as methods for sequencing DNA and for isolating and working with unbroken eukaryote DNA improve.

Note added in proof: Work recently published by Wilson and Thomas (*J. molec. Biol.*, **84**, 115-144 (1974)) reveals numerous nearly perfect palindromes in eukaryote DNA, but does not indicate whether any are terminally located as my model would predict.

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- ¹ Klein, A., and Bonhoeffer, F., *A. Rev. Biochem.*, **41**, 301-332 (1973).
- ² Wickner, W., Brutlag, D., Schekman, R., and Kornberg, A., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 965-969 (1972).
- ³ Keller, W., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1560-1564 (1972).
- ⁴ Sugino, A., Hirose, S., and Okazaki, R., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1863-1867 (1972).
- ⁵ Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K., and Sugino, A., *Proc. natn. Acad. Sci., U.S.A.*, **59**, 598-605 (1968).
- ⁶ Watson, J. D., *Nature new Biol.*, **239**, 197-201 (1972).
- ⁷ Bellet, A. J. D., and Younghusband, H. B., *J. molec. Biol.*, **72**, 691-709 (1972).
- ⁸ Laird, C. D., *Chromosoma*, **32**, 378-406 (1971).
- ⁹ Petes, T. D., and Fangman, W. L., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 1188-1191 (1972).
- ¹⁰ Kavenoff, R., and Zimm, B. H., *Chromosoma*, **41**, 1-27 (1973).
- ¹¹ Thomas, C. A., *A. Rev. Genet.*, **5**, 237-256 (1971).
- ¹² Callan, H. G., *Proc. R. Soc. B.*, **181**, 19-41 (1972).
- ¹³ Kelly, T. J., and Smith, H. O., *J. molec. Biol.*, **51**, 393-409 (1970).
- ¹⁴ Swanson, C. P., *Cytology and Cytogenetics* (Macmillan, London, 1958).
- ¹⁵ Schrader, F., *Mitosis* (Columbia University Press, New York, 1953).
- ¹⁶ White, M. J. D., *Animal Cytology and Evolution* (Cambridge University Press, 1948).
- ¹⁷ Davidson, E. H., Hough, B. R., Amenson, C. S., and Britten, R. J., *J. molec. Biol.*, **77**, 1-23 (1973).
- ¹⁸ Dupraw, E. J., *DNA and chromosomes* (Holt, Rinehart and Winston, Inc., New York, 1970).
- ¹⁹ John, B., and Lewis, K. R., *The Meiotic System* (Protoplasmatologia VI, F1, Springer-Verlag, Wien, 1965).
- ²⁰ Lima-de-Faria, A., and Bose, S., *Chromosoma*, **13**, 315-327 (1962).
- ²¹ Darlington, C. D., *Recent Advances in Cytology* (2nd ed.) (J., and A. Churchill Ltd., London, 1937).
- ²² White, M. J. D., *Am. Nat.*, **95**, 315-321 (1961).
- ²³ Wilson, E. B., *The Cell in Development and Heredity* (3rd ed.) (New York: Macmillan, 1925).
- ²⁴ Ammermann, D., *Chromosoma*, **33**, 209-238 (1971).
- ²⁵ Prescott, D. M., Murti, K. G., and Bostock, C. J., *Nature*, **242**, 576, 597-600 (1963).

letters to nature

X-ray observations of the Coma and Virgo clusters from Copernicus

THE X-ray sources in both the Virgo and Coma clusters of galaxies are known to be extended. We report here observations to determine the spatial characteristics of each source using the two grazing incidence reflecting telescopes and the collimated proportional counters on board Copernicus¹.

The observations were made on January 6 and 7, 1973, and January 26 and 27, 1973, for the Virgo and Coma clusters respectively. In both cases only the widest field of view on the X-ray telescopes was used (effective beam diameter about 12') to observe seven regions, one centred on the source, and six spaced equally 10' from the centre position. The two telescopes covered the energy ranges 1.5 to 4.6 keV (3 to 8 Å) and 0.48 to 1.45 keV (6 to 18 Å) and the collimated counters the range 2.5 to 7.5 keV. A total of 254.7 min of usable data was acquired from the Virgo cluster and 382.6 min from the Coma cluster; the total observation time was approximately equally divided between the seven observing regions.

Coma cluster: It is now well established that the Coma cluster is an extended X-ray source²⁻⁵. The Uhuru results³⁻⁵ were originally analysed in terms of a uniform emitting disk, with a resulting diameter of $36' \pm 4'$ (ref. 5). A reanalysis of the data⁴ by Lea *et al.*⁶ in terms of more realistic models, including an isothermal gas sphere, resulted in smaller source dimensions. For the isothermal sphere models Lea *et al.* deduced a best-fit core radius $a = 16' \pm 3'$, and for a model in which the gas density $\rho \propto r^{-2}$ (the asymptotic approach to an isothermal sphere) they obtained $a = 7' \pm 2'$. Models for which the emission is proportional to the first power of the density, predicted for some nonthermal mechanisms, were excluded by their analysis.

The Uhuru observations could not distinguish between thermal or power-law X-ray spectra, but the low energy results from the rocket flight of Gorenstein *et al.*⁷ show that the spectrum is best fitted by thin source bremsstrahlung with $kT = 8.1 \pm 1.5$ keV (including an energy-dependent Gaunt factor), taking into account the absorption of 2.10^{20} hydrogen atoms cm^{-2} along the line of sight to Coma. A power-law spectrum appears to be excluded by the need for a hydrogen column density lying outside the acceptable range 1.5 to 2.5×10^{20} hydrogen atoms cm^{-2} (for the galactic latitude of Coma).

The X-ray telescopes on Copernicus observed a central region of Coma and six adjacent regions with 12' effective diameter⁸. A total of 63 min observing time was spent on the central region, which contains the cluster kinematic centre, the Uhuru X-ray centroid and the two prominent galaxies NGC4874 and NGC4869. In the 0.48 to 1.45 keV range, the 2σ upper limit on the flux from the central region corresponds to 1.3×10^{-2} photons $\text{cm}^{-2} \text{s}^{-1}$, and in the 1.5 to 4.6 keV band the recorded count, at 3σ above background, corresponds to a flux of 9.4×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$, assuming the spectrum described here. In the outer regions, the average signal over the six bins gave 3.3×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ in the higher energy band (a 2.7σ result).

We have compared these observations with the counts predicted by spectral fits to the combined data from Uhuru and Gorenstein *et al.*⁶:

$$dN/dE = (0.044/E) G e^{-E/kT} \text{ photons cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$$

where the Gaunt factor $G = 0.8 (E/kT)^{-0.4} E/kT > 0.1$
and $G = 1 - \log_{10} (E/kT) E/kT < 0.1$
with $kT = 8.1 \pm 1.5$ keV.

For the efficiencies of the telescope system covering the 0.48 to 1.45 keV band, the predicted count from the whole cluster is 375 ± 41 . The 2σ upper limit from the central bin was 54 counts and we can therefore state with 95% confidence that less than 14% of the total emission comes from the central 12' for this energy interval. For the higher energy band (1.5 to 4.6 keV) the predicted count from the whole cluster is 287_{-15}^{+17} . The observed count after background subtraction was 57 ± 27 (at 3.2σ above the mean background level, or $20 \pm 10\%$ of the predicted total).

Assuming an isothermal gas distribution $\rho = \rho_0 (1 + r^2/a^2)^{-3/2}$, the above result leads to a lower limit of $19.5'$ for the core radius in the 0.48 to 1.45 keV band, and a value $a = 15_{-3}^{+7}$ from the higher energy band. The positive error on the latter result is reduced by noting the ratio between the counts in the outer and central bins, giving finally $a = 15' \pm 3'$. This agrees well with the value $a = 16' \pm 3'$ from Lea *et al.*⁶ for a similar energy interval.

Following Lea *et al.* we have also fitted the density function $\rho = \rho_0 (1 + r^2/a^2)^{-1}$, or emission $\epsilon \propto (1 + r^2/a^2)^{-2}$, approached asymptotically by an isothermal sphere. For this function, the upper limit for a becomes $10'$ in the low energy band, and $a = 8' \pm 2'$ for the higher energy band, the latter measurement again agreeing with $a = 7' \pm 2'$ from ref. 6.

As a test on the reliability of the Copernicus results quoted here, we have compared them with predictions based on the Uhuru and Gorenstein results. The count observed in the 2.5 to 7.5 keV detector, which viewed the whole cluster throughout the observations was $2,873 \pm 192$ counts at 19σ above background. The total predicted count was $2,716 \pm 275$, the agreement to 5% accuracy being well inside the statistical uncertainties involved.

Thermal models for the origin of the Coma cluster X-ray emission are presently divided between those which invoke accretion for the origin of the gas⁹⁻¹¹ and those which explain the intracluster gas as a wind emanating from the cluster centre¹² with the energy coming from violent activity (see ref. 13), or by frictional heating. The inverse Compton models^{14,15} seem to be ruled out by the thermal shape of the X-ray spectrum, supported by the present observations, as well as by difficulties with the emission mechanism itself when applied to Coma^{16,17}.

The temperature distribution in the gas both for the accretion models and the wind models (heating by mechanisms inside galaxies, and by friction) is predicted to fall with angular distance from the cluster centre. The tentative results obtained in the present observations clearly show this trend, whilst not allowing a preference among these individual thermal models to be firmly stated. But the 'heating inside galaxies' model of Yahil and Ostriker¹² is given some support as the dominant mechanism, since the core radii found here agree well with those predicted.

Virgo cluster: Several rocket flights have observed a source at 1 to 10 keV in the direction of M87 and the Virgo cluster, as summarised by Lampton *et al.*¹⁸ for flights before 1971. Since then, the Lockheed Group have observed the

source between 0.2 and 1.6 keV (ref. 19) and found it to consist of a point source within a few arc mins of M87 and an extended source with its centroid displaced $\sim 0.2^\circ$ in the direction of the optical jet. The point source was found to contain $60 \pm 30\%$ of the total low-energy X-ray emission; the Uhuru data are consistent with up to 35% of the emission coming from a point source.

The best fit to the Uhuru spectral data²⁰ is a power law, supposedly arising from the inverse Compton effect. Extrapolation of this spectrum to lower energies, combined with the spectral points of R.C. Catura *et al.* (unpublished results consistent with the upper limits of Gorenstein *et al.*²¹) leads to a necessity for ageing of the electrons in the ambient photon field; self absorption, suggested by Kellogg *et al.*²⁰, is ruled out by the spectral data of R. C. Catura *et al.* (unpublished). Alternatively both sets of data are consistent with a thermal bremsstrahlung from a plasma at 36×10^6 K, with only 1.5×10^{20} hydrogen atoms cm^{-2} along the line of sight, consistent with radio observations in this direction.

A reanalysis by Ricketts²² of previous Leicester rocket flights which observed M87 shows agreement with the above bremsstrahlung spectrum in temperature and normalisation, without strong evidence for any variability (see also ref. 18). Uhuru observed no variability greater than 30% between observations four months apart²⁰.

As with Coma, the Copernicus telescopes were pointed at a central bin and six neighbouring regions of the Virgo cluster, where the central bin contained M87. The counts obtained were compared with those predicted from the thermal spectrum above:

$$dN/dE = 0.31/E \exp(-E/3.1) \exp(-\sigma N_H) \text{ photons cm}^{-2}\text{s}^{-1}\text{keV}^{-1} \text{ with } N_H = 2.5 \times 10^{20} \text{ hydrogen atoms cm}^{-2}.$$

In the 0.48 to 1.45 keV band we observed 71 ± 21 counts in 63 min of observation on the central bin (a signal 4.1σ above background), compared with $1,032 \pm 62$ counts predicted from the whole cluster; only $7 \pm 2\%$ of the predicted count was in the central bin. In the 1.5 to 4.6 keV band, we can set an upper limit of 10% of the predicted total cluster emission being in the central region containing M87 and the jet. These percentages are clearly inconsistent with the $\sim 60\%$ found by Catura *et al.*, and are well below the 35% upper limit set by Uhuru.

Assuming the isothermal gas distribution $\rho = \rho_0 (1 + r^2/a^2)^{-3/2}$, our results imply a core radius $a > 23'$ for the 1.5 to 4.6 keV band, and $a = 28' \pm 4'$ for the 0.48 to 1.45 keV band. Lea *et al.*⁶ found $a = 25' \pm 4'$ for the 2 to 6 keV range.

In the 2.5 to 7.5 keV detector with a wide field of view, the total observed count was $1,934 \pm 153$, compared with $1,711 \pm 174$ from the Lockheed/Uhuru spectrum quoted above. So our observed flux is consistent with Uhuru to an accuracy of $\approx 10\%$. We therefore infer that the total X-ray flux from the Virgo cluster in this energy range did not change by more than 10% between early 1971 and January 1973.

The OAO Copernicus observations of the Coma and Virgo clusters are consistent with the high-temperature isothermal gas sphere models already proposed for the emission, with some indication that the low-energy X-ray emission is more extended, as hypothesised for the Perseus cluster²³. There is no strong galactic X-ray source at the centre of either cluster, in contrast to the bright X-ray emitting Seyfert galaxy NGC 1275 at the centre of the Perseus cluster²³. We note that all three clusters contain active galaxies (strong radio sources) near their centres, but only Perseus has a strong infrared emitting Seyfert galaxy.

The absence of a strong point source in the Virgo cluster is in disagreement with Lockheed rocket flight data. We do find good agreement, however, with Uhuru data from the whole cluster (including M87) in the 2.5 to 7.5 keV range.

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- ¹ Bowles, J. H., Patrick, T. J., Sheather, P. H., and Eiband, A. M., *J. Phys. E.*, **7**, 183 (1974).
- ² Meekins, J. F., Gilbert, F., Chubb, T. A., Friedman, H., and Henry, R. C., *Nature*, **231**, 101 (1971).
- ³ Gursky, H., Kellogg, E., Murray, S., Leong, O., Tananbaum, H., and Giacconi, R., *Astrophys. J. Lett.*, **167**, L81 (1971).
- ⁴ Giacconi, R., Gursky, H., Kellogg, E., Murray, S., Schreier, E., and Tananbaum, H., *Astrophys. J.*, **178**, 281 (1972).
- ⁵ Forman, E., Kellogg, E., Gursky, H., Tananbaum, H., and Giacconi, R., *Astrophys. J.*, **178**, 309 (1972).
- ⁶ Lea, S., Silk, J., Kellogg, E., Murray, S., *Astrophys. J. Lett.*, **184**, L105 (1973).
- ⁷ Gorenstein, P., Bjorkholm, P., Harris, B., and Harndex, F. R. Jun., *Astrophys. J. Lett.*, **183**, L57 (1973).
- ⁸ Fabian, A. C., Zarnecki, J. C., and Culhane, J. L., *Nature phys. Sci.*, **242**, 18 (1973).
- ⁹ Gott, J. R., III, and Gunn, J. E., *Astrophys. J. Lett.*, **169**, L13 (1971).
- ¹⁰ Gunn, J. E., and Gott, J. R., III, *Astrophys. J.*, **176**, 1 (1972).
- ¹¹ Hunt, R., and Sciamia, D. W., *Nature*, **238**, 320 (1972).
- ¹² Yahil, A., and Ostriker, J. P., *Astrophys. J.*, **185**, 787 (1973).
- ¹³ Solinger, A. B., and Tucker, W. H., *Astrophys. J. Lett.*, **175**, L197 (1972).
- ¹⁴ Brecher, K., and Burbidge, G. R., *Nature*, **237**, 440 (1972).
- ¹⁵ Perola, G. C., and Reinhardt, M., *Astr. Astrophys.*, **17**, 432 (1972).
- ¹⁶ Bridle, A. H., and Feldman, P. A., *Nature phys. Sci.*, **235**, 168 (1972).
- ¹⁷ Okoye, S. E., *Mon. Not. R. astr. Soc.*, **160**, 339 (1972).
- ¹⁸ Lampton, M., Bowyer, S., Mack, J., and Margon, B., *Astrophys. J. Lett.*, **168**, L1 (1971).
- ¹⁹ Catura, R. C., Fisher, P. C., Johnson, H. M., and Meyerott, A. J., *Astrophys. J. Lett.*, **177**, L1 (1972).
- ²⁰ Kellogg, E., Gursky, H., Tananbaum, H., Giacconi, R., and Pounds, K., *Astrophys. J. Lett.*, **174**, L65 (1972).
- ²¹ Gorenstein, P., Harris, B., and Gursky, *Astrophys. J. Lett.*, **172**, L41 (1972).
- ²² Ricketts, M. J., thesis, Univ. Leicester (1973).
- ²³ Fabian, A. C., Zarnecki, J. C., Culhane, J. L., Hawkins, F. J., Peacock, A., Pounds, J. A., and Parkinson, J. H., *Astrophys. J. Lett.*, **189**, L1 (1974).

Prediction of radio structure in the two largest redshift QSOs

THE discovery that the optical objects identified with OH471 (0642+449) and OQ172 (1442+101) are QSOs with redshifts of 3.40 (ref. 1) and 3.53 (ref. 2), respectively, has stimulated considerable interest in their optical^{3,4} and radio properties⁵.

Here we present predicted angular sizes for the radio sources based on incoherent electron synchrotron emission. Such angular sizes, combined with a cosmological interpretation of the redshifts, determine the minimum variability timescales consistent with the absence of relativistic effects.

Figure 1 shows the radio spectral data of OH471 and OQ172 (ref. 5) and decompositions of the spectra into canonical self-absorbed synchrotron components. We have used the minimum number of canonical components consistent with the data and have made the simplifying assumption that all components of a given source have the same spectral index, α . Uncertainties in the data preclude a meaningful determination of the spectrum at the highest radio frequencies.

Table 1 lists the resulting properties of each component. The frequency ν_n and the spectral flux $F\nu_n$ connote the intersection of the extrapolated opaque and transparent portions of the spectrum of each component. The $\nu_n = 6.5$ GHz and the $\nu_n = 18.4$ GHz components of OH471 are not very well established, but any combination of canonical components in this frequency range results in similar angular sizes.

Table 1 Theoretically expected angular radii and timescales

Source		OH471 (0642+449)		OQ172 (1442+101)	
z		3.40		3.53	
ν_n [GHz]	0.84	6.5	18.4	0.26	0.94
F_{ν_n} [Jy]	3.0	0.63	0.83	2.6	2.5
	0.89	0.89	0.89	0.63	0.63
α					
$\theta(F_V^c = F_V^s)$ [10^{-3} arc s]	3.8	0.28	0.13	6.1	1.9
$\theta(u_r = u_m)$ [10^{-3} arc s]	4.9	0.33	0.14	12	3.5
$\theta(u_e = u_m)$ [10^{-3} arc s]	5.1 (4.8)*	0.31 (0.29)	0.12 (0.12)	14 (13)	3.7 (3.5)
$\theta(u_r = u_e)$ [10^{-3} arc s]	5.1 (4.4)	0.27 (0.23)	0.10 (0.08)	17 (14)	3.9 (3.4)
D_1 [Gpc]		20† (55)		21 (59)	
$R(F_V^c = F_V^s)$ [pc]	19 (50)	1.4 (4)	0.6 (1.7)	30 (80)	10 (26)
$t_v(F_V^c = F_V^s)$ [yr]	90 (250)	6 (18)	3 (8)	150 (400)	50 (130)
t_v^{-1} [% per yr]	1.1 (0.4)	15 (5)	30 (12)	0.7 (0.24)	2.1 (0.8)
$B(u_e = u_m)$ [mgau]	15 (12)	130 (100)	300 (260)	6 (5)	19 (15)
$U(u_e = u_m)$ [$M_\odot c^2$]	50 (600)	1 (20)	1 (15)	100 (1000)	30 (300)

*Two values appear for each parameter dependent upon the cosmological model: They are calculated for standard Friedmann cosmologies with $q=1$ and with $q=0$ (in parentheses).

†The luminosity distance is based upon $(c/H)=6$ Gpc ($H=50$ km/s Mpc) ($H=50$ km/s Mpc) $^{-1}$.

The angular size of a compact nonthermal source depends only weakly on assumptions about physical properties or distance, if the radiation is incoherent electron synchrotron emission with no relativistic bulk motions¹¹⁻¹³. Angular sizes and assumed distances therefore imply a minimum timescale of variability in the absence of highly relativistic effects^{14,15}.

1 Jy = 1 f.u.

Estimation of the angular size of a compact radio source with known spectral form rests upon the establishment of its maximum brightness temperature. Compact nonthermal radio sources show a rather narrow range of maximum brightness temperatures, 10^{11} K $< T_n < 10^{12}$ K (refs 6, 7). Although this results partly from observational selection, it also follows as a direct consequence of synchrotron theory^{6,7}. For a given radio

spectrum, the small dispersion in brightness temperature allows an accurate estimate of angular radius θ , since $\theta(\nu_n, F_{\nu_n}) \propto T_n^{-1/2}$. Table 1, which lists angular radii calculated according to assumed values of four different physical source parameters, demonstrates the insensitivity of the angular radius (brightness temperature) to physical conditions in a source. The parameter (F_V^c/F_V^s) represents the ratio of spectral flux in Compton-scattered synchrotron photons to (extrapolated) synchrotron spectral flux. The other three ratios involve the energy densities in relativistic electrons (u_e), magnetic field (u_m), and radiation (u_r).

The insensitivity of θ to each of these parameters follows from readily derivable relationships (see Table 4, ref. 8):

$$\theta(F_V^c/F_V^s) \propto (F_V^c/F_V^s)^{-1/(2(3+2\alpha))} (1+z)^{(2+\alpha)/(3+\alpha)}, \quad (1)$$

$$\theta(u_r/u_m) \propto (u_r/u_m)^{-1/10} (1+z)^{3/5}, \quad (2)$$

$$\theta(u_e/u_m) \propto (u_e/u_m)^{-1/17} (1+z)^{9/17} D_1^{-1/17}, \quad (3)$$

$$\theta(u_e/u_r) \propto (u_e/u_r)^{-1/7} (1+z)^{3/7} D_1^{-1/7}, \quad (4)$$

with D_1 the luminosity distance. So even crude bounds to any of these ratios greatly restrict the angular sizes. In many cases, optical and X-ray observations require $(F_V^c/F_V^s) < 1$ (ref. 8) and the radio data alone generally suggest $(F_V^c/F_V^s) < 1$ (refs. 9, 10). If $u_r > u_m$, Compton energy losses dominate synchrotron losses; thus, to avoid catastrophic luminosities, $(u_r/u_m)^{1/10} \lesssim 1$. Although equipartition ($u_e = u_m$) seems unlikely in evolving compact sources and experience suggests $u_e > u_m$ (refs. 7, 8) it seems reasonable to expect that $(u_e/u_m)^{1/17} \approx 1$. Finally, (u_e/u_r) represents the weighted radiative lifetime of electrons normalized to the travel time of light across the source; consequently, in the absence of noncentral accelerations, $(u_e/u_r) \gtrsim 1$.

The canonical incoherent synchrotron model admits angular sizes smaller than those given in Table 1 only in the presence of relativistic bulk motions (for example, small pitch angles or relativistic expansion)^{8,9}. These minimum angular radii range in the lower frequency components between 2 and 6×10^{-3} arc s, and even the $\nu_n = 18.4$ GHz component of OH471 should have an angular radius in excess of 0.1×10^{-3} arc s. Very long baseline interferometric (VLBI) observations could confirm, therefore, the consistency of the canonical assumptions.

The source angular radius indicates a physical size

$$R = D_1 \theta (1+z)^{-2} \quad (5)$$

and, for variable sources, an index of variability

$$i_v = (R/c t_v) (1+z) \approx (D_1 \theta / c t_v) (1+z), \quad (6)$$

where the variability timescale $t_v = |d \ln F_V / dt|^{-1}$. In the absence of highly relativistic effects, $i_v \lesssim 3$ (ref. 9); conversely, for $i_v \lesssim 3$, there is a minimum t_v consistent with the assumed angular size and distance. Table 1 lists for each source com-

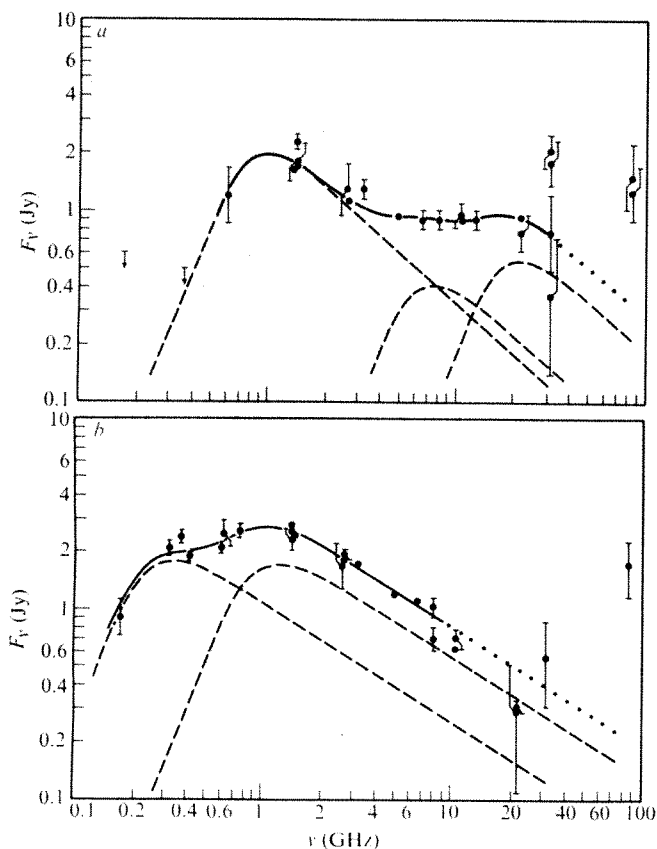


Fig. 1 Radio spectral data of: a, OH471 (0642+449); b, OQ172 (1442+101) from ref. 5. Points with no error bars have errors within the solid circles. Dashed curves represent best fit decompositions of those portions of the spectra indicated by solid lines into canonical self-absorbed synchrotron components.

ponent values of R and t_v , based on luminosity distances D_l calculated (assuming $c/H=6$ Gpc; $H=50$ km/(s Mpc)) for a Friedmann cosmological model with $q=1$ and for one with $q=0$. We note that for redshifts as large as three, the choice of cosmological model (q) significantly influences the allowed values of t_v and t_v^{-1} as well as some derived parameters such as the equipartition (minimum) energy content.

Present observations do not firmly establish the existence of variability in either of these sources, although Gearhart *et al.*⁵ suggest the possibility of a 20% decrease in OH471 at 1.4 GHz during a 4 yr span, as compared to a value $t_v^{-1} \lesssim 1\%$ per yr given in Table 1 for the relevant component. If such rapid variations are present, VLBI observations could ultimately decide among possible alternatives to the canonical assumptions made here^{8,9}.

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- ¹ Carswell, R. F., and Strittmatter, P. A., *Nature*, **242**, 394–395 (1973).
- ² Wampler, E. J., Robinson, L. B., Baldwin, J. A., and Burbidge, E. M., *Nature*, **243**, 336–337 (1973).
- ³ Oke, J. B., *Astrophys. J. Lett.*, **189**, L47–L49 (1974).
- ⁴ Baldwin, J. A., Burbidge, E. M., Burbidge, G. R., Hazard, C., Robinson, L. B., and Wampler, E. J., *Astrophys. J.* (in the press).
- ⁵ Gearhart, M. R., *et al.*, *Nature*, **249**, 743 (1974).
- ⁶ Kellermann, K. I., and Pauliny-Toth, I. I. K., *Astrophys. J. Lett.*, **155**, L71–L78 (1969).
- ⁷ Burbidge, G. R., Jones, T. W., and O'Dell, S. L., *Astrophys. J.* (in the press).
- ⁸ Jones, T. W., O'Dell, S. L. and Stein, W. A., *Astrophys. J.* (in the press).
- ⁹ Jones, T. W., O'Dell, S. L., and Stein, W. A., *Astrophys. J.*, **188**, 353–368 (1974).
- ¹⁰ Jones, T. W., *Astrophys. J. Lett.* (in the press).
- ¹¹ Shklovsky, I. S., *Soviet Astr.*, **8**, 132–133 (1964).
- ¹² Woltjer, L., *Astrophys. J.*, **146**, 597–599 (1966).
- ¹³ Rees, M. J., *Mon. Not. R. astr. Soc.*, **135**, 345–360 (1967).
- ¹⁴ Rees, M. J., *Nature*, **211**, 468–470 (1966).
- ¹⁵ Noerdlinger, P. D., *Astrophys. Lett.*, **4**, 233–234 (1969).

How long do radio galaxies emit at radio wavelengths

MANY clusters of galaxies from Abell's catalogue¹ have recently been observed at 1,400 MHz with the 300-foot radio telescope of the NRAO (ref. 2 and F. Owen, to be published). On the Palomar Sky Survey prints we inspected 355 clusters of galaxies (111 of them were observed by H.M.T.², the remaining 244 by F. Owen, to be published) which belong to distance group 5 and thus are situated at almost the same distance. Radio emission in excess of 0.1 flux units (f.u.) was detected from 95 out of the 355. A radio source was identified with a cluster of galaxies if it was within 5 arc min from the cluster centre, the diameter of which is about 25 arc min.

The study showed that:

(a) There are 27 clusters of galaxies in which the brightest is a cD-type galaxy (cluster type I according to Bautz and Morgan³). Eighteen of them emit at radio frequencies. Since the probability of detecting a radio source at the position of a cluster of galaxies is one in the case of observations of 22 clusters, if objects of both types are distributed randomly², the most probable number of

incorrect identifications is one or two.

(b) There are four clusters in which the brightest galaxy is a compact galaxy and two of them are radio emitters.

(c) Fifty-one clusters have two or three bright members, one of which is either a cD-type or a compact galaxy, or a peculiar galaxy, or a close double galaxy. Radio sources are found at the positions of 39 such clusters. Two or three of the detected sources may be located there by chance.

(d) There are 33 clusters in which a bright elliptical galaxy dominates. Radio emission is detected at the positions of only three clusters and two of these identifications could be by chance.

(e) There are 45 clusters which contain two or three bright E galaxies. Radio sources are found at the positions of six of them and two of these identifications are most probably spurious.

Since the angular resolution of the survey is greater than the diameters of the clusters it is possible to go one step further and check whether the dominant galaxy in the cluster is within the error box of a radio source position. In almost all the above cases this is so. Since radio sources are generally not resolved this means that radio emission originates in one galaxy of the cluster rather than in many members of it. Thus one can with great confidence identify the radio sources in the clusters considered with centrally located, bright cD compact, peculiar, close double or elliptical galaxies. The radio luminosities of these galaxies are of the same order as that of NGC4486 (Vigo A) and higher. The Hubble constant $H = 75$ km s⁻¹ Mpc⁻¹ and the mean redshifts of clusters of galaxies in distance group 5, given by Abell, were used to determine the radio luminosities.

(f) Most numerous are the clusters of galaxies which do not contain any one dominant galaxy or even 2 or 3 members sharply differing in brightness from the rest. In such clusters the differences in magnitude of bright member galaxies are small (cluster type III according to Bautz and Morgan³). There are 195 such clusters and radio emission is detected at the positions of only 27 of them. Moreover nine radio sources could be located at the positions of clusters purely by chance.

Thus, about 70% of clusters of the first three groups have radio emission associated with them and hence the bright galaxies of these clusters, which are either cD-type, compact, peculiar or close double galaxies, emit at radio wavelengths during at least two-thirds of their lives, or more precisely during the interval of time when they look like galaxies of the named optical types. The duration of the radio emitting phase may be even longer if not all such galaxies pass through this phase.

In clusters of galaxies of groups d, e and f, where the dominating bright member is of type E or there is no outstandingly bright galaxy, the occurrence of radio emission is seven or eight times less frequent than in clusters of the first three groups. Consequently these galaxies emit radio waves for not less than 10% of their lives.

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¹ Abell, G. O., *Astrophys. J. Suppl.*, **3**, 211 (1958).

² Tovmassian, H. M., *Astrofizika*, **9**, 177 (1973).

³ Bautz, L. P., and Morgan, W. W., *Astrophys. J.*, **162**, L149 (1970).

H₂O, O₃, N₂O and HNO₃ in the arctic stratosphere

MEASUREMENTS of the total amounts and average mixing ratios of a number of stratospheric trace gases at altitudes above 15 km have previously been published¹⁻³. The measurements were derived from submillimetre wavelength emission spectra, recorded on board Concorde 002 during high altitude test flights.

Here we report the preliminary results of further measurements from this aircraft, using an improved experimental system which permitted us to carry out a limb-scanning experiment to deduce not only the total amounts but also the vertical profiles of several gases.

The results were obtained during flights made in May 1973 into the Arctic circle. Prestwick international airport was used as an operations centre, and flights took place during both day and night, reaching a most northerly latitude of 72.5°N. The flight paths were generally triangular, with a rather short northernmost east-west or west-east leg. The present data were obtained on flights on May 16 and 16/17 (night), 1973. Flight altitude was a constant 50,200 feet (15.25 km). Take-off times were 1500 GMT and 2400 GMT. The results reported here were obtained between 65 and 70°N.

The interferograms were recorded once per 8 min, up to an optical path difference equivalent to 0.04 cm⁻¹. These were later Fourier transformed at the National Physical Laboratory (NPL). The atmospheric temperature profiles were obtained from the meteorological sounding network, with kind cooperation from the Meteorological Office. The analysis and spectroscopic assignments of the spectra have been described elsewhere^{1,2,4}. The method used for analysing the concentration profile was a standard limb-scanning method^{5,6}.

Figure 1 shows the initial results obtained, using spectral data from May 16/17, 1973. The diagram shows mixing ratio profiles for H₂O, O₃, HNO₃ and N₂O, a tentative column density for NO₂ and an estimated upper limit for SO₂; it also shows the volume mixing ratios evaluated for each species below 15 km. It is not possible to deduce unambiguously a mixing ratio distribution profile above the flight altitude and so we have quoted only the total

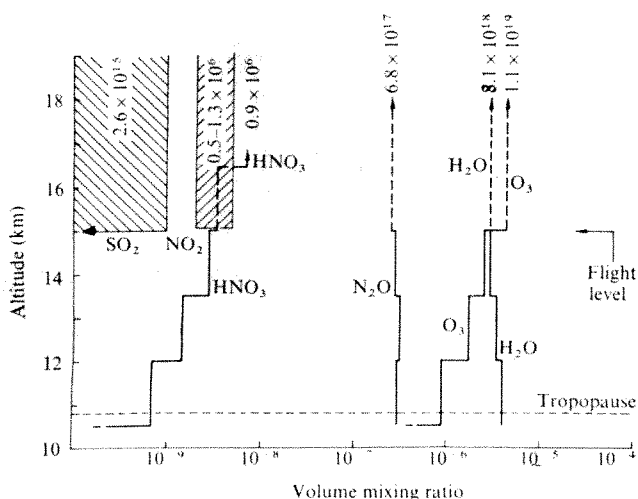


Fig. 1 The vertical distributions of H₂O, O₃, N₂O and HNO₃, at 65–70°N, measured in the limb-scanning experiment on Concorde 002, May 1973, from an altitude of 15 km. Below 15 km, concentrations are quoted explicitly in volume mixing ratio units; above 15 km, total column number densities (cm⁻²) are quoted at the top of each curve. The curve above 15 km represents the (assumed constant) volume mixing ratio equivalent to the quoted column density. At the top left the range of column densities observed for NO₂ is given, and an upper limit to the amount of SO₂.

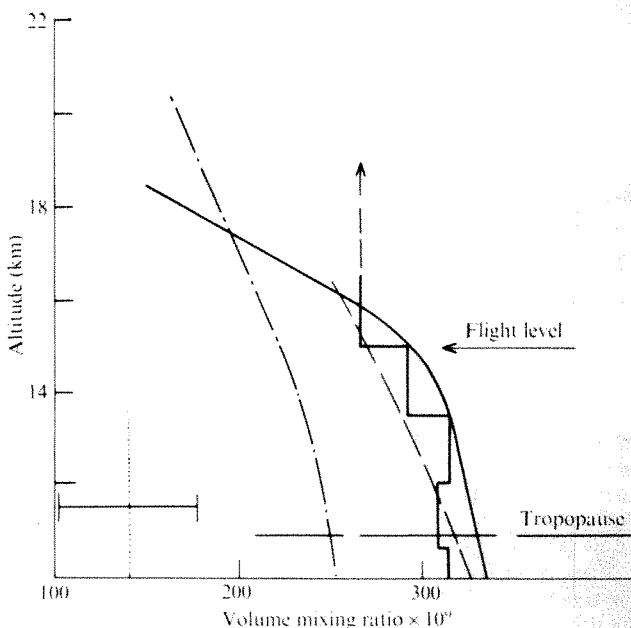


Fig. 2 Results of the inversion procedure for N₂O (May 16/17 at 65–70°N). The stepped curve shows the present results; broken curve, our previous results²; solid curve, some recent results by Murcray *et al.*¹²; bar-dash curve, results by Schutz *et al.*¹³; dotted curve, some earlier results by Murcray *et al.*¹⁴.

column number density above this level. These are the numbers shown at the top of each curve; the values range from 10¹⁹ molecules cm⁻² to less than 10¹⁵ molecules cm⁻². This range of column density may be obtained from a single spectrum, demonstrating the power and dynamic range of radiative methods such as ours. The broken lines with arrows represent the equivalent effective mixing ratios, if constant mixing is assumed, and are shown for comparison.

The shaded areas at the left are for NO₂ and SO₂. In the case of NO₂ we have measured the Q branch at 37.78 cm⁻¹; this, unfortunately, does not yield any information below the horizon, because nearby strong H₂O lines become so intense that the NO₂ Q branch is 'obscured'. Thus information on the column density only has been obtained. The box shown represents the range of values of column number density observed, 0.5 to 1.3 × 10¹⁶ cm⁻² converted to an effective average volume mixing ratio (see above). Some limited evidence is available of a variation between day and night, but more evidence is necessary before it can be regarded as conclusive. In the case of SO₂ there is still uncertainty in our assignments and, moreover, in the value of integrated line strength which should be used. The box thus indicates that we can place an upper limit on the amount of SO₂ above 15 km of 2.6 × 10¹⁵ cm⁻², corresponding to an effective average volume mixing ratio of 1 × 10⁻⁹. This is not an absolute measurement and the true value could be much lower than this.

Below 15 km, we give mixing ratio profiles for H₂O, O₃, HNO₃ and N₂O. The results are given as the average volume mixing ratio in 1.5 km thick layers.

For water vapour, the mixing ratio drops from a value of about 4 × 10⁻⁶–5 × 10⁻⁶ at the tropopause to about 3 × 10⁻⁶ at 15 km and seems to stay near this value above 15 km. We have previously obtained a large amount of information in this region of the stratosphere on the humidity distribution and have invariably found similar behaviour (see Fig. 2 of ref. 1). No diurnal variation was observed.

The ozone profile yields a total amount in good agreement with the limited meteorological network data that were available³ and indicates fairly high mixing ratios in the 11–15 km layer, as might be expected for polar spring maximum

conditions. We are at present analysing similar data taken in October 1973 and hope to compare the spring and autumn profiles. A variation in the amount of ozone between the day and the night flights was observed, which agrees fairly well with meteorological data.

The N_2O profile is basically a constant mixing result, with some indication of a falloff above 15 km. The average mixing ratio in the lower stratosphere is, however, approximately 300×10^{-9} , somewhat higher than the accepted value of 250×10^{-9} . The N_2O results have been replotted in Fig. 2 on a linear scale of mixing ratio and the results of several other workers are shown for comparison. C. B. Farmer and R. A. Toth (private communication) report results similar to Murcray's and our own.

Thus there still seems to be uncertainty about the N_2O profile in the lower stratosphere and it is an open question whether the observed variabilities are a consequence of real atmospheric variations or instrumental effects. It is not possible in the present work to obtain any information about N_2O above about 17–18 km. We did not detect any diurnal effects.

Finally in Fig. 1 we consider the mixing ratio profile obtained for HNO_3 . The mixing ratios seem slightly but significantly higher than those given by Murcray^{8,9} for the same levels but obtained at lower latitudes. Murcray does, however, report higher concentrations in results he has obtained over Alaska at similar latitudes to our own¹⁰ and what is observed may be an effect based on tropopause height and seasonal variations, similar to that found for O_3 . No diurnal variation was detected.

Figure 3 shows the HNO_3 data replotted on a linear mixing ratio scale and compared with other results (the column density above 15 km has been arbitrarily contained below

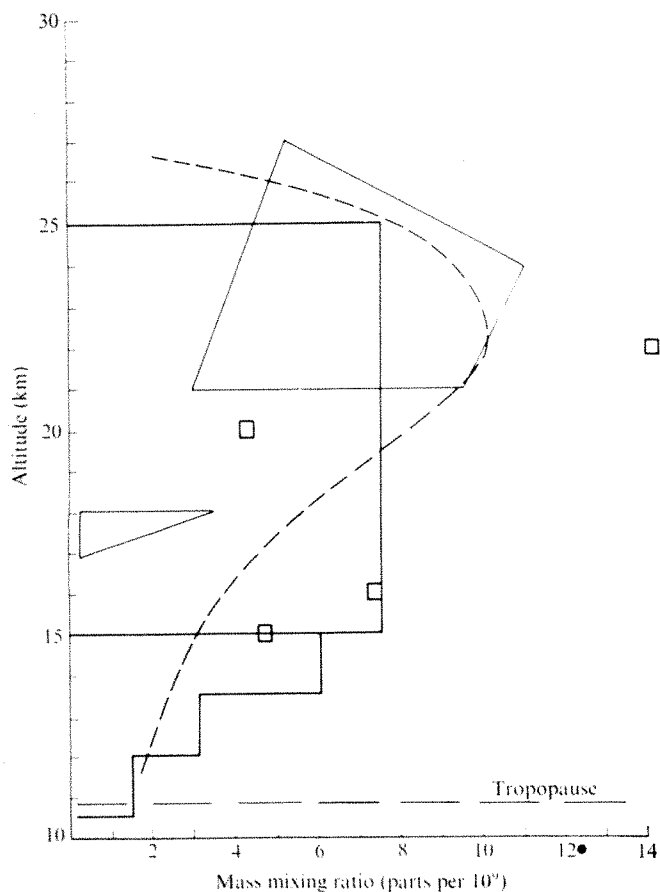


Fig. 3 The results for HNO_3 (May 16/17, 65–70°N) compared with previous measurements. The broken curve is by Murcray *et al.*⁸ as are the squared points⁹; the light triangle and quadrangle enclose points measured by Lazarus *et al.*¹¹. Total column density (10km–top) = 3.14×10^{-4} cm atm.

25 km in order to simulate a layer for the purposes of the diagram. See Fig. 1 for the corresponding column density.) The broken curve is a result by Murcray's group in mid-latitudes⁸, the squares represent the extreme values of a "double-layer" observed by the same group⁹ and the two areas enclosed by polygons circumscribe the range of values obtained, again in mid-latitudes, by Lazarus and coworkers¹¹.

We are at present analysing data for October 1973 and we hope to compare spring and autumn results for HNO_3 in this way.

The picture emerging for HNO_3 seems to be reasonably consistent between the various groups of workers, although at the moment unexplained variations in the total amount do occur, ranging over about a factor of 2. The mean pressure column density seems from our work to be about 2.5×10^{-4} cm atm (in agreement with Murcray *et al.*).

The results reported here are some of the first to be obtained in the Arctic stratosphere, an important part of the atmosphere *vis-à-vis* stratospheric contamination by aircraft because of the typically low tropopause, which means that transpolar and North Atlantic flights spend a large fraction of their time well above the tropopause. They are also the first results from a submillimetre wave limb-scanning experiment.

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- ¹ Harries, J. E., *Nature*, **241**, 515 (1973).
- ² Harries, J. E., *NPL Report*, DES 16 (1972).
- ³ Harries, J. E., Stone, N. W. B., Birch, J. R., Moss, D. G., Swann, N. R. W., and Neill, G. F., *Proc. First Int. Symp. IAMAP/IAPSO, Melbourne* (January 1974).
- ⁴ Harries, J. E., Birch, J. R., Fleming, J. W., Stone, N. W. B., Moss, D. G., Swann, N. R. W., and Neill, G. F., *Proc. Third Conf. on CIAP, Boston* (February 1974).
- ⁵ Gille, J. C., and House, F. B., *J. atmos. Sci.*, **28**, 1427–1442 (1971).
- ⁶ Williams, W. J., Brooks, J. N., Murcray, D. G., Murcray, F. H., Fried, P. M., and Weinman, J. A., *J. atmos. Sci.*, **29**, 1375–1379 (1972).
- ⁷ Craig, R. A., *The Upper Atmosphere* (Academic Press, London, 1965).
- ⁸ Murcray, D. G., Goldman, A., Murcray, F. H., Williams, W. J., Brooks, J. N., and Barker, D. B., *Proc. Second Conf. CIAP*, 86–98 (November 1972).
- ⁹ Williams, W. J., Brooks, J. N., Murcray, D. G., Murcray, F. H., Fried, P. M., and Weinman, J. A., *J. atmos. Sci.*, **29**, 1375–1379 (1972).
- ¹⁰ Murcray, D. G., *et al.*, *Proc. First Int. Symp. IAMAP/IAPSO, Melbourne* (January 1974).
- ¹¹ Lazarus, Gandrud, B., and Cadle, R. D., *J. appl. Met.*, **11**, 389 (1972).
- ¹² Goldman, A., Murcray, D. G., Murcray, F. H., and Williams, W. J., *J. opt. Soc. Am.*, **63**, 843–864 (1973).
- ¹³ Shutz, K., Junge, C., Beck, R., and Albrecht, S., *J. geophys. Res.*, **75**, 230 (1970).
- ¹⁴ Goldman, A., Murcray, D. G., Murcray, F. H., Williams, W. J., Kyle, T. G., and Brooks, J. N., *J. opt. Soc. Am.*, **60**, 1466 (1970).

'Cold spot' in West Africa: anchoring the African plate

VERY low heat flow values of $18 \pm 2 \text{ mW m}^{-2}$ ($0.42 \pm 0.04 \text{ } \mu\text{calorie cm}^{-2} \text{ s}^{-1}$) and $22 \pm 2 \text{ mW m}^{-2}$ ($0.51 \pm 0.05 \text{ } \mu\text{calorie cm}^{-2} \text{ s}^{-1}$) have been determined for two sites 85 km apart on the West African Precambrian shield in the Niger Republic (Table 1).

Temperature surveys were carried out with a thermistor probe in March 1972 in three boreholes, 245 m, 406 m, and 315 m deep, respectively, in western Niger. The thermal

conductivities of solid rock disks, saturated with water before measurement, were subsequently determined on a conventional divided bar apparatus.

Figure 1 shows temperature profiles with depth, temperature gradients calculated for 20-m intervals, and thermal conductivities for the analysed portion of three holes. Two sets of temperature data are shown for site K15. Heat flows were calculated using both K15 gradients; the mean is recorded in Table 1 and agrees very well with the heat flow determined from the nearby site, K6B.

The increasing gradient with depth in K6B (Fig. 1) is puzzling in view of the apparently uniform conductivity of the samples from that hole, and suggests the possibility of recent climatic warming. The consensus of climatological opinion¹, however, is that the region was, if anything, cooling rather than warming during recent times. Furthermore, the Donkolo site does not confirm the speculation of a recent regional warming.

The results of radioactive heat production measurements on aggregate samples of core chips are given in Table 1. The mean of $1.2 \text{ } \mu\text{W m}^{-3}$ for the basement rock at the Kourki site is taken to be representative of the region.

The two sites of the heat flow determinations are both located in Precambrian terrain on the eastern edge of the exposed West African craton. Six K-Ar ages of rock within 100 km of the heat flow sites² range from 2,487–1,206 Myr. The heat flow values reported here substantiate the general observation of low heat flow in Precambrian shields, a result anticipated by Beck and Mustonen³ from temperature measurements in boreholes in Ghana. These west Niger heat flow values are, however, considerably less than the mean heat flows of any of the other shields. The Southern Africa craton provides a useful comparison; there, fifteen measurements range from 36 to 59 mW m^{-2} with an average of 49 mW m^{-2} . The worldwide average of all shields is near 40 mW m^{-2} .

When heat flow data are coupled with information about the distribution of radioactive heat sources, reasonable estimates of temperatures at depth can be made. For a continental shield, Sclater and Francheteau⁴ outline a heat production model based on the petrological concepts of Ringwood⁵ and compatible with observed heat flow and surface heat production data. A surface heat flow in the Sclater-Francheteau model of 40 mW m^{-2} is comprised of 28 mW m^{-2} arising from radioactive heat sources in the outer 400 km of the Earth and 12 mW m^{-2} originating at greater depth.

It is clear that the Niger heat flow could be satisfied entirely by the flux originating above 400 km in the Sclater-Francheteau model, or with appropriate fractions of both the shallow and deeper flux. We reject the former option because it possibly implies a nearly isothermal lower mantle, a condition we think unlikely. Therefore, we have calculated temperature models that include some flux from below 400 km, consistent with two constraints: first, the measured surface heat flow, 20 mW m^{-2} ; and second a near surface radioactive heat source distribution that diminishes exponentially downward from the measured surface value of $1.2 \text{ } \mu\text{W m}^{-3}$. The logarithmic decrement of the near surface heat source function and the temperature dependence of the thermal conductivity remain variable parameters of the models.

Two temperature models for Niger, along with a typical 40 mW m^{-2} shield geotherm and a melting point curve, are shown in Fig. 2. One model, which we have called an 'upper limit' model, represents the likely upper limit for the temperature distribution beneath western Niger, consistent with the listed constraints. It is characterised by a logarithmic decrement of the heat source of $(5 \text{ km})^{-1}$ and a uniform thermal conductivity of $2.5 \text{ W m}^{-1} \text{ K}^{-1}$. We prefer the second model which uses a logarithmic decrement of $(8 \text{ km})^{-1}$ and a modest increase of thermal conductivity

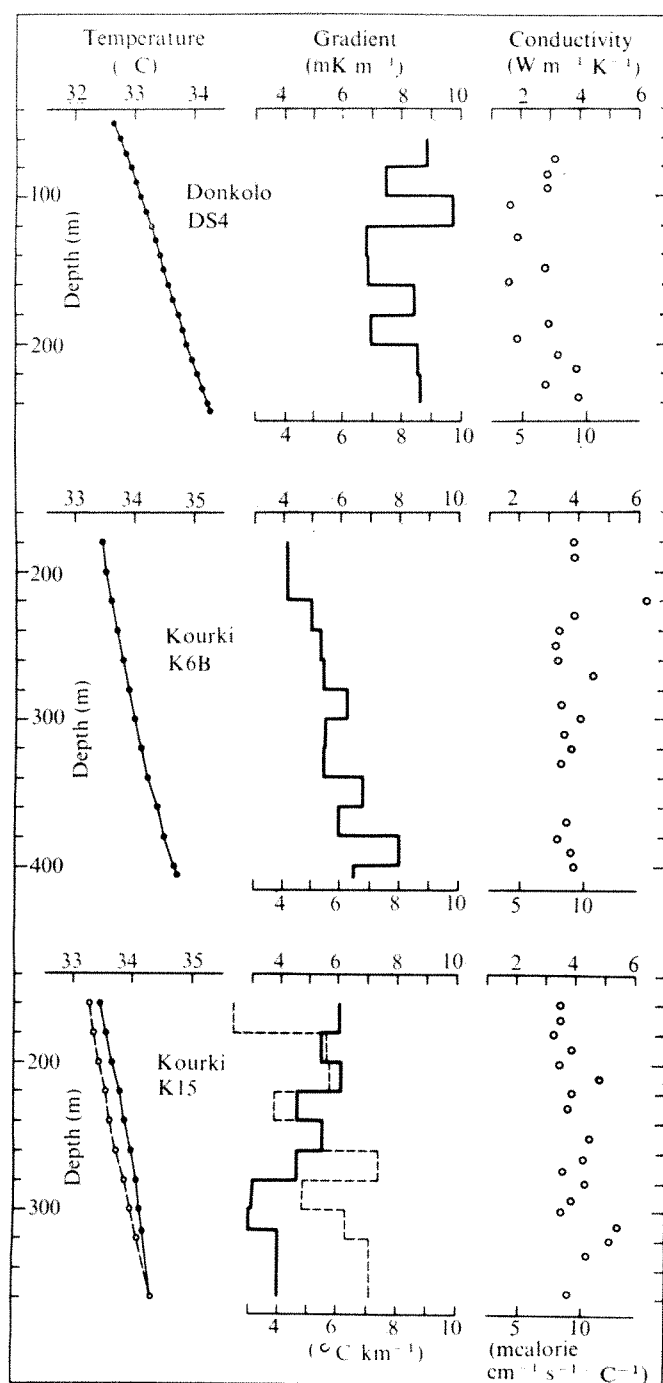


Fig. 1 Temperatures, gradients and conductivities for the analysed portions of the boreholes. In the graphs for K15 the solid curve represents a survey made nine days after drilling had stopped at 315 m, and the dashed line represents equilibrium temperatures predicted from two additional surveys made soon after the hole was deepened to 360 m. The nine-day curve and the predicted equilibrium curve bracket the measured equilibrium profile for K6B which was only 200 m away.

Table 1 Heat flow and heat production data

Site	Latitude	Longitude	Borehole	Depth interval (m)	Heat production ($\mu\text{W m}^{-2}$)	Heat flow (mW m^{-2})
Donkolo	14° 53'N	0° 55'E	DS4	60-245		18 (0.42)
			DS3	140-250	1.8 (4.3)*	
Kourki	14° 25'N	0° 20'E	K6B	180-406		22 (0.52)
			K15	160-358		21 (0.51)
			K6B	60-190	1.3 (3.2)	
			K6B	210-410	1.2 (2.8)	
			K15	50-190	1.1 (2.6)	
			K15	200-360	0.96 (2.3)	
			K16	50-100	1.2 (2.9)	

* Numbers in brackets refer to heat production in 10^{-13} calorie $\text{cm}^{-3} \text{s}^{-1}$ and heat flow in $\mu\text{calorie cm}^{-2} \text{s}^{-1}$.

with temperature, following Schatz and Simmons⁶. Neither of the Niger models reaches the temperatures of the average shield geotherm. Furthermore, unless the entire section of the upper mantle below the West African shield is severely depleted in heat producing isotopes, the heat flow originating below a depth of 400 km must be only 7 mW m^{-2} , about half the corresponding value for the average shield. We therefore must conclude that the Precambrian crust and underlying upper mantle of western Niger probably comprise one of the coldest regions in the outer 400 km of the Earth.

The mechanical consequences of this 'cold spot' are worth brief consideration. The plate tectonic model of Earth dynamics envisions a rigid, mechanically strong lithosphere overlying a weak and deformable asthenosphere. The base of the lithosphere has no rigorous definition; it is commonly equated to the top of the upper mantle seismic low velocity zone. This zone usually begins at depths of 50-150 km, depending on the tectonic setting. We suggest another working definition for the boundary between the lithosphere and the asthenosphere: that depth at which the viscosity of the Earth has diminished from its high surface value to $10^{20} \text{ kg m}^{-1} \text{s}^{-1}$ (10^{21} poise). Such a viscosity for the asthenosphere is suggested by postglacial rebound, and also

by the velocities of lithospheric plate motion over the asthenosphere.

We have calculated the viscosity as a function of temperature, and thus of depth, for the temperature models shown in Fig. 2. We have followed the development of Weertman⁷, which relates the viscosity to temperature-dependent creep and dislocation glide. The calculations assume a stress of 10^4 N m^{-2} (0.1 bar) and the melting curve shown in Fig. 2. The calculated viscosity profiles also are shown in Fig. 2. The model for the average shield temperature implies a lithospheric thickness of some 175 km for a typical shield, a value quite consistent with seismically determined values reported for various shields. The two models for West Africa suggest that the lithosphere there extends to depths well below 400 km; whether an asthenosphere is developed at greater depths is somewhat uncertain. Our conclusion is that the lithosphere is very thick, and that the asthenosphere is very thin or absent beneath West Africa.

The logical consequence of a thick lithosphere and a poorly developed or absent asthenosphere would be that the motion of the lithosphere would be impeded. In the extreme, the plate would be rendered immobile. Burke and Wilson⁸ have indeed suggested that such has been the case for the African plate since the early Miocene.

Has the African plate run aground?

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- ¹ Van Zinderen Bakker, E. M., *Palaeoecol. Afr. surround. Isles Antarct.*, 7 (1972).
- ² Brunnschweiler, R. O., *Geology*, **22**, 1 (1974).
- ³ Beck, A. E., and Mustonen, E., *Nature phys. Sci.*, **235**, 61 (1972).
- ⁴ Slater, J. G., and Francheteau, J., *Geophys. J. R. astr. Soc.*, **20**, 509 (1970).
- ⁵ Ringwood, A. E., in *The Earth's Crust and Upper Mantle* (edit. by Hart, P. J.), (Am. geophys. Union. Geophys. Monogr., 13, Washington, DC, 1969).
- ⁶ Schatz, J. F., and Simmons, G., *J. geophys. Res.*, **77**, 35 (1972).
- ⁷ Weertman, J., *Rev. Geophys. Space Phys.*, **8**, 1 (1970).
- ⁸ Burke, K., and Wilson, J. T., *Nature*, **239**, 5372 (1972).

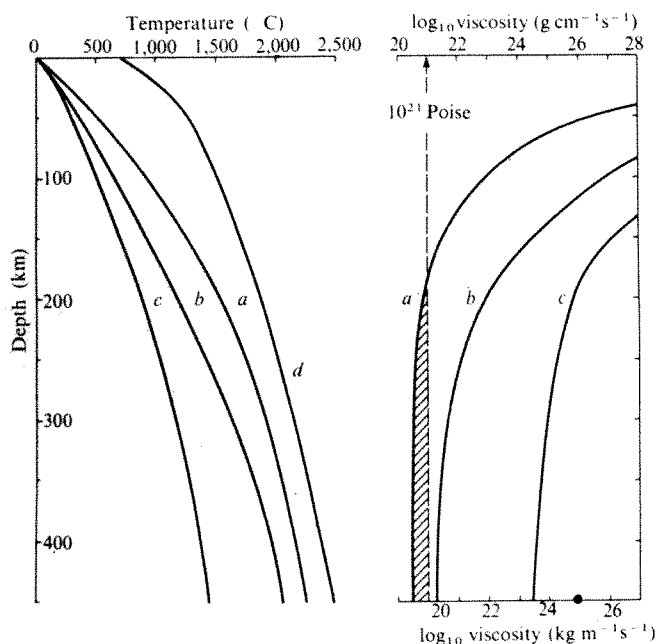


Fig. 2 The variation of temperature and viscosity with depth for three heat flow-heat production models: a, Average shield; b, Niger upper limit; c, the preferred Niger model, d, melting point.

Magnetic behaviour of some partially unmixed titanomagnetites

THE remanent magnetisation of igneous rocks is mainly carried by titanomagnetites. To obtain reliable information about the Earth's field during the past it is necessary to understand the mechanisms involved in the acquisition of remanence and in any subsequent changes. Much information has been obtained by studying basalts, but this approach is limited because of unknown factors such as grain size, degree of subsequent oxidation and impurities present. Synthetic, sintered samples of known composition have been used by many workers in order to avoid these difficulties.

In our present work, sintered titanomagnetite, $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$ (with $x=0.5$, denoted TM50) was used to prepare simulated average basalt by dispersing a known quantity of a selected particle size into a silicate nonmagnetic matrix (powdered Pyrex or silica). This study was undertaken in order to investigate reversals of magnetisation observed in natural basalts¹. The simulated sample showed a partial reversal of remanence when cooled in zero field. The study was then extended to the compositions TM40 ($x=0.4$), and TM60 ($x=0.6$).

The samples were prepared by a double sintering procedure² at $1,350^\circ\text{C}$ in a sealed mullite tube filled with a mixture of CO_2 -CO which acted as an oxygen buffer and at the same time aided the oxygen transfer between Fe and Fe_2O_3 in the initial mixture consisting of Fe, Fe_2O_3 , and TiO_2 . All samples were cooled at approximately the same rate given by the normal cooling rate of a massive furnace. It took about 15 h for the furnace to cool from $1,350^\circ\text{C}$ to room temperature. The samples were chemically analysed (% of Fe^{2+} by wet chemical analyses, Fe:Ti ratio by electronprobe microanalyses) and their phase uniformity checked by X-ray analyses using Debye-Scherrer and Guinier-de Wolff cameras. All samples were nearly stoichiometric and free of stray phases. Initial susceptibility, and saturation magnetisation-temperature curve confirmed this. The size of individual crystallites (grains) in the densely sintered tablets ranged from about 20 to $160\text{ }\mu\text{m}$, grains of

$40\text{ }\mu\text{m}$ being most abundant.

A part of the TM50 material was powdered and fractionated into various particle sizes by means of a centrifugal dust classifier. But, in this study only the fraction containing particles from 20 to about $50\text{ }\mu\text{m}$ was used. It was mixed with specially dried powdered Pyrex to give 3.5% by volume of the titanomagnetite in the non-magnetic matrix. The mixture was enclosed in a Pyrex capsule. This was evacuated and outgassed and the capsule sealed off in such a way that the magnetic particles were immobilised.

The sample was given an isothermal remanent magnetisation (IRM) at room temperature in a direct field of 5 kOe. The result of continuous thermal demagnetisation in zero field obtained by means of an astatic magnetometer

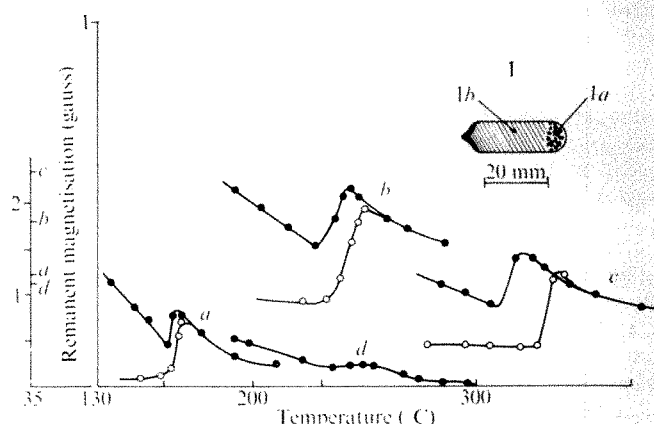


Fig. 2 Magnetic remanence as a function of temperature for *a*, TM60, *b*, TM50, and *c*, TM40. *d*, TM50 annealed at $1,100^\circ\text{C}$. Heating curve (●), cooling curve (○). The values of remanence at 35°C are shown on the left vertical axis (reduced four times). The Curie temperatures determined from susceptibility measurements are indicated on the temperature axis as bold marks. Inset 1: Pyrex (quartz) capsule with TM40, 50, and 60; 1*a*, coarse particles of titanomagnetite; 1*b*, powdered Pyrex (quartz).

next to the heating furnace³ is shown in Fig. 1. The two Curie temperatures show that while the bulk of the material is TM50, magnetite is also present. The amount of magnetite proved to be under the detection range of X-ray analyses, while low field susceptibility and saturation magnetisation-temperature curves suggest a very small quantity of magnetite (possibly under 1% by weight). If the demagnetisation was stopped at about 280°C (after the Curie temperature of TM50 was reached) and the sample was then cooled in zero field the remanent magnetisation showed a partial reversal on cooling through the Curie point. The same result was observed after prolonged heating to higher temperatures.

To investigate further the partial reversal, samples of TM40, TM50 and TM60 were prepared in a time-saving way as indicated in the inset to Fig. 2. All the magnetic sample in the form of relatively large pieces broken off from the original tablet (ranging in size from about 0.1 to 1.0 mm) was confined to the bottom space of a Pyrex (or quartz) ampoule. The three samples were then given an IRM in a field of 5 kOe. The results of continuous thermal demagnetisation are shown in Fig. 2. The curves exhibit a sudden increase of remanence at the Curie temperature of each composition respectively and the final Curie temperature of magnetite. If the samples were cooled after the first Curie point had been reached and before total demagnetisation occurred, a very sharp decrease in remanence was observed at a slightly higher temperature than the 'jump' observed on heating. The coarsely crushed sample of TM50 was then annealed at $1,100^\circ\text{C}$ for 3 h in an attempt to resorb the small quantity of magnetite. To avoid any appre-

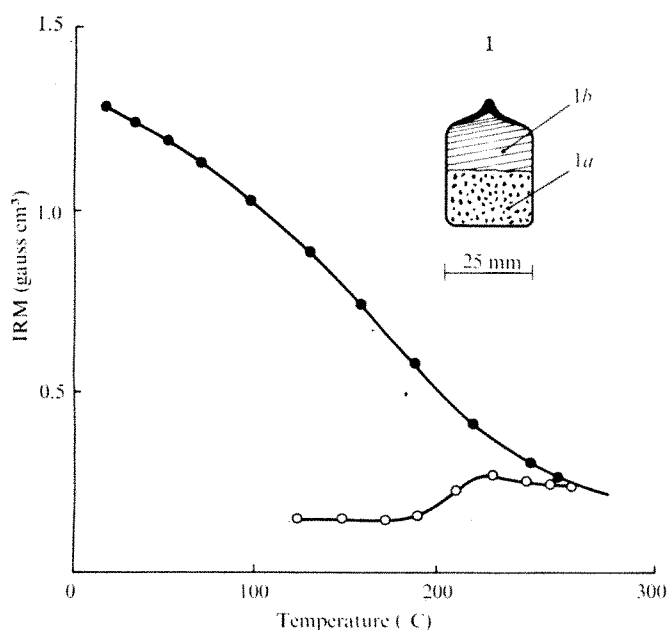


Fig. 1 Magnetic remanence as a function of temperature for simulated basalt. Heating curve (●), cooling curve (○). Inset 1: Pyrex capsule with the simulated basalt; 1*a*, layer of TM50 particles dispersed in powdered Pyrex; 1*b*, protective layer of powdered Pyrex.

ciable unmixing occurring on cooling, the sample was rapidly removed from the hot furnace into a water-cooled brass jacket. This was successful, as in the repeat demagnetisation experiment the strange behaviour in the vicinity of the Curie temperature of TM50 practically disappeared (Fig. 2 curve *d*). It is noteworthy that the initial value of IRM exhibited by the sample at room temperature (see point *d* on the left vertical axis in Fig. 2) was very much smaller than previously. All these results suggest the possibility of a strong coupling between the magnetite regions present and the original titanomagnetite matrix.

The partial reversal observed on cooling of our simulated basalt seems to be similar to partial reversals demonstrated in natural basalts^{1,4-6} and in synthetic TM60 (ref. 7). This effect has been explained by negative magnetostatic coupling between the original spinel and a partially-oxidised cation-deficient spinel with higher T_c . In our samples, however, oxidation was ruled out. So, this explanation cannot account for the features of the curves shown in Fig. 2. We believe that all our observations are related to the same phenomenon—the presence of segregated magnetite and the resulting coupling. Small regions of magnetite are most likely to be the product of subsolvus segregation (in petrological terminology usually called ‘unmixing’) from the originally homogeneous single phase titanomagnetite. The curve confining the miscibility gap in the magnetite–ulvöspinel solid solution series is reported to be of the simple ‘hoop-shaped’ type⁸. It is known, however, that the ‘two spinels’ field in the appropriate phase diagram⁸ is still rather tentatively marked and much less is known about the kinetics of unmixing. Nevertheless, the phase diagram shows that the critical temperature for unmixing for the three compositions studied here should lie between 500° and 700° C. Unmixing in our samples is therefore likely to have taken place during the relatively slow cooling after their final sintering.

The correlation between the position of the jump and the Curie temperatures is obvious. The temperatures marking the onset of the jump on the heating curves correspond approximately to 160°, 230°, and 315° C for TM60, 50, and 40 respectively. The corresponding Curie temperatures determined for the latter samples from the susceptibility-temperature curves were 159°, 227° and 320° C. The reproducibility of the curves in Fig. 2 shows that no unmixing is taking place during the thermal demagnetisation. The jump is also reversible on cooling except for a hysteresis which grows with increasing x value.

The magnetic behaviour of the samples in our experiments can be phenomenologically explained in the following way. Below the Curie point of the matrix its permeability is high and some of the magnetic lines of force emanating from the magnetite regions are trapped in the matrix of the parent titanomagnetite. This results in a reduced amount being sensed by the astatic magnetometer. On passing the Curie point the permeability of the matrix drops, so that the lines of force emanating from the magnetite inclusions spring out of the sample and the magnetometer records a sudden increase in moment. When the sample is cooled the permeability rises sharply at the Curie point of the matrix and the lines of force from the inclusions are once more partially trapped and the decrease in moment is produced. The onset of these downward jumps which occurs at slightly higher temperatures may indicate that the regions adjacent to the magnetite inclusions could be slightly richer in iron than the bulk of the matrix. Some other mechanism might account for these hystereses, however, such as temperature-dependent reordering of cations. Research into this possibility is under way. In fact, we have observed similar hystereses on the susceptibility-temperature curves.

The simulated rock does not show the relaxation on heating through the Curie point. This may be associated with the different history of this sample, in that the titanomagnetite particles are smaller, mutually separated by non-magnetic material and strained because of grinding. More experimental data are needed.

It seems that measurements of magnetic remanence provide a very sensitive method of studying the process of unmixing in titanomagnetites from the very initial stage. It may also prove valuable for studying the kinetics of unmixing. We believe that further research carried along these lines will have petrological as well as palaeomagnetic significance as the process of unmixing is undoubtedly very common in natural basalts.

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¹ Creer, K. M., Petersen, N., and Petherbridge, J., *Geophys. J. R. astr. Soc.*, **21**, 471 (1970).

² Hauptman, Z., *Geophys. J. R. astr. Soc.*, **37** (1974).

³ Petherbridge, J., de Sa, A., and Creer, K. M., *J. Phys. E.*, **5**, 579 (1972).

⁴ Creer, K. M., and Petersen, N., *Z. Geophys.*, **35**, 501 (1969).

⁵ Havard, A. D., and Lewis, M., *Geophys. J. R. astr. Soc.*, **10**, 59 (1965).

⁶ Ozima, M., and Ozima, M., *Earth planet. Sci. Lett.*, **3**, 213 (1973).

⁷ Petersen, N., and Bleil, U., *Z. Geophys.*, **39**, 965 (1973).

⁸ Vincent, E. A., Wright, J. B., Chevallier, R., and Mathieu, S., *Min. Mag.*, **31**, 624 (1957).

Humic substances from seawater

SINCE the first spectroscopic observation of humic substances in seawater¹, it has been presumed that they constitute a considerable fraction of the dissolved organic matter in the sea²⁻⁴. Base-soluble organics were first isolated from seawater in 1958 (ref. 5), but the chemical composition and source of these materials has remained speculative and controversial^{3,6-9}. We here present evidence that humic substances in the Sargasso Sea display bulk characteristics which differ from those of humics from coastal waters and especially from those of humic substances isolated from soils and marine sediments.

Water from the north-western Sargasso Sea (surface and 1,500 m), and coastal water off Woods Hole, Massachusetts, has been analysed. Batches of seawater (400 l each) were acidified (pH 2, HCl) and passed through Amberlite XAD-2 resin¹⁰. (The resin was cleaned prior to use by batch extraction in refluxing benzene until NH₄OH, ethanol, and methylene chloride concentrated eluents showed no ultraviolet absorption. An extraction efficiency of 95.2% was determined when isolated humic substances were redissolved and adsorbed on Amberlite XAD-2 from salt water (pH 2) at 1.7 bed volumes per minute.) Salt was eluted with distilled water and humic materials were eluted with NH₄OH (pH 11.6) and recovered by freeze-drying. The humic materials were further fractionated by dissolving the fulvic acid (FA) in 0.01N HCl and the humic acid (HA) in NH₄OH. Freeze-drying these fractions yielded FA:HA ratios of 97:3 (by weight) in Sargasso surface water, 78:22 in

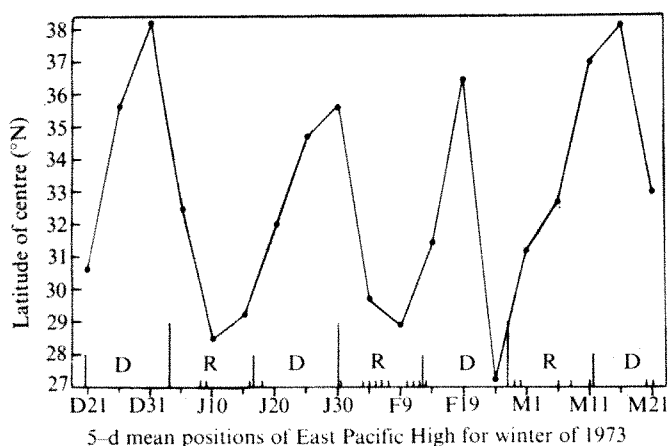


Fig. 1 Latitude time plot of 5-d mean position of surface East Pacific high pressure cell for winter of 1973. D, J, F, and M are abbreviations for the months (December through March). The symbol R denotes rainier halves and D drier halves of successive 27-d intervals. The upper tick marks indicate days it actually rained in Los Angeles.

graph of the 5-d mean positions, plotted on the third day, of the principal centre, or cell, of highest pressure of the semipermanent East Pacific high pressure cell in the region 19 to 50°N and 120 to 165°W for the 1973 winter (Los Angeles is at 34°3'N and 118°14'W). On average, the cell was more southward during rainier halves and more northward during drier halves of the 27-d intervals. The oscillation seems to be greater and more regular in winter than in other seasons.

This rainfall cycle might be related to a cyclic latitudinal oscillation of the jet stream at west coast longitudes. This oscillation might, in turn, cause a cyclic latitudinal oscillation of the resident East Pacific high pressure cell. The oscillation might then modulate the rainfall. The oscillation's phase would then determine whether Pacific storms move inland and eastward across Northern California or whether the storms move southwards. The respective positions about which the jet stream and Pacific high cell oscillate move farther south¹ as the rain season progresses with greater effects on conditions in Southern California (population 12×10^6) as the season progresses.

Records of daily precipitation for 1921 to 1971 for Los Angeles, Santa Barbara (34°25'N, 119°42'W) and San Diego (32°43'N, 117°9'W) were obtained from the National Climatic Center, Asheville, North Carolina. Additional Los Angeles Civic Center data from 1900 to 1920 were obtained from the Los Angeles office of the National Weather Service. The mean annual rainfall for Santa Barbara is 17.63 inches compared with 14.42 inches for Los Angeles (Table 2) and 9.76 inches for San Diego. The lower frequency part of the power spectrum for each of these cities is plotted in Fig. 2. The time series used for each spectrum was the cube root of the daily rainfall²⁻⁴. The entire continuous record was used to make each power spectrum and the data were also detrended. The Blackman-Tukey

method⁵ and the Tukey spectral window were used to compute the power spectrum, with 250 lags (equal maximum lag number). Twice as many spectral estimates as lags were computed⁶. The spectral estimates are statistically correlated in a band but greater resolution is obtained.

The 80% confidence interval is used as the standard of significance⁶. For the five curves the heights of the peaks at 54 d and 27 d (and for the 13.5-d peak in most cases) are equal to or larger than the 80% confidence interval and are therefore considered to be significant. There is a significant peak at almost exactly 27.0 d. The frequency for that spectral estimate is 0.037 cycles d⁻¹ which gives

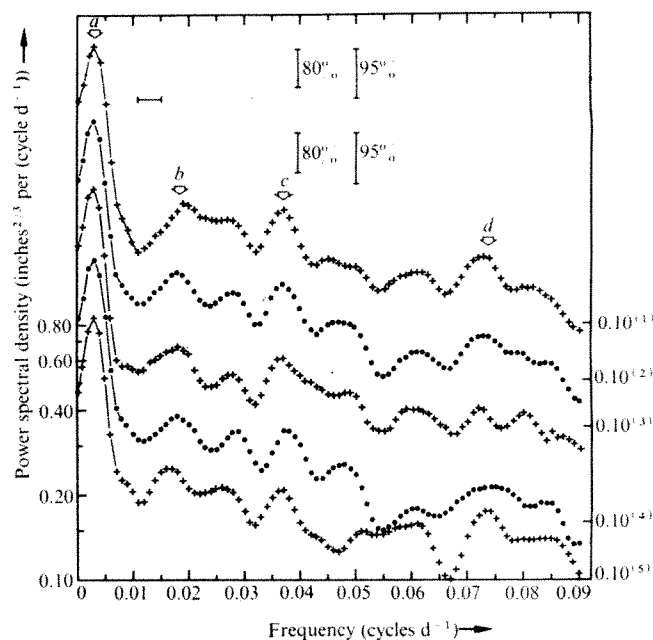


Fig. 2 Power spectra of Southern California rainfall records. The five superimposed curves, numbered 1 to 5 from top to bottom, give lower frequency part of power spectral density as a function of frequency. The left-side ordinate scale of 0.10 to 0.80 is for curve 5. The 0.10 level for each curve is shown on the right side. Curves 1, 2, and 3 represent, respectively, spectra of Santa Barbara, Los Angeles, and San Diego, for the interval December 1927 through 1971 and each have 129 degrees of freedom. Curves 4 and 5 are spectra for Los Angeles for years 1936-1971 and 1900-1935, respectively, and each have 105 degrees of freedom. The vertical bars at very top are 80% and 95% confidence intervals for curves 1, 2, and 3. Below them are confidence intervals for curves 4 and 5. The horizontal bar in upper left corner is spectral bandwidth. Arrows a, b, c and d, denote peaks at 365, 54, 27 and 13.5 d, respectively.

$1/0.037 = 27.03$ d. The spectra for Los Angeles in curves 4 and 5 were obtained for time intervals completely independent from each other. San Diego is 180 miles to the south-east of Santa Barbara. The 27-d and 54-d peaks at Santa Barbara meet the very stringent 95% confidence interval standard⁶. Santa Barbara is 80 miles west and 30 miles north of downtown Los Angeles and is closer to the central longitude of the semi-permanent, East Pacific high cell. Thus, the storm blocking capability of this cell may be more effective at Santa Barbara, producing relatively larger 27- and 54-d peaks there. This possibility suggests that the closer the cell's average longitudinal position is to the west coast, the more dominant is the 27-d rainfall cycle.

The curves suggest two sets of harmonically related bands. The harmonics of "Set A" are separated by 0.009 cycles d⁻¹ (fundamental period ~ 108 d). The harmonics of "Set B" are separated by 0.012 cycles d⁻¹ (fundamental 81 d). In

Table 2 Monthly rainfall, in inches, at downtown Los Angeles station, averaged over the years 1921 through 1971.

January	2.84	July	0.00
February	2.93	August	0.03
March	2.11	September	0.25
April	1.34	October	0.38
May	0.22	November	1.56
June	0.07	December	2.69

neithercase is the fundamental marked by a peak. The first apparent peak in Set A is at 54 d and in Set B it is at 27 d. The peaks at 27 d and 13.5 d are effectively common to both sets. There are no peaks at Set A harmonics at 0.056 cycles d^{-1} and 0.065 cycles d^{-1} . Curves 1, 2, and 3 contain a mixing of both sets. The Los Angeles spectrum for 1936–1971, curve 4, is dominated by Set A and that for 1900–1935, curve 5, contains more of Set B than curve 4. On curve 5 there are indications of contributions but no peak by the Set B fundamental at 0.012 cycles d^{-1} and by its first harmonic at 0.024 cycles d^{-1} . These contributions partially fill in regions that are troughs at those same locations on curve 4 and seem to shift the two nearby peaks to lower frequencies on curve 5.

The Los Angeles computer tape was used in simulating forecasts for the past 50 yr (Table 3). The oscillation of the Pacific high cell (Fig. 1) suggests the primacy of the 27-d cycle. The other two large significant peaks, at 54 d and 13.5 d, are related to the 27-d cycle by factors of 2. The forecast was simulated by finding the early trend in the

27 cycle days (using two 27-d periods) that revealed the pattern that might continue. The first day after September 23 with ≥ 0.26 inches of rain or ≥ 0.26 inches total in two adjacent days was taken as point 1. The area in the next 27-d period under point 1 (23 to 30 d later) was searched for nearby days on at least one of which there was ≥ 0.16 inches. If this condition was not met, then a second point was searched for. When the day with a repeating pattern was found, it was taken as the "centre" day of the 13.5 days in a rainier half cycle. Only rain in this half cycle in cycles (to June 30) after the ones used in the trend establishment was totalled, and the ratio to rain in the other half cycle was calculated and printed in column B. The summation of the rains for the ratio in column B was started on the first day of the first drier half in the cycles to be included. This day was the eighth day after the centre day of the second 27-d interval used in establishing the early trend. If there was no more than 0.25 inches on any day between September 24 and December 31, then no forecast was made (Next to column B and 0.0 in column B). In a real-time forecast in the early autumn, one must distinguish and eliminate the very infrequent local thunderstorms and Sonoras (storms from Baja, Mexico). The average for the ratio in column B is 2.40 and 31 yr, or 67.4%, exhibited a ratio of 1.10 or greater, the criterion used for "success". Most forecasts (58.7%) were started (column E) before January 1.

Climatological rainfall statistics for Los Angeles, based on 1919–1973, were available at UCLA. We have examined climatological daily averages and determined the positive skill score over climatology from the formula^{7,8} $(C-S)/(T-S)$, where C is the number of correct forecasts (ratio in column B ≥ 1.10), and T is the total number of forecasts made. S is the number of those correct forecasts in column B that were also correct when the climatology statistics were used in column C or D in place of actual rainfall. If we take 1.05 or above, instead of 1.10, as an indication of success in columns C and D, then $S=6$ for column D and S is still zero for column C. For column C (using the number of rainfall days) the positive skill over climatology is 0.67 and for column D (using rainfall calendar date averages), it is then 0.63. The average of the two comparisons is 0.65. Using the same method as above, real-time forecasts were made for the 1972–73 and 1973–74 Los Angeles rain seasons and gave, respectively, 3.01 and 4.76 times as much rain for the predicted rainier halves as for the predicted drier halves of the 27-d cycles. The 1973–74 forecast was monitored by the Los Angeles office of the National Weather Service.

Rainfall may occasionally be scant within an entire 27-d interval. This may occur if the 54-d cyclic component is then more effective or if storms are blocked by a strong ridge of high pressure which sometimes forms over the western states and persists for a few weeks. When the southern part of the landward extension of the East Pacific high is over Los Angeles, as the cell moves northward during the drier half, the wind direction is from the east since the high circulates in a clockwise sense. Then the East Pacific high over Los Angeles can help create the Santa Ana wind condition (foehns) and higher temperatures from compressional effects as air flows downhill toward the coast. Rain washes pollutants out of the atmosphere and cold fronts associated with storms do not favour formation of low-temperature inversion layers. For the 1972–73 rain season figures obtained from the Los Angeles County Air Pollution Control District show that, on average, the ozone level was 43.5% higher and the nitrogen oxides 11.3% higher during the predicted drier halves of the five principal 27-d rainfall cycles.

Our analysis indicates that the phase of the 27-d cycle usually remains the same for most or all of a particular rain season, and that the phase changes usually occur be-

Table 3 Results of simulated forecasts using 27-d cycles for each of 50 Los Angeles rain seasons ending in approximately mid-summer of year shown in column A

A	B	C	D	E
1922	1.57	0.96	1.03	306
1923	0.94	0.89	0.83	348
1924	1.76	0.77	0.68	35
1925	0.73	0.90	0.79	349
1926	2.42	0.93	1.09	337
1927	1.73	0.93	1.09	337
1928	0.47	0.92	0.97	362
1929	0.34	0.87	0.83	347
1930	0.0 N	0.0	0.0	0
1931	9.90	0.94	0.97	16
1932	1.12	0.97	0.99	355
1933	3.73	0.86	0.79	40
1934	0.01	0.70	0.49	62
1935	3.12	1.01	0.98	327
1936	0.16	0.70	0.49	62
1937	1.31	0.86	0.88	2
1938	5.68	0.86	0.88	42
1939	2.11	0.98	1.08	46
1940	0.07	0.77	0.64	54
1941	1.27	1.00	1.06	332
1942	1.22	0.86	0.81	346
1943	1.16	0.89	1.00	364
1944	6.64	0.85	0.81	8
1945	1.49	0.84	0.79	344
1946	0.47	0.88	0.97	365
1947	1.13	0.91	1.01	308
1948	0.0 N	0.0	0.0	0
1949	1.96	0.95	0.95	21
1950	0.91	0.90	0.79	349
1951	2.29	0.85	0.68	65
1952	1.67	1.00	1.06	332
1953	0.13	0.92	0.97	362
1954	0.97	0.77	0.70	53
1955	1.45	0.91	0.77	350
1956	9.90	0.84	0.77	4
1957	3.40	0.84	0.75	37
1958	1.40	0.87	0.83	347
1959	0.0 N	0.0	0.0	0
1960	7.52	0.98	1.08	46
1961	9.90	0.87	0.87	341
1962	2.29	0.98	1.03	20
1963	0.0 N	0.0	0.0	0
1964	0.44	1.01	0.91	326
1965	0.59	1.01	0.98	327
1966	0.27	0.97	0.99	355
1967	2.11	0.86	0.81	346
1968	3.50	0.98	1.04	333
1969	2.54	0.73	0.57	32
1970	0.10	0.86	1.03	71
1971	3.95	0.88	0.97	365

Column B gives rainier-to-drier half ratio for predicted intervals. Column C gives same ratio using climatology statistics consisting of total number of days of rainfall on each calendar date for 1919–1973. Column D gives the ratio using statistics of average amount of rainfall on each calendar date from 1919–1973. Column E gives day of year (1 to 365 or 366) on which each forecast began. 0 in column E indicates no forecast made.

tween rain seasons. Some very small effects on rainfall are associated with both the full moon and new moon which occur every 14.7 d (refs 4, 9). This particular lunar phase effect would give roughly equal rainfall contributions (14.7 d apart) to the drier and rainier halves of the Los Angeles 27-d rain cycle and is probably not related to it. The Sun and solar sector structure also produce atmospheric effects¹⁰. But our studies of the rainfall cycle's phase indicate that it is not simply driven by either solar or lunar processes. If the periodicity is of solar or lunar origin, then its phase must be determined by another mechanism.

Just off the northwest coast of the United States, confluence (narrow, strong jet stream) is associated with the Gulf of Alaska surface low surmounting the eastern cell of the Pacific high¹¹. These unusual circumstances provide very good conditions for high zonal index so storm tracks can stay north of Southern California a long time. We now consider the possibility that the spectral peaks in Fig. 2 arise from resonances of the atmospheric subsystem regulating the weather, including the Gulf low and the Pacific high, in the Eastern Pacific region at northern hemispheric mid-latitudes. The spectra suggest the possibility that this resonance is excited by time dependent processes with energy at frequencies closer to higher harmonics than to the fundamental of the subsystem and closer to some harmonics than to others. Since atmospheric systems are "deformable", we might expect a somewhat different fundamental and a different set of harmonics in years when the atmospheric conditions were different. The differences in the spectra in curves 4 (1936-1971) and 5 (1900-1935) may be the result of a long term change in the state of the atmosphere. Thus, Conover¹², citing work by Petterssen¹³, states that the global trend of rising temperatures diminished after about 1937-1940. He relates changes in temperature trends to changes in the mean atmospheric state of meridional flow relative to zonal circulation.

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- ¹ Riehl, H., Alaka, M. A., Jordan, C. L., and Renard, R. J., *Meteorol. Monogr.*, **2**, 23-45 (1954).
- ² Stidd, C. K., *Tran. Am. geophys. Un.*, **34**, 31-35 (1953).
- ³ Howell, W. E., *J. Meteor.*, **17**, 684 (1960).
- ⁴ Brier, G. W., and Bradley, D. A., *J. atmos. Sci.*, **21**, 386-395 (1964).
- ⁵ Blackman, R. B., and Tukey, J. W., *The Measurement of Power Spectra*, 22 (Dover, New York, 1958).
- ⁶ Jenkins, G. M., and Watts, D. G., *Spectral Analysis and Its Applications*, 290 (Holden-Day, San Francisco, 1968).
- ⁷ Brier, G. W., and Allen, R. A., in *Compendium of Meteorology* (edit. by Malone, T. F.), 841-848 (Am. Meteor. Soc., Boston, 1951).
- ⁸ Panofsky, H. A., and Brier, G. W., *Some Applications of Statistics to Meteorology*, 191-207 (Pennsylvania State Univ. Press, University Park, 1958).
- ⁹ Adderley, E. E., and Bowen, E. G., *Science*, **137**, 749-750 (1962).
- ¹⁰ Wilcox, J. M., Scherrer, P. H., Svalgaard, L., Roberts, W. O., Olson, R. O., and Jenne, R. L., *J. atmos. Sci.*, **31**, 581-588 (1974).
- ¹¹ Namias, J., *J. Meteor.*, **7**, 130-139 (1950).
- ¹² Conover, J. H., in *Climatic Change* (edit. by Shapley, H.), 229 (Harvard Univ. Press, Cambridge, 1953).
- ¹³ Petterssen, S., in *Glaciers and Climate*, *Geografiska Annaler*, **31**, 212 (1949).

Concentrating solutes with membranes containing carriers

We have developed membranes which can rapidly concentrate a specific solute against its concentration gradient. These membranes depend on mobile carriers and are examples of *in vitro* systems which exhibit characteristics of biological transport. When combined with the 'liquid surfactant membrane' geometry, they offer a method for wide classes of large scale separations. We show here how our specific results provide a blueprint for designing additional membrane systems.

The membranes operating in our laboratories at present are summarised in Table 1. These membranes depend on mobile carriers which react rapidly and selectively with the solutes being transported. Two mechanisms are involved. In the first (Fig. 1), the flux of the protons which supply the energy is in the opposite direction to the flux of the solute being moved against its gradient, resulting in counter-transport^{1,2}. The second mechanism is analogous to that shown in Fig. 1, except that both the protons and the solute being separated combine with the carrier on the same side of the membrane. As a result, the two fluxes are in the same direction, resulting in co-transport. Moreover, because all of these membranes are chemically well defined, they can be understood on a molecular basis, and the diffusion and chemical reaction responsible for their operation can therefore be studied in detail³.

Additional membrane systems can be developed in a straightforward manner. An organic solution containing a complexing agent for the relevant solute must be chosen. This solution and the complexing agent will become the membrane material and the mobile carrier, respectively. The degree of complex formation must vary strongly with pH, and both carrier and complex must be soluble in the membrane solution but insoluble in aqueous acid and base. One then runs two extractions sequentially. For example, a solution of 500 parts per million (p.p.m.) mercuric ion in 2 M NaCl and 1 M HCl is mixed with a membrane solution of 10% trioctylamine in *o*-xylene. The mercuric ion and the protons complex with the amine and dissolve in the organic phase. When this organic phase is

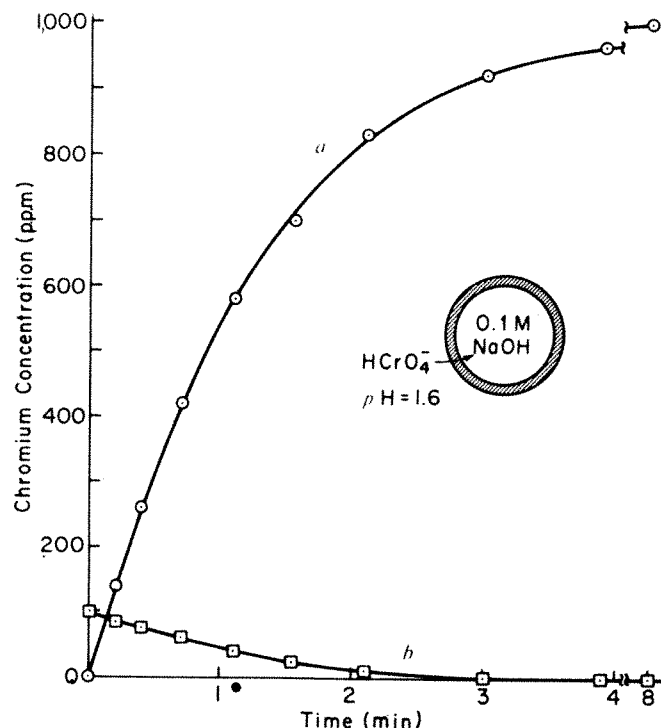


Fig. 1 Counter-transport mechanism involving a mobile carrier.

Table 1 Existing membrane systems^a

Solute	Carrier	Transport mechanism	Energy	Remarks
Na ⁺ , K ⁺ , Li ⁺ , Cs ⁺	Monensin in octanol	counter-	HCl into NaOH	Selective for Na ⁺ ; strong biological parallel
Na ⁺ , K ⁺ , Li ⁺ , Cs ⁺	Cholanic acid in octanol	counter-	HCl into NaOH	Non-selective analogue of above
Cu ²⁺	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \phi\text{CCH}_2\text{CCH}_3 \end{array}$ in chloroform or in carbon tetrachloride	counter-	HCl into NH ₄ OH	Works best at 0.01 M Cu ²⁺ ; selective for Cu ²⁺ over Ni ²⁺ and Co ²⁺ ; carriers poisoned by Fe ³⁺
	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \phi\text{CCH}_2\text{CCF}_3 \end{array}$ in xylene	counter-	HCl into NaOH	
Zn ²⁺ Pb ²⁺ Hg ²⁺	$\begin{array}{c} \text{O} \quad \text{O} \\ \quad \\ \phi\text{CCH}_2\text{C}\phi \end{array}$ in chloroform Dithizone in carbon tetrachloride Trioctylamine in xylene	counter- co-	0.5 N HCl into pH 8.5 citrate HCl into NaOH	Works best at high dilution Selectivity depends on pH difference Works best at 1,000 p.p.m. with 2 M Na ⁺ on both sides Selectivity depends on pH difference
Cl ⁻ SO ₄ ²⁻ Cr ₂ O ₇ ²⁻	Trioctylamine in xylene Trioctylamine in xylene Tridodecylamine in hydrocarbon oils	co- co- co-	HCl into NaOH H ₂ SO ₄ into NaOH H ₂ Cr ₂ O ₇ into NaOH H ₂ SO ₄ into NaOH	Potential for treatment of plating wastes

* All these systems have been checked by double extraction and in a membrane geometry.

extracted with 500 p.p.m. mercuric ion in 2 M NaCl and 0.3 M NaOH, the mercury concentration of the basic solution is increased. The two extractions produce a net transfer of

material, and therefore this chemical system provides a potential separation process. As a thin layer dividing two aqueous solutions, it will function as a membrane capable of concentrating a specific solute.

The real interest in these membranes, however, arises when they are converted into the geometry of synthetic vesicles or 'liquid surfactant membranes'⁴. This geometry consists of small drops of solution coated with the liquid membrane and suspended in a solution of different concentration. Because these bubbles have such a large area per volume, diffusion in and out of the bubbles is much faster than is possible with classical membrane geometries. Separation has been achieved on an industrial scale, but the selectivity of these separations was controlled by solubility differences alone.

Bubbles coated with membranes containing carriers, like those in Table 1, have three advantages over those based on solubility alone: they can concentrate as well as separate a given solute; they have inherently much greater selectivity; and they are applicable to a much wider variety of chemical systems. These advantages accrue from the specific chemical reactions between carrier and solute. Because these reactions include a much wider spectrum of effects than solubilities do, a much greater variety of phenomena are possible.

An example of the properties of the bubbles coated with membranes containing carriers is shown in Fig. 2. In this experiment, 10 ml of 0.1 M NaOH was prepared. The membranes were made of light mineral oil containing 4 weight % trioctylamine and 1 weight % sorbitol monooleate. These bubbles were added, whilst stirring, to 100 ml of solution containing 100 p.p.m. chromium at pH = 1.6. The concentration of chromium in the bubbles rose from an initial value of zero, past the concentration in the bulk solution, to a value of 900 p.p.m. after 4 min, and the chromium concentration in the bulk solution decreased correspondingly. The very rapid uptake with very little amine provides an intriguing alternative to conventional ion exchange.

We have shown here how the membrane mechanisms postulated⁵ in biology can be generalised to produce a variety of chemically well defined analogues to living membranes. Although we have reported only data for inorganic ions, these principles can be extended to other solutes, including antibiotics, detergents, and amino acids. When these transport systems are used in conjunction with the technology developed for liquid surfactant membranes, separations of considerable

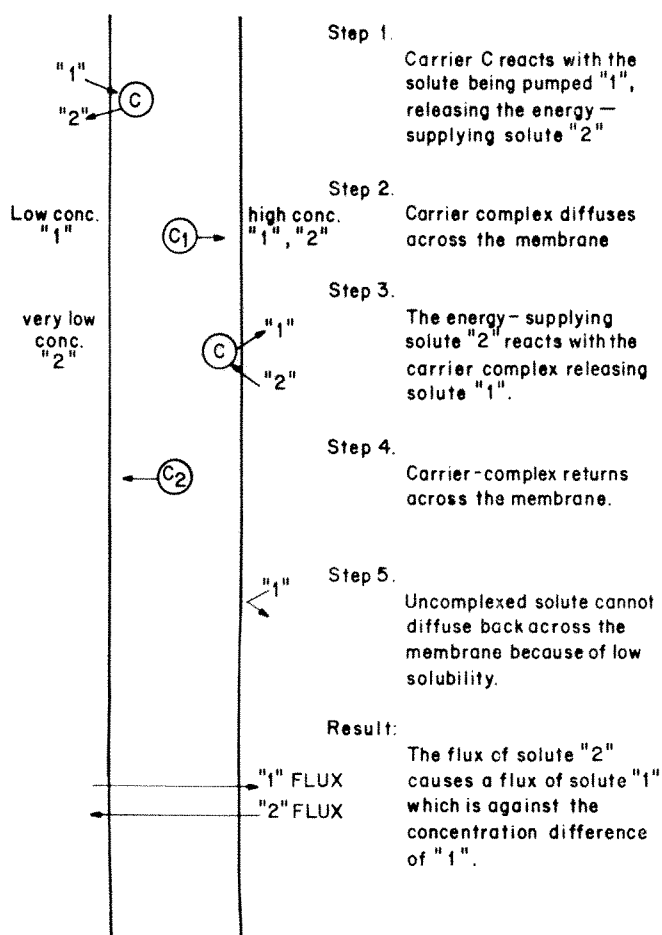


Fig. 2 Concentration of chromium against its concentration gradient using liquid surfactant membranes. a, In bubbles; •, b, in bulk solution.

practical potential are obtained.

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- ¹ Stein, W. D., *Movement of Molecules Across Cell Membranes* (Academic Press, New York, 1967).
- ² Cussler, E. L., Evans, D. F., and Matesich, M. A., *Science*, **172**, 377 (1971).
- ³ Choy, E. M., Evans, D. F., and Cussler, E. L., *J. Am. chem. Soc.* (in the press).
- ⁴ Li, N. N., *A.I.Ch.E.J.*, **17**, 459 (1971); *Ind. Eng. Chem., Process Des. Dev.*, **10**, 215 (1971).
- ⁵ Li, N. N., and Shrier, A. L., in *Recent Advances in Separation Science* (edit. by Li, N. N., CRC Press, Cleveland, 1972).
- ⁶ Li, N. N., and Asher, W. J., *Adv. Chem. Ser.*, **118**, 1 (1973).

Removal of ozone from the atmosphere by soil and vegetation

OZONE is the principal constituent of the photochemical smog that plagues many cities in the United States. Produced by the action of sunlight on the hydrocarbons and oxides of nitrogen emitted by vehicles and industry, concentrations of ozone greater than 25×10^{11} molecules cm^{-3} (1×10^{11} molecules $\text{cm}^{-3} = 0.4$ parts per hundred million $= 8 \mu\text{g m}^{-3}$), used as evidence of photochemical smog¹, have been observed in Los Angeles for more than two decades². Because of lower air temperatures, less sunshine and fewer vehicles, photochemical pollution was considered unlikely to occur in Western Europe but concentrations indicative of photochemical smog have now been reported from Germany³, the Netherlands^{4,5} and southern England^{6,7} on calm, sunny days. High concentrations of ozone cause respiratory difficulties in humans⁸ and damage many plants⁹ including crops¹⁰.

The Earth's surface acts as a sink for ozone produced in the upper atmosphere¹¹. The rate of removal of ozone varies with the surface: water, snow, grass, and a juniper bush remove it increasingly quickly^{12,13}. Since soil bare of vegetation also removes ozone from the atmosphere at rates similar to those previously observed over land¹⁴, the role of vegetation is not clear. Laboratory studies with individual plants have indicated that vegetation removes ozone from the air when the stomata are open¹⁵⁻¹⁷.

We have examined the role of vegetation and soil as sinks for atmospheric ozone in the field. For this we constructed a mathematical simulator of ozone removal by vegetation and soil. In the laboratory, the rate at which bean plants removed ozone from the air corresponded to a resistance to ozone removal about the same as that encountered by water leaving the leaves through the stomatal pores¹⁵. After taking into account the different rates of diffusion of water vapour and ozone, we obtained similar results for young maize plants. The resistances to ozone removal and evaporation were 271 and 270 s cm^{-1} respectively in the dark and only 3 and 4 s cm^{-1} respectively in the light. This similarity of resistance to ozone removal and transpiration of water implies that at the cell walls beneath the stomata, where the air is saturated with water, the concentration of ozone must be essentially zero. Thus ventilation and

stomata alone control ozone removal by a leaf. This inference has suggested that simulators of evaporation can be modified to calculate the ozone removal by vegetation¹⁸.

Our simulator of ozone removal is based on a simulator of evaporation^{19,20}. The salient features of the simulator of evaporation are its conception of evaporation as an energy exchange process, the predominance of vertical exchange between the canopy and air above over horizontal exchange, the division of the canopy of leaves into strata, the use of stomatal and atmospheric resistances to evaporation, and the use of boundary conditions of temperature and humidity above and below the leaf canopy. The exchange of ozone can be compared with the flow of a direct electrical current through a series of resistances. This clarifies the concept of exchange and simplifies the algebra. The maize canopy may be depicted with eight strata of leaves and a basal stratum of stems.

The flux of ozone into each stratum of leaves or into the soil is opposed by vertical resistances to exchange from one stratum to the next, and within each stratum the exchange is opposed by the resistances of the boundary layer and stomata. The vertical transfer resistances and boundary layer resistances are the same as encountered by sensible heat or vapour transfer, and the stomatal resistance only needs to be modified for the different rates of diffusion of ozone and water vapour. Using the rules of electrical currents, as shown elsewhere¹⁸⁻²¹, we can relate the products of the fluxes of ozone into the leaves and soil, and the resistance to these fluxes to the differences in concentration of ozone at the canopy top and near the ground. Thus, the simulator enables us to calculate the ozone removal by soil and vegetation from the concentration of ozone at the top and bottom of the canopy if we know the vertical transfer resistances, the resistances to removal by the stomata and leaf boundary layer, and the leaf area distribution.

We first calculated the evaporation from measurements of temperature and humidity above a canopy of known leaf area per stratum, the temperature and humidity near the ground, the radiation absorbed by the canopy, the stomatal

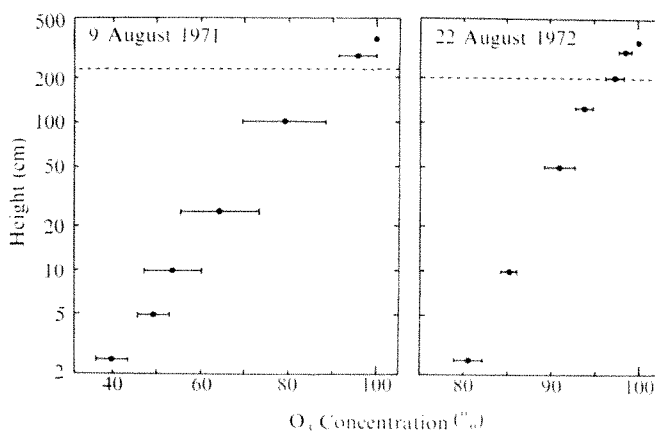


Fig. 1 Relation between ozone concentration and logarithm of height within and above a maize field. The concentration is presented as a percentage of that at 350 cm: 11×10^{11} mol cm^{-3} in 1971 and 31×10^{11} mol cm^{-3} in 1972. The dashed line indicates the height of the maize and the bars denote twice the standard error of the mean of four observations in 1971 and eight observations in 1972. Ozone concentrations were measured with a model 724-2 Mast (Mast Development Company, Davenport, Iowa) ozone meter fitted with a trap for sulphur dioxide. The meter was suspended from a vertical tower, so that it could be rapidly moved between pre-set heights from 2.5 to 350 cm above the soil. Each observation at a particular height was quickly compared with the concentration at 350 cm. Concentrations at seven heights were usually measured during a 20 to 30 min sampling period.

Table 1 Calculated removal of ozone by maize and soil

	August 9, 1971					August 22, 1972				
	Time (est.)									
	1330-1415	1415-1500	1500-1530	1630-1730	1730-1830	1500-1530	1530-1555	1630-1650	1715-1740	1740-1800
Ozone concentration* ($\times 10^{11}$ molecules cm^{-3})	9	9	11	12	10	31	34	36	32	30
Stomatal resistance† (s cm^{-1})	4	4	6	12	25	4	4	7	8	11
Ozone removal ($\times 10^{11}$ molecules $\text{cm}^{-2} \text{s}^{-1}$)										
(a) Foliage	8	7	5	4	2	27	24	16	13	9
(b) Soil (crop present)	6	6	6	8	4	7	8	8	6	9
(c) Total	14	13	12	12	6	34	32	24	19	18
(d) Bare soil‡	8	8	10	18	11	11	11	13	11	14
Soil and air layer§ resistance (s cm^{-1})	0.5	0.5	0.8	0.6	1	4	3	3	5	3

* At canopy top.

† Mean of two leaves per stratum for all strata.

‡ Assuming no crop present.

§ Layer 2.5 cm thick above soil.

and boundary layer resistances within each stratum and the vertical diffusivities within the canopy. We then compared the evaporation calculated by the simulator with actual observations of evaporation from a portion of the crop growing in a pair of weighing lysimeters.

Our test site was a 1 hectare field of a cultivar of *Zea mays* at the Lockwood Farm, Hamden, Connecticut. The maize was planted in mid-May to give a final population of 75,000 or 93,000 plants per hectare and a leaf area index (LAI) of 4.3 and 3.0 in 1971 and 1972 respectively. On August 9, 1971 and August 22, 1972, both clear sunny days with abundant ozone throughout the afternoon, the ozone concentration, net radiation, temperature, humidity, ventilation, stomatal resistance and evaporation were measured between 1330h and 1930h Eastern Standard Time (EST).

August 9, 1971 was slightly warmer and drier than August 22, 1972, and the combination of days and diurnal changes provided a range of weather conditions. The concentration of ozone varied markedly between the two days but not during a particular sampling period. The mean ozone concentration at 350 cm was 11×10^{11} molecules cm^{-3} in 1971 compared with 31×10^{11} molecules cm^{-3} in 1972; the decrease in concentration of ozone with height (Fig. 1) indicated that ozone was diffusing downwards into the canopy and soil. The comparison of evaporation from the lysimeters with that calculated from our observations of weather and plant factors for a range of conditions (Fig. 2; data obtainable from authors) showed that our estimates were realistic. We had to adopt the same rules as in ref. 24 to calculate the vertical resistances within the canopy and avoid impossibly rapid evaporation from the soil.

Assured that the vertical, boundary and stomatal resistances to vapour exchange in the different strata were realistic, we used them in the simulator of ozone removal. The simulated profiles of ozone concentration within the leaf canopies were then compared with the actual profiles measured in the field (Fig. 3). The good agreement gave us confidence in the simulator of ozone removal.

The calculated removal of ozone by the foliage plus soil at five times in both 1971 and 1972 is shown in Table 1. The calculated removal by the foliage was markedly affected both by the ozone concentration and by the aperture of the stomata. The flux of ozone into the soil beneath the maize was similar to that measured earlier¹⁴ over bare soil on several days when the ozone concentration was about 12×10^{11} molecules cm^{-3} .

We also calculated the removal of ozone by bare soil

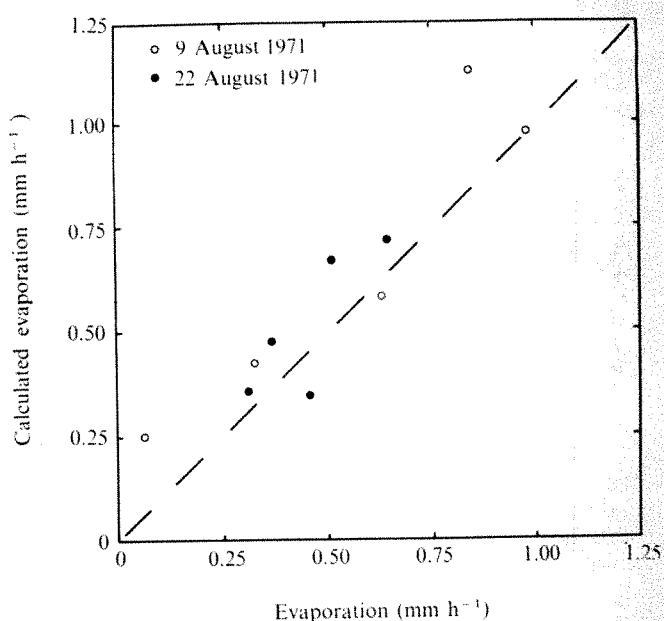


Fig. 2 Comparison of calculated and measured evaporation. To calculate evaporation, the net radiation above the crop was measured continuously with a Beckman and Whitley (San Carlos, California, Model 188-01) net radiometer. The coefficient of extinction of net radiation within the canopy was calculated from the leaf area and the logarithm of net radiation measured at five heights within the canopy with a portable net radiometer (Thorntwaite, Centerton, New Jersey, Model MNR540). The temperature and humidity at the canopy top (225 cm in 1971 and 200 cm in 1972) and at 10 cm above the soil were measured with a ventilated wet-bulb psychrometer (Bendix, Friez Instrument Division, Baltimore, Maryland, Model 566-2). The temperature, humidity, and net radiation within the canopy were measured near the beginning and end of each period. The horizontal wind was observed at four heights above and one height within the canopy with sensitive cup anemometers (Casella, London, Model T16112), and the mean windspeeds for the sampling period were then used to calculate the vertical transport coefficient, or diffusivity, at the canopy top. During each sampling period, the stomatal resistances of the upper and lower leaf surfaces of two leaves per stratum were measured with a ventilated diffusion porometer²². The resistances were measured on two plants growing in one of a pair of lysimeters²³ that measured the evaporation during each period. The lysimeter, ozone meter, and micrometeorological towers were approximately 7 m from the northerly edge and 150 m from the southwestern, leading edge of the field.

for the same concentrations of ozone but assuming that the canopy was not present. For this we used vertical resistances appropriate to bare soil¹⁴. The calculated removal of ozone by the soil had the crop been absent varied from 11 to 17×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$ (Table 1). By difference, the additional removal of ozone by the foliage over that removed by bare soil was 0 to 23×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$. Thus, even this more conservative estimate of the contribution of the foliage indicated that the crop increased the removal of ozone from the atmosphere while the stomata were open, but the decrease in ventilation by the crop decreased the flux of ozone into the soil and hence decreased the net impact of the crop. On the two occasions on August, 9 1971 when the calculation implied that the removal by bare soil would have been greater than by the crop and soil together, the stomata were closed, thus the foliage was removing little ozone, but it was slowing the ventilation and thereby slowing the removal of ozone by the soil.

The combined resistance of the soil and 2.5 cm air layer above it varied from 0.5 to 5 s cm^{-1} (Table 1), and was similar to the 2 s cm^{-1} observed previously¹⁴. Since the resistance of soil increases with moisture¹⁴, the variation in the resistance from 1971 to 1972 may simply reflect the greater amount of water in the soil in 1972. During the 7 d before measurement the rainfall was 3.5 mm in 1971 compared with 23.9 mm in 1972. Alternatively the differences may also reflect our inability to specify accurately the diffusivity in the air near the ground²⁴.

The calculations of ozone removal by the soil and maize are, however, reasonable, and clearly indicate that both soil and vegetation removed ozone when the stomata were open. It is known that in some species ozone closes the stomata^{25,26}, but our measurement of stomatal resistance were similar to those we had reported previously for maize^{24,27}, indicating that the maize stomata were not closed by the ozone. Clearly, however, closure of stomata or leaf injury by ozone will reduce the removal of ozone by vegetation.

Finally we determined whether a forest also cleanses ozone from the air. We chose a mixed hardwood and coniferous forest where the trees were about 25 m tall and had a leaf area index of about 3. We measured the concentration of ozone with a portable meter at a height of 0.3 m over a nearby lake and at seven stations within the forest along a 150 m line parallel to the prevailing southwest wind. Since the ozone concentration was similar all over the lake, only one station was established over the lake, 1 m from the shore. The meter was then moved

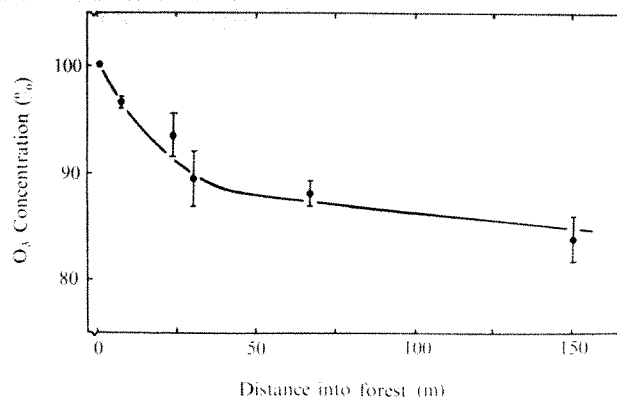


Fig. 4 Ozone concentrations measured at various distances from the shore of a lake into a forest. Concentrations are related to the 30×10^{11} molecules cm^{-3} at the shore. The bars denote twice the standard error of the mean of five observations between 1330 and 1850 h EST on August 23, 1972.

quickly between the stations, and the concentration observed for 1 to 2 min at each station. The decrease of ozone at stations within the forest (Fig. 4) indicated that the forest was removing ozone from the atmosphere, even when observation were made in the direction of the 450 cm s^{-1} wind.

Soil is a major sink for the carbon monoxide²⁸ and vegetation is an important sink for the sulphur dioxide²⁹ emitted into the atmosphere by man's activity. Since ozone is not emitted directly into the atmosphere, but is generated from other pollutants, a knowledge of its production in the atmosphere is required for a similar evaluation to be made for ozone. Recent estimates^{30,31} indicate an ozone production rate of nearly 10×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$ in the lowest 2 km of the atmosphere. Clearly, a rate of removal or destruction of ozone of 6×10^{11} – 34×10^{11} molecules $\text{cm}^{-2} \text{s}^{-1}$ by soil and vegetation together could account fully for this estimated rate of production, and indicates that the soil and vegetation represent primary sinks for photochemically produced ozone.

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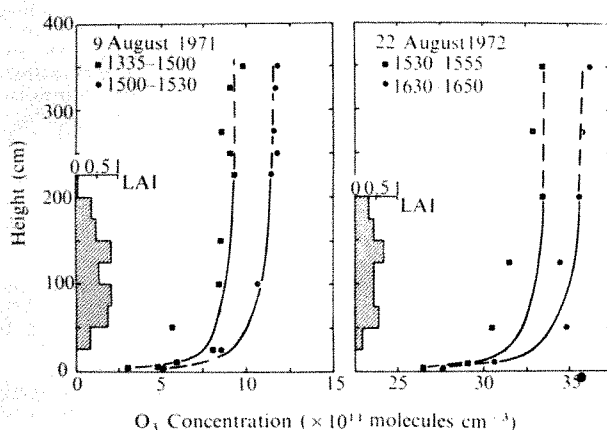


Fig. 3 Simulated (—) and observed (■, ●) concentrations of ozone in and above a maize field. The simulated profiles are extrapolated (—) above the canopy and below 10 cm. The leaf area index (LAI) of each stratum is also shown.

- ¹ Air Quality Criteria for Photochemical Oxidants, chapter 3 (US Department of Health, Education and Welfare, Washington, 1970).
- ² Haagen-Smit, A. J., *Ind. engng Chem.*, **44**, 1342 (1952).
- ³ Jost, D., *Pure appl. Chem.*, **24**, 643 (1970).
- ⁴ Wisse, J. A., and Velds, C. A., *Atmos. Environ.*, **4**, 79 (1970).
- ⁵ Wisse, J. A., and Velds, C. A., *Atmos. Environ.*, **4**, 187 (1971).
- ⁶ Atkins, D. H. F., Cox, R. A., and Eggleton, A. E. J., *Nature*, **235**, 372 (1972).
- ⁷ Derwent, R. G., and Stewart, H. N. M., *Nature*, **241**, 342 (1973).
- ⁸ Palm, P. E., and Nick, M. S., in *Environmental Biology* (edit. by Altman, P. L., and Dittwer, D. S.), 281 (Fedn Am. Soc. exp. Biol. Bethesda, Md. 1966).
- ⁹ Rich, S., *A. Rev. Phytopath.*, **2**, 253 (1964).
- ¹⁰ Heggestad, H. E., and Heck, W. W., *Adv. Agron.*, **23**, 111 (1971).
- ¹¹ Regener, V. H., *J. geophys. Res.*, **62**, 221 (1957).
- ¹² Aldaz, L., *J. geophys. Res.*, **74**, 6943 (1969).

- ¹³ Galbally, I., *Q. Jl. R. Met. Soc.*, **97**, 18 (1971).
¹⁴ Turner, N. C., Rich, S., and Waggoner, P. E., *J. environ. Quality*, **2**, 259 (1973).
¹⁵ Rich, S., Waggoner, P. E., and Tomlinson, H., *Science*, **169**, (1970).
¹⁶ Hill, A. C., *J. Air Pollut. Control Ass.*, **21**, 341 (1971).
¹⁷ Thorne, L., and Hanson, G. P., *Environ. Pollut.*, **3**, 303 (1972).
¹⁸ Waggoner, P. E., *BioScience*, **21**, 455 (1971).
¹⁹ Waggoner, P. E., and Reifsnnyder, W. E., *J. appl. Met.*, **7**, 400 (1968).
²⁰ Waggoner, P. E., Furnival, G. M., and Reifsnnyder, W. E., *Forest Sci.*, **15**, 37 (1969).
²¹ Waggoner, P. E., in *Physiological Aspects of Crop Yield*, (edit. by Eastin, J. D., Haskins, F. A., Sullivan, C. Y., and Van Bavel, C. H. M.), 343 (American Society of Agronomy, Madison, Wis., 1969).
²² Turner, N. C., and Parlange, J. Y., *Pl. Physiol.*, **46**, 175 (1970).
²³ Waggoner, P. E., Begg, J. E., and Turner, N. C., *Agric. Met.*, **6**, 227 (1969).
²⁴ Waggoner, P. E., and Turner, N. C., *Agric. Met.*, **10**, 113 (1972).
²⁵ Engle, R. L., and Gabelman, W. H., *Proc. A. Soc. hort. Sci.*, **39**, 423 (1966).
²⁶ Rich, S., and Turner, N. C., *J. Air Pollut. Control Ass.*, **22**, 718 (1972).
²⁷ Turner, N. C., and Begg, J. E., *Pl. Physiol.*, **51**, 31 (1973).
²⁸ Inman, R. E., Ingersoll, R. B., and Levy, E. A., *Science*, **172**, 1229 (1971).
²⁹ Garland, J. A., Clough, W. S., and Fowler, D., *Nature*, **242**, 256 (1973).
³⁰ Crutzen, P., *Pure appl. Geophys.*, **106-108**, 1385 (1973).
³¹ Leuy, H., *Planet. Space Sci.*, **21**, 575 (1973).

How sea snakes may avoid the bends

MARINE snakes and turtles are probably the deepest diving reptiles. Sea snakes dive to maximum depths of at least 40 m with air in the lung¹. Pressure at this depth potentially produces the formation of N₂ bubbles in the blood and other tissues during decompression, yet sea snakes ascend to the surface rapidly and do not seem to be affected by caisson disease. Here I show that N₂ concentrations in the blood and tissues may remain much lower than that in the pulmonary blood because of a large venous shunt and a low but significant permeability of the skin to dissolved gas.

When a snake dives, the pressure in the lung increases, causing N₂ to move into the blood and from there into the tissues, tending toward equilibrium with pulmonary N₂. Because the N₂ concentration in seawater varies insignificantly with depth, diving creates a N₂ gradient between the blood and the seawater, and N₂ is lost at a rate dependent on the magnitude of this gradient and the resistance to diffusion through the skin. N₂ increases in the blood and tissues until the rate of gain from the lung equals the rate of loss through the skin. The N₂ content of the blood when equilibrium is reached is the maximum value that will occur at any particular depth. This value may be roughly estimated from the N₂ pressure in the lung, the diffusion coefficient of the skin, the cardiac output, and the degree of shunting of venous blood past the lung.

Because of the incompletely divided ventricle, shunting of venous blood from the right atrium to the systemic circuit (right to left shunt) may occur. Right to left shunting may also occur, in effect, if the blood in the pulmonary circuit does not fully equilibrate with the lung gas. For this discussion, it does not matter where right to left shunting occurs, in the heart or the lung. It is useful only to partition the systemic outflow into two fractions: one with the gas concentration equal to the venous return (that is, the shunted blood) and one having a gas concentration equal to that in the lung (that is, the effective pulmonary flow).

The net N₂ flow from the lung in ml min⁻¹ (\dot{V}_L) depends on the effective pulmonary blood flow in ml min⁻¹ (\dot{Q}_L) and the difference in N₂ concentration in ml ml⁻¹ between the blood functionally entering the lung (C_{sv}) and leaving it (C_{lv}).

$$\dot{V}_L = \dot{Q}_L (C_{lv} - C_{sv}) \quad (1)$$

The N₂ loss from the surfaces of the snake (\dot{V}_s) may be considered to depend on the diffusion coefficient D , and the gradient between the blood (C_{sa}) and the seawater. For this discussion, D is defined as the rate of gas movement through the entire gas permeable surface of a snake in response to a gradient of 1 atmosphere absolute (ATA) between the seawater and the arterial blood (ml min⁻¹ ATA⁻¹). Thus D varies with the size of the snake. This simplification permits conversion of the observed rate of extrapulmonary O₂ uptake to an estimate of N₂ diffusion out. A N₂ content (STPD) of 1 ml ml⁻¹ in blood is equivalent to a N₂ pressure of about 70 ATA at 30° C (ref. 2) and the N₂ pressure in seawater is about 0.78 ATA.

$$\dot{V}_s = D(70C_{sa} - 0.78) \quad (2)$$

At equilibrium, $\dot{V}_L = \dot{V}_s$.

$$\dot{Q}_L (C_{lv} - C_{sv}) = D(70C_{sa} - 0.78) \quad (3)$$

The N₂ concentration in the venous systemic blood (C_{sv}) is related to the systemic cardiac output (\dot{Q}_a), the rate of N₂ loss (\dot{V}_s), and the concentration in the arterial systemic blood (C_{sa}). Any heterogeneity in C_{sa} due to unequal distribution of shunted blood to the left and right aortic arches is ignored but, as will become evident, this does not greatly affect the results.

$$C_{sv} = C_{sa} - (\dot{V}_s / \dot{Q}_a) \quad (4)$$

Substituting (2) into (4)

$$C_{sv} = C_{sa} - [D(70C_{sa} - 0.78)] / \dot{Q}_a \quad (5)$$

Substituting (5) into (3) and solving for C_{sa}

$$C_{sa} = (0.78D(\dot{Q}_a - \dot{Q}_L) + \dot{Q}_a \dot{Q}_L C_{lv}) / (70D(\dot{Q}_a - \dot{Q}_L) + \dot{Q}_a \dot{Q}_L) \quad (6)$$

At equilibrium C_{sa} is maximal and is the highest N₂ concentration in the blood except in the pulmonary vein. The internal tissues may eventually equilibrate with the arterial blood so C_{sa} is important in determining the potential for N₂ bubble formation. Although C_{lv} may be much greater than C_{sa} , the small amount of N₂ in the pulmonary vein may be quickly diluted in the systemic circuit during decompression. C_{sa} increases with higher \dot{Q}_L or lower D . If there is no N₂ loss ($D = 0$) or if no shunting occurs ($\dot{Q}_a = \dot{Q}_L$), the N₂ pressure in the blood and tissues approaches that in the lung ($C_{sa} = C_{lv}$).

It is possible to estimate equilibrium C_{sa} in a hypothetical 100 g sea snake diving to various depths (Fig. 1). Systemic cardiac output (\dot{Q}_a), estimated with the Fick principle from the arterial-venous O₂ difference of *Hydrophis belcheri* during spontaneous diving (R.S.S., and M. E. D. Webster, unpublished) and the rates of pulmonary and extrapulmonary O₂ uptake of a 100 g *Pelamis platurus*³, is assumed to be constant at 6 ml min⁻¹. The rate of cutaneous O₂ uptake is related directly to the O₂ gradient across the skin of *Pelamis platurus* (J. B. Graham, unpublished) and *Pseudemys scripta*⁴ which indicates that gas exchange is limited by diffusion rather than blood flow. At 30° C the O₂ uptake through the skin of *Pelamis platurus* is about 0.13 ml min⁻¹ with an O₂ gradient of one ATA (ref. 3). Because N₂ diffuses through tissue at about 55% of the rate of O₂ (ref. 5), the N₂ diffusion coefficient (D) becomes 0.07 ml min⁻¹ ATA⁻¹. Assuming 1 ml of blood holds 0.014 ml N₂ ATA⁻¹ (ref. 2), C_{lv} is calculated from the pulmonary N₂ pressure at depth. As not all of the O₂ absorbed from the lung is replaced by CO₂, N₂ accounts for about 95% of the lung volume after long breath holding (R. S. S., and M. E. D. Webster, unpublished).

The overall venous contribution to systemic cardiac output has been estimated to average 66% of the total in two sea snake species; about 80% of the blood in the left aortic arch and 50% of that in the right arch effectively bypasses the lung (R. S. S., and M. E. D. Webster, unpublished). If the blood N₂ threshold for bubble formation for cats (3.3 ATA at sea level)⁶ applies in sea snakes, decompression sickness cannot occur at depths less than about 50 m (Fig. 1). If no shunting occurred, the critical limit in the arterial blood would be reached at only 25 m,

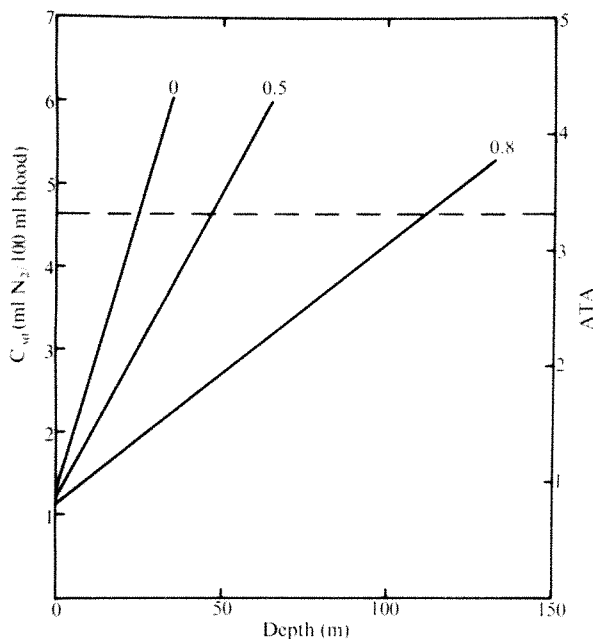


Fig. 1 Maximum blood N_2 concentration (C_{sa}) in a hypothetical 100 g sea snake when the rate of N_2 gain from the lung equals the rate of loss to the seawater at various depths. The effective shunting of venous blood past the lung is indicated as the fraction of systemic cardiac output. — — — Near the threshold for N_2 bubble formation in the blood of cats at sea level⁶.

a depth which produces caisson disease in humans following repeated breath-hold dives⁷. Lung volumes in diving *Pelamis platurus* (~ 9.5% of the body volume)⁸ and green turtles, *Chelonia mydas*, (~ 12% (ref. 9)) indicate that enough N_2 is available to saturate the tissues to dangerous levels. Equilibrium C_{sa} may never be reached, however, if voluntary dives are terminated before enough N_2 can be removed from the lung. Right to left shunting and cutaneous N_2 loss nevertheless decrease the risk of bubble formation during repeated or deep diving.

Because some degree of shunting may occur in other reptiles, it seems that sea snakes were preadapted for avoiding caisson disease when they invaded the ocean. Right to left shunting seems to be higher in sea snakes than in other reptiles breathing normally (R. S. S., and M. E. D. Webster, unpublished). Most reptiles show little or no intraventricular shunting when O_2 remains in the lung¹⁰. It is perhaps significant that all other measurements come from terrestrial or fresh water species which would not normally encounter great depths. Shunting should be measured in marine turtles which may dive to 290 m (ref. 11). Cutaneous gas exchange has been demonstrated in several species of turtles⁴. Berkson¹² observed that the arterial N_2 concentration in *Chelonia mydas* stabilises at values which are considerably less than those predicted by the pulmonary N_2 concentration at various ambient pressures. This occurred at pressures less than that required to compress the pulmonary gas into the rigid nonabsorptive air passages. Berkson suggested that shunting might account for this but did not consider cutaneous N_2 loss.

The mechanism described here may not be the only adaptation of sea snakes for avoiding caisson disease. It is possible that the poorly vascularised sacculus portion of the lung limits N_2 absorption in the manner proposed for the tracheal and bronchial dead spaces in diving turtles¹² and mammals¹³. If this occurs in sea snakes, however, O_2 uptake would necessarily be inhibited and one would wonder why they dive with a considerable amount of air in the lung. Moreover, horizontal or vertical swimming might be difficult if the gas were retained only in the posterior sacculus portion of the lung.

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- ¹ Heatwole, H., and Minton, S. A., in *Biology of Sea Snakes* (edit by Dunson, W. A.) (in the press).
- ² Van Slyke, D. D., Dillon, R. T., and Margaria, R., *J. biol. Chem.*, **105**, 571–596 (1934).
- ³ Graham, J. B., *Respir. Physiol.* (in the press).
- ⁴ Belkin, D. A., *Respir. Physiol.*, **4**, 1–14 (1968).
- ⁵ Krogh, A., *J. Physiol. Lond.*, **52**, 391–408 (1919).
- ⁶ Harvey, E. N., McElroy, W. D., Whiteley, A. H., Warren, G. H., and Pease, D. C., *J. cell comp. Physiol.*, **24**, 117–132 (1944).
- ⁷ Paulev, P., *J. appl. Physiol.*, **20**, 1028–1031 (1965).
- ⁸ Graham, J. B., Gee, J. H., and Robison, F. S., *Comp. Biochem. Physiol.* (in the press).
- ⁹ Berkson, H., *Comp. Biochem. Physiol.*, **18**, 101–119 (1966).
- ¹⁰ White, F. N., *Fedn. Proc.*, **29**, 1149–1153 (1970).
- ¹¹ Landis, A. T., *Undersea Tech.*, **6**, 21 (1965).
- ¹² Berkson, H., *Comp. Biochem. Physiol.*, **21**, 507–524 (1967).
- ¹³ Kooyman, G. L., *S.E.B. Symposium*, **26**, 295–311 (1972).

On Allen's suggestion for long-distance translocation in phloem of plants

N. S. ALLEN has made a film of the streaming of cytoplasm in *Nitella*, in which she used laser light through chloroplast-free 'windows' in the walls of the giant alga cell to take a cine film under Nomarski optics. This film shows that the cytoplasm, which seems to flow steadily and spirally along the axis of the cell, contains undulating filaments that apparently provide the motive force for streaming. It is postulated¹ that endoplasmic filaments undulate in a sinusoidal fashion and thereby drive the cytoplasm. There is some supporting evidence that these filaments are branches originating from microfilament bundles which in turn are normally anchored and parallel to the fixed chloroplast rows. The waves passing along the semi-free microfilament branches cause them to act somewhat like beating flagella in other organisms.

Allen has suggested that some similar systems may be operating in sieve tubes in the phloem of plants. Two possible ways in which this might occur are given in Fig. 1. In Fig. 1a the microfilament material (m.f.m.) is shown attached to the plasma membrane and lateral walls of the sieve tubes. The swishing of these microfilaments would presumably continue through the sieve tube pores in sieve plates. In Fig. 1b (closer to the Allen proposal for *Nitella*) some fibrils persist as axial bundles from plate to plate, through the pores and lumina, though some may be anchored to the plates near the pores. Attached to these by one end only are loose branches of microfilaments which, it is postulated, can swish sinusoidally and hence propel sucrose solution linearly along channels through the lumen and sieve plate pores. We have carefully considered these suggestions in the light of the cine film study of D. R. Lee, D. S. F., and J. W. Costerton. Either model should produce an apparent mass flow with the profile of an activated diffusion², but model b fits more closely the evidence of the cine film study. Neither model will, however, account for a bimodal type of translocation whereby small pulses of sucrose precede the mass flow mode^{3,4}. We therefore propose a slight modification of Allen's hypothesis shown in Fig. 2, whereby the axial fibrils are thought to have flagella-like branches, but also to have hollow

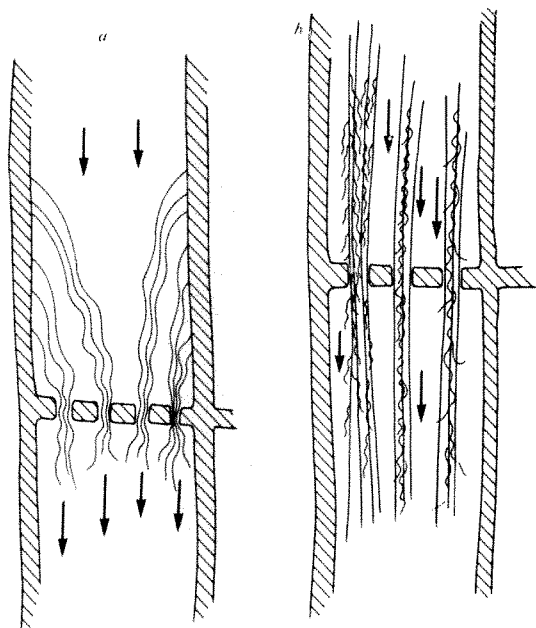


Fig. 1 Sieve tube and plate illustrating mass flow activated by swishing microfilament material (m.f.m.) attached *a*, to the sieve tube walls; *b*, to axial fibrils.

centres so that a small amount of sucrose can be moved ahead of the mass flow by a kind of microperistaltic movement. Such a model overcomes a difficulty in the proposals of D. S. F. whereby many parallel bundles 20–60 nm diameter would be required to operate in phase and in parallel through sieve plate pores. This new model agrees much more closely with the electron micrographs of Robidoux *et al.*⁵ and also with the freeze-etching work of Johnson⁶. It does require the existence of some transcellular material which an increasing number of investigators now believe to exist. But this axially oriented material could not be in the form of membrane-bound tubules of 5–0.5 μm in diameter, at least in *Heracleum*, for these would be clearly visible in living sieve tubes using Nomarski optics nor would a mass flow mode then be possible⁷.

The reasonable nature of these proposals can be demonstrated when Allen's hypothesis¹ is applied to calculate the probable order of magnitude of the energy dissipation in sieve tubes of *Heracleum*. We assume that the motive force causing flow operates throughout the length of the sieve tubes, that is, along the channels in the lumina and across the sieve-plate pores and we can write¹ the expression for the propulsive force per wavelength of a single filament as

$$F_p = 2\pi^2 b^2 V_m (C_N - C_T) / \lambda$$

where V_m is the measured velocity, about $3 \times 10^{-2} \text{ cm s}^{-1}$ (ref. 5); b is the amplitude of the vibration, about $2.3 \times 10^{-6} \text{ cm}$; λ is the wavelength, about $1.1 \times 10^{-5} \text{ cm}$.

The parameters b and λ are not based on *Heracleum*, for good micrographs by freeze-etching technique are not yet available for this plant. But Johnson⁶ has published electron micrographs of the sieve elements of *Nymphaeoides* using freeze-etching techniques. One of these (his Fig. 2) seems to show wave-like structures of repeating pattern suggestive of helical forms. Johnson has interpreted these as filaments, for they are more regular in pattern than the ramifications left between ice crystals and they appear as an array of dots. Tentatively assuming that these waveforms are the vestiges of filaments and that the pattern in *Nymphaeoides* would be reasonably similar to that in *Heracleum*, we have obtained the mean measurements given above.

C_N and C_T are the surface coefficients of resistance acting, respectively, normal and parallel to the axis of the tube. The

values of C_N and C_T for small fibrils can be calculated from the equations

$$C_N = 2C_T \text{ and } C_T = 2\pi \nu / (0.5 + \ln(r/\lambda))$$

where ν , the viscosity, is taken as 2×10^{-2} poise (20% sucrose at 20°C) and r the radius of the filaments is taken as $5 \times 10^{-7} \text{ cm}$ (ref. 5). The numerical values of C_N and C_T are then 0.26 and 0.13 poise, respectively.

Hence $F_p = 1.7 \times 10^{-8}$ dyne per wavelength. The energy dissipation for mass flow will be $F_p \times V_m = 0.5 \times 10^{-9}$ erg per s per wavelength in a single filament. But in one sieve tube there are between 5×10^4 and 5×10^5 filaments in the cross section³ and 6.7×10^4 wavelengths per cm of filament. Hence in 1 cm of sieve tube the energy dissipation would be expected to be in the range 1.7 to 0.17 erg s⁻¹. These values are more conveniently expressed in terms of the rate of consumption of sucrose. For this we assume the number of individual sieve tubes per cm² of sieve tube cross section to be 3×10^5 (ref. 5) and take a value of 4×10^3 calorie per g of sucrose metabolised. Hence, for mass flow alone, the expected rate lies between 1.1×10^{-2} and $1.1 \times 10^{-3} \text{ g sucrose h}^{-1} \text{ cm}^{-2}$ which corresponds to only 1/9,000 to 1/90,000 of the sucrose carried by specific mass transfer, based on sieve tube cross section alone.

Of course, allowance must be made for the pulse flow moiety which seems also to be present. If such a pulse flow travelled by a microperistaltic process as suggested by D. S. F., then the number of transcellular fibrils conducting by this mode need only be perhaps 1/20 of the number previously calculated³ when it was envisaged that all the fibrils were potentially tubular in nature. In other words, we can consider that any one axial tubular fibril complex has at least 20 swishing filament tail units operating around it. Thus the sucrose consumption by this mode, and additional to that required for mass flow, would be about $2.3 \times 10^{-3} \text{ g h}^{-1} \text{ cm}^{-2}$.

Hence we conclude that the mechanisms operating either by undulating filaments alone or by filaments accompanying a

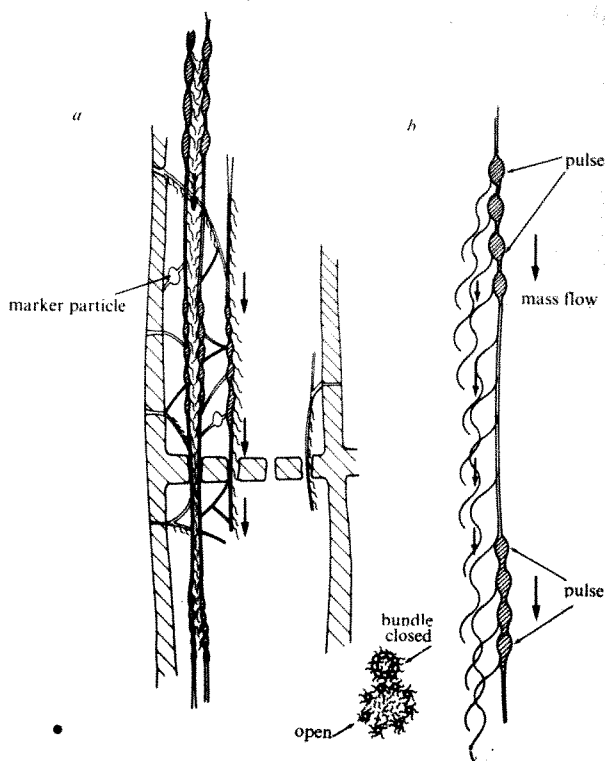


Fig. 2 Diagram of sieve tube and plate illustrating a bimodal system ('activated' mass flow + pulsatory flow) in which the m.f.m. are anchored to axial fibrillar bundles capable of microperistaltic action. *a*, Sieve tube; *b*, individual axial fibril with attached filaments (m.f.m. helices with branches in sinusoidal movement anchored to axial bundle).

microperistaltic moiety in the supporting fibrils are both energetically feasible and seem to be at least an order of magnitude lower than one using microperistalsis in 60-nm diameter tubules alone.

The strength of Allen's suggestion to us is that it provides a motive force through the lumen and sieve plate which assists rather than impedes flow past filamentous material, without invoking large membrane-bound tubules for which there is much less evidence than for transcellular fibrils or bundles of filaments. We hope that other workers in the field will be alerted to see whether further experimental evidence can be found to support it in other plants or to test it. Meanwhile it could be a very important concept in unravelling the problem of the mechanism of long distance sugar translocation in plants.

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¹ Allen, N. S., *J. Cell Biol.* (in the press).

² Canny, M. J., *Phloem Translocation* (Cambridge University Press, London, 1973).

³ Fensom, D. S., *Can. J. Bot.*, **50**, 479 (1972).

⁴ Knight, B. W., Mitton, G. D., Davidson, H. R., and Fensom, D. S., *Can. J. Bot.* (in the press).

⁵ Rohidoux, J., Sandborn, E. B., Fensom, D. S., and Cameron, M. L., *J. exp. Bot.*, **24**, 349 (1973).

⁶ Johnson, R. P. C., *Nature*, **244**, 464 (1973).

⁷ Barclay, G. F., and Fensom, D. S., *Acta bot. neerl.*, **22**, 228 (1973).

Effect of vasopressin on the isolated human collecting duct

THE study of human kidney function has been largely indirect, based on clearance studies and interpretation of data from more direct approaches on other animal species. Extrapolation from one species to another, however, is not always justified and the need for accurate information on human kidney function is still great. Obviously, several ethical and practical factors severely limit the use of direct studies (such as micropuncture) on the human kidney.

If some portion of an intact human kidney was isolated *in vitro*, however, renal tubular transport could be studied directly. We have had the opportunity to carry out such a study which has involved isolation and microperfusion of human kidney tubules.

The kidney was from a male foetus 5.5 months old, which was aborted through hysterotomy. The indication for abortion was a D₁ trisomy¹ as diagnosed by karyotype analysis of cultured amniotic cells. The congenital defects included agenesis of olfactory lobes, pulmonary artery stenosis, ventricular septal defects and a single umbilical artery. The kidneys appeared normal in all respects.

The experimental procedure for the renal study was as follows. After removal of the left kidney, one slice was cut and immersed in chilled Ringer's solution. The slice was teased with small forceps and needles under a dissecting microscope². The tubules in the cortex were hard to separate. Eventually, a few fragments could be isolated from the medullary region and were identified as collecting ducts. One of them, after transfer to a special incubation chamber was successfully pump perfused using techniques previously described³. The bathing solution was a Ringer bicarbonate buffer with 5.5 mM glucose pH 7.4, gassed with a mixture of 95% O₂ and 5% CO₂ (ref. 2). The luminal fluid was a phosphate buffer containing 60 mM NaCl (125 mOsmol

kg⁻¹ water), to which ¹²⁵I-iothalamate was added as a volume marker³. This enabled accurate computation of the perfusion rate. Osmotic water permeability was calculated as previously described³. Perfusion was initiated within 90 min after hysterotomy. The length of the tubule exposed to the bathing medium was 400 µm.

The osmotic water permeability after 80 min incubation was 2.48 µl cm⁻² osmol⁻¹ min⁻¹. After the addition of vasopressin (2.13 mU ml⁻¹) to the medium bathing the tubule it rose to 11.98 µl cm⁻² osmol⁻¹ min⁻¹. The values are of the same order of magnitude as those found in the isolated collecting tubule of the rabbit in the presence and absence of vasopressin³. The data are not strictly comparable, however, as the rabbit collecting tubules were obtained from the cortex and the nephron segment in the present study is from the medulla.

During the first hour of perfusion, the epithelial cells appeared cuboidal with barely visible intercellular spaces. The outer diameter of the tubule was 43 µm and the inner diameter 21 µm. After the addition of vasopressin, striking morphological changes occurred. These included cell swelling, vacuolisation and conspicuous dilatation of intercellular spaces (Fig. 1). These changes accompanied the increase in osmotic water permeability and indicated that outward net water movement occurs through intercellular channels as well as through the cell membranes. The structural changes are quite similar to those previously found in the isolated rabbit collecting tubule⁴.

Additional experiments are needed to substantiate the results of the present study. Nevertheless, our first data

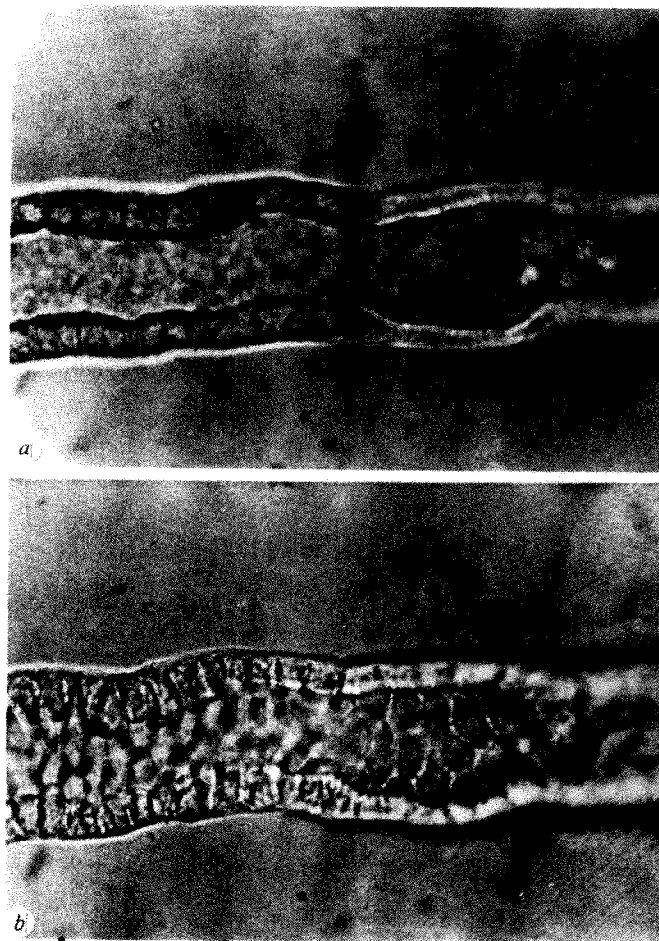


Fig. 1 Micrograph of living perfused human collecting duct. Focal plane at the central axis on the right and at apical surface of the cells on the left. *a*, Before vasopressin; *b*, after vasopressin ($\times 520$).

suggest that in man the collecting tubule receptors for vasopressin are well developed during prenatal life. It is likely therefore that the failure of the infant to excrete a concentrated urine cannot be attributed to a lack of end-organ responsiveness to antidiuretic hormone, as previously proposed³.

The inadequate renal concentrating ability in the newborn presumably reflects a poorly developed osmotic gradient in the medulla⁶.

The present study provides the first reported evidence that the function of the human nephron can be directly evaluated with the help of *in vitro* microperfusion techniques. Further work with similar material or with fresh, undamaged portions of surgically removed kidneys will be required to gain direct insight into renal tubular transport mechanisms in man.

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- ¹ Smith, D. W., *Am. J. Obstet. Gynec.*, **90**, 1055 (1964).
- ² Burg, M. B., Grantham, J. J., Abramow, M., Orloff, J., *Am. J. Physiol.*, **210**, 1293 (1966).
- ³ Abramow, M., *J. clin. Invest.*, **53**, 796 (1974).
- ⁴ Ganote, C., Grantham, J. J., Moses, H. L., Burg, M. B., Orloff, J., *J. cell Biol.*, **36**, 355 (1968).
- ⁵ Heller, H., *J. Physiol., Lond.*, **102**, 429 (1944).
- ⁶ Edelmann, C. M., Barnett, H. L., Troupkou, V., *J. clin. Invest.*, **39**, 1062 (1960).

Mutagenicity of chromates in bacteria and its relevance to chromate carcinogenesis

THERE is abundant experimental evidence that certain chromium compounds are carcinogenic in animals^{1,2}. There are also epidemiological data which suggest that chromium compounds are carcinogenic in man^{3,4}. Calcium chromate especially, produces epithelial lung tumours both by intra-bronchial implantation¹ and sarcomata by intramuscular administration to rats³. In epidemiological studies Bidstrup and Case⁴ reported a significantly high lung cancer mortality in men who worked in chromate-producing factories, and concluded that the most likely explanation of this increased risk was due to an occupational carcinogen.

The mechanism of chromate carcinogenicity is not understood. But considerable evidence exists which suggests that chemical carcinogens are also mutagens although the converse is not necessarily true⁵. We have, therefore, tested the hypothesis that some chromium compounds, including a known carcinogen, are mutagenic. We have used a simple bacteriological spot-test mutagenicity assay⁶ which has convincingly demonstrated that simple hexavalent chromium salts of Na, K and Ca are indeed mutagenic under conditions where related heavy metal salts show no mutagenic activity. There is no evidence, however, that the highly soluble chromates such as Na and K used in this study are carcinogenic to experimental animals (ref. 7 and L. S. Levy, unpublished data), nor are there reports that they cause cancer in man.

The organisms used in this study were: *Escherichia coli* B/r WP2; WP2uvrA; WP2exrA, which differ in their sensitivity to mutability by ultraviolet and ionising irradiation and a variety of alkylating and arylalkylating agents. All

strains require tryptophan for growth due to an *ochre* mutation in the *trpE* locus⁸.

E. coli K12(λ) CA165 (containing *SupB* (ref. 1) was used in conjunction with bacteriophage T4 *ochre* 427 for characterising the WP2 mutants obtained after chromate treatment⁹.

E. coli WP2 strains were grown overnight in nutrient broth at 37° C and diluted 1:50 in M9 medium containing 0.4% (w/v) glucose and 10 μg ml⁻¹ L-tryptophan. Cells were collected by filtration during mid-logarithmic phase, washed in M9 salts and resuspended at 5 × 10⁸ cells ml⁻¹ in M9 salts and kept on ice until used. For mutagenicity assays by the spot-test method 5 × 10⁷ bacteria were spread on agar plates containing M9 medium, 0.4% (w/v) casamino-acids and 1 μg ml⁻¹ L-tryptophan. Test compounds (Analar where available, otherwise General Purpose Reagent Grade, obtained from Hopkin and Williams, Romford, Essex, England) were dissolved in deionised water, filter-sterilised, and 5 μl applied to the centre of each plate. Pre-existing revertants were assayed on agar plates containing M9 medium, and never exceeded 2-3 colonies per five replicate plates. Plates were incubated at 37° C and the maximum yield of mutants (that is, revertants to tryptophan prototrophy) was obtained 4-5 d after plating.

Table 1 shows the results of such an assay using WP2 and three doses of Na₂CrO₄, K₂CrO₄, and CaCrO₄ respectively. Chromate-induced mutant colonies grew more slowly than the larger spontaneous colonies and at the higher doses a central zone of growth inhibition was visible. It is clear from the data presented in Table 1 that all three chromates were equally effective in inducing statistically significant increases (of the order of threefold) in the yield of prototrophic revertants over control levels. The values obtained are of course minimum estimates of the mutant yield since in this method of screening lethality cannot be assessed accurately.

Table 1 Reversion of *E. coli* WP2 (try⁻) to prototrophy after exposure to chromates*

Dose of compound, (μmol per plate)	Revertants per plate (mean of three plates ± s.d.)					
	Na ₂ CrO ₄	P†	K ₂ CrO ₄	P	CaCrO ₄	P
Control	37 ± 10		37 ± 10		37 ± 10	
0.05	125 ± 8	< 0.001	114 ± 16	< 0.01	133 ± 26	< 0.01
0.10	105 ± 10	< 0.01	125 ± 7	< 0.001	99 ± 40	< 0.1
0.20	55 ± 6	< 0.1	127 ± 17	< 0.01	113 ± 34	< 0.05

* Spot-test method.

† P refers to probability in *t* test.

In order to confirm the results obtained using the spot-test method we performed a number of experiments in which bacteria in suspension were treated with Na₂CrO₄, after which the chromate was removed and mutation and lethality then assayed simultaneously (Fig. 1). In both *E. coli* WP2 and WP2uvrA the yield of induced revertants increased linearly with increasing chromate concentration, and there was no significant difference in mutability between the two strains. *E. coli* WP2uvrA was slightly more sensitive to the cytotoxic effect of Na₂CrO₄ than was WP2 (which is wild type with respect to excision repair), the 1/*e* doses being 2 mg ml⁻¹ and 2.7 mg ml⁻¹ respectively.

We obtained negative results with soluble salts of tungsten and molybdenum (the two metals closest to chromium in the periodic table) and also with a soluble trivalent chromium compound, Cr₂SO₄·K₂SO₄·2H₂O. Salts of zinc, cadmium and mercury were also tested and found to be inactive. From the present data, therefore, it seems that of the metals tested, only hexavalent chromium is mutagenic in this system, of which CaCrO₄ alone has been shown to be carcinogenic *in vivo*^{1,2}.

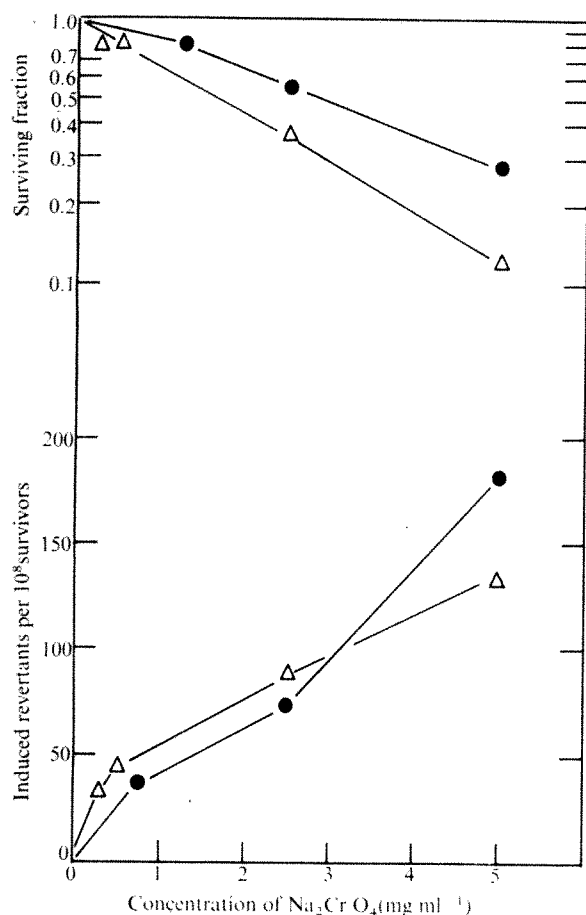


Fig. 1 Dose against survival and mutation of *E. coli* WP2 and *E. coli* WP2uvrA following treatment with Na₂CrO₄ in suspension culture. Bacteria were grown to mid-logarithmic phase (2×10^8 ml⁻¹) in M9 medium containing $10 \mu\text{g ml}^{-1}$ L-tryptophan and collected by filtration and washed in M9 salts. Washed cells were resuspended in M9 salts containing 10 mM Mg^{2+} and 1 mM Ca^{2+} and treated with graded doses of Na₂CrO₄ for 2 h at 37° C. The treated bacteria were washed free of chromate by three cycles of centrifugation in ice-cold M9 salts and resuspended in M9 salts. For assay of viable counts, triplicate 0.1 ml samples at appropriate dilution were plated. Reversion to tryptophan independence was assayed by plating quintuplicate 0.2 ml samples of undiluted bacteria (5×10^7 ml⁻¹) per plate. The plating medium for both viable-count and reversion assays contained 1.5% agar in M9 medium, 0.4% casamino acids, and $1 \mu\text{g ml}^{-1}$ L-tryptophan. Viable counts were scored after 18 h at 37° C, and the full yield of revertants was scored after 4 d at 37° C. Pre-existing revertants were assayed on M9 medium plates. The frequency of induced mutations was calculated according to the formula of Sedgwick and Bridges¹⁵. Each point represents the mean number of colonies of three plates (viable counts) and five plates (mutation frequency). ●, WP2; △, WP2uvrA.

The absence of either the *exrA* repair pathway ('error-prone') or the *uvrA* repair pathway ('excision-repair') did not significantly modify the mutagenic response to chromate (Fig. 1 and Table 2) in this test system. We therefore conclude that chromates fall into that class of mutagens exemplified by hydroxylamine¹⁰, bisulphate¹¹ and ethyl methanesulphonate (EMS) (ref. 14 and E. M. Tarmy, personal communication) which exert their effect by directly modifying DNA bases in such a way that base-pair errors arise at subsequent cell divisions. They do not fall into the second category of mutagens, such as methyl methanesulphonate¹², ultraviolet irradiation¹³, 7-bromomethylbenz[a]anthracene⁹ and nitroquinoline-*N*-oxide¹⁴, whose mutagenicity can be drastically modified or even abolished by the presence or absence of DNA repair processes.

Table 2 Mutagenicity of K₂CrO₄ in three DNA-repair strains of *E. coli* WP2*

Bacterial strain	Revertants per plate (mean of three plates ± s.d.)			<i>P</i>
	Control	Treated (0.05 μmol K ₂ CrO ₄ per plate)		
WP2	Exp. (a) 37 ± 10	114 ± 16		< 0.01
	Exp. (b) 28 ± 6	155 ± 20		< 0.001
WP2 _{exrA}	Exp. (a) 22 ± 8	65 ± 5		< 0.001
	Exp. (b) 30 ± 9	70 ± 6		< 0.001
WP2 _{uvrA}	1 Exp. 37 ± 7	74 ± 11		< 0.02

* Spot-test method.

We investigated the specificity of the postulated base-pairing error by determining the ratio of true revertants (that is, structural reversions at the *trpE* locus) to *ochre*-suppressor-containing revertants in a population of chromate-induced mutants (Table 3). We found that in the chromate-treated group, 98% of revertants contained *ochre* suppressors compared with 45% in the untreated (spontaneous) group. The reversion from tryptophan auxotrophy to prototrophy in this system involves mutation at an *ochre* triplet, UAA (containing only AT base-pairs in DNA)*.

Table 3 Characterisation of *E. coli* WP2 revertants obtained after treatment with K₂CrO₄

	No. of revertants lysed by T4 <i>ochre</i> 427	No. of revertants not lysed by T4 <i>ochre</i> 427	Total no. of revertants tested
Spontaneous revertants (untreated)	9	11	20
Induced revertants (chromate treated)	98	2	100

100 revertant colonies arising from a K₂CrO₄-treated plate, and 20 revertant colonies from an untreated plate, were purified by repeated subculture on minimal medium agar, and overnight nutrient broth cultures were prepared. A lysate of bacteriophage T4 *ochre* 427 was prepared by growth in and lysis of *E. coli* K 12 CA 165 in M9 containing 1% lactose and 0.4% casamino acids. This lysate, after centrifugation, contained less than 0.04% revertant bacteriophage when assayed on *E. coli* WP2. 0.2 ml of each purified revertant culture was mixed with 0.1 ml T4 *ochre* 427 (2×10^4 PFU ml⁻¹) in 0.6% agar at 46° C and poured on to plates containing nutrient agar supplemented with 1% glucose. *E. coli* WP2 which is not lysed by T4 *ochre* 427, and *E. coli* CA 165 which is, were used as controls¹⁶.

Since the treated population contained only 2% true reversions (of which some may well be spontaneous in origin), it is highly likely that chromate does not modify AT base-pairs, but specifically attacks GC base-pairs, causing GC to AT transitions at a subsequent round of DNA replication. In this respect, as well as in its mutagenicity in *Exr*⁻ bacteria, chromate closely resembles EMS, hydroxylamine and bisulphite, which have also been shown to specifically induced GC at AT transitions^{10,11}.

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¹ Laskin, S., Kushner, M., and Drew, R. J., in *Inhalation*

- Carcinogenesis* (edit. by Hanna, M. G., jun., Nettesheim, P., and Gilbert, J. R.), 327 (US Atomic Energy Commission, 1970).
- ² IARC *Monograph on the Evaluation of Carcinogenic Risk of Chemicals to man*, 2, 120 (International Agency for Research on Cancer, Lyon, 1973).
 - ³ Roe, F. J. C., and Carter, R. L., *Br. J. Cancer*, **23**, 172-176 (1969).
 - ⁴ Bidstrup, P. L., and Case, R. A. M., *Br. J. industr. Med.*, **13**, 260-264 (1956).
 - ⁵ Miller, E. C., and Miller, J. A., in *Chemical Mutagens: Principles and Methods for their Detection* (edit. by Hollaender, A.), **1**, 83-119 (Plenum Press, New York and London, 1971).
 - ⁶ Ashwood-Smith, M. J., Trevino, J., and Ring R., *Nature*, **240**, 418-420 (1972).
 - ⁷ Hueper, W. C., and Payne, W. W., *Archs environ. Health*, **5**, 445-462 (1962).
 - ⁸ Bridges, B. A., Dennis, R., and Munson, R. J., *Genetics*, **57**, 897-908 (1967).
 - ⁹ Tarmy, E. M., Venitt, S., and Brookes, P., *Mutat. Res.*, **19**, 153-166 (1973).
 - ¹⁰ Phillips, J. H., and Brown, D. M., *Progr. Nucleic Res., molec. Biol.*, **7**, 349-368 (1967).
 - ¹¹ Mukai, R., Hawryluk, I., and Shapero, R., *Biochem. biophys. Res. Commun.*, **39**, 983-988 (1970).
 - ¹² Bridges, B. A., Mottershead, R. P., Green, M. H. L., and Gray, W. J. H., *Mutat. Res.*, **19**, 295-303 (1973).
 - ¹³ Witkin, E. M., *Brookhaven Symp. Biol.*, **20**, 17-55 (1967).
 - ¹⁴ Kondo, S., Ichikawa, H., Iwo, K., and Kato, T., *Genetics*, **66**, 187-217 (1970).
 - ¹⁵ Sedgwick, S. G., and Bridges, B. A., *Molec. gen. Genet.*, **119**, 93-102 (1972).
 - ¹⁶ Osborn, M., Person, S., Phillips, S., and Funk, F., *J. molec. Biol.*, **26**, 437-447 (1967).

Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion

KOEFOED-JOHNSEN and Ussing proposed¹ that the transepithelial potential difference (PD) in frog skin was due to the sum of two diffusion potentials, one at the outside border due to sodium, and one at the inside due to potassium. Others have shown somewhat similar results in toad urinary bladder^{2,3}. Although there is evidence to support the notion that such a mechanism cannot account for the entire PD⁴⁻⁶, the hypothesis is still favoured by many investigators.

The hypothesis might be true if when the sodium pump is inhibited or stimulated, the same changes in PD occur

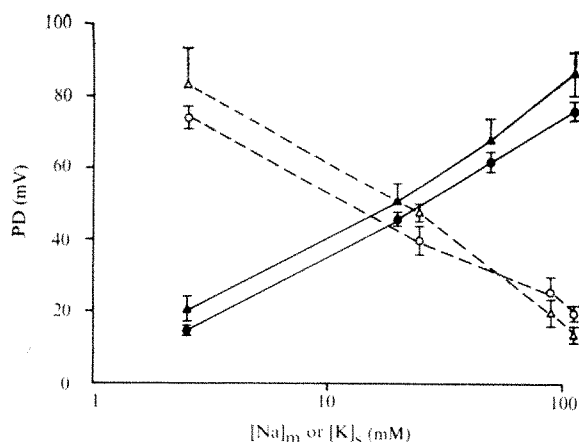


Fig. 1 Response of control bladders to ionic changes. The transepithelial potential is plotted against the mucosal sodium or serosal potassium (— — —) concentration. ●, ○, Experiments performed with chloride as the major anion; ▲, △, Experiments done in sulphate media. In each case progressive Na for K substitutions were made in the appropriate medium and the resulting PD measured. Vertical lines indicate \pm s.e.m.

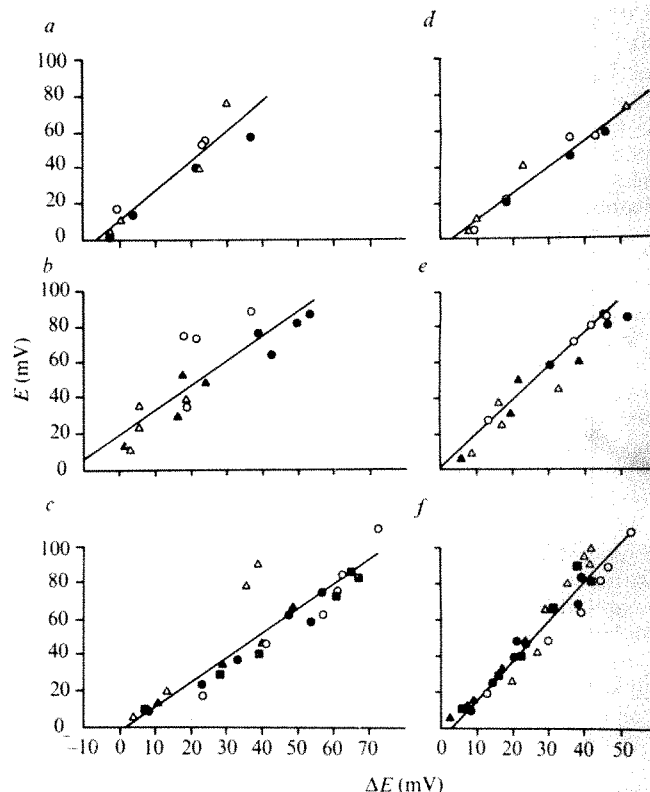


Fig. 2 Effect of inhibition of the transepithelial potential on the response to changes in ionic constituents. Each panel represents a series of studies on several bladders, and studies on a single bladder are shown for each panel as like symbols. *a* and *d* show the result of mucosal (*a*) and serosal (*d*) ionic changes in ouabain experiments. *b* and *e* show mucosal (*b*) and serosal (*e*) ionic changes in experiments in which the temperature was changed. *c* and *f* show mucosal (*c*) and serosal (*f*) ionic changes. *a*, $y = 1.65 (\pm 0.17) x + 10.3 (\pm 3.2)$, $r = 0.95$; *b*, in amiloride experiments, $y = 1.40 (\pm 0.21) x + 20.1 (\pm 5.8)$, $r = 0.88$; *c*, $y = 1.35 (\pm 0.12) x - 1.59 (\pm 5.16)$, $r = 0.91$; *d*, $y = 1.50 (\pm 0.10) x - 5.5 (\pm 2.9)$, $r = 0.98$; *e*, $y = 1.79 (\pm 0.13) x + 0.90 (\pm 4.1)$, $r = 0.97$; *f*, $y = 2.19 (\pm 0.11) x - 5.86 (\pm 3.28)$, $r = 0.97$.

when medium Na or K concentrations are changed as are seen in the control state, even though cell Na and K may have reached a new steady state value. That is, for example, whatever the cell K concentration, a tenfold change in serosal medium K would result in a more or less instantaneous tenfold change in the concentration ratio $K_{\text{cell}}/K_{\text{medium}}$, and the PD, a logarithmic function of the concentration ratio, should decrease by the same absolute amount.

Bladders were dissected from Colombian toads, (*Bufo marinus*), and none were used whose PD was less than 50 mV. The standard Ringer solution used contained (mmol l⁻¹): NaCl, 109; CaCl₂, 0.9; NaHCO₃, 2.4; KCl, 2.5; glucose, 5.5, and was stirred by means of a bubble lift with room air. When sulphate was used as the anion, the solution contained (mmol l⁻¹): Na₂SO₄, 55; K₂SO₄, 1.25; CaSO₄, 0.9; NaHCO₃, 2.4; glucose, 60. When step-wise changes in Na and K concentrations (carried out by K-for-Na substitutions) in the mucosal or serosal medium were made, results were similar to those previously described for this tissue^{2,3}, and were consistent with the interpretation that the mucosal barrier is more permeable to Na than to K, while the opposite is true for the serosal barrier (Fig. 1). Note also that there are only small differences between the results with Cl and those with SO₄²⁻ as the major anion. In all cases, the PD chosen was that seen 2 min after the previous solution was removed; each time a change was made, the chamber was washed three times with the new solution.

When the PD was reduced in seven experiments to 3 ± 1 mV with ouabain (10^{-3} M), however, the decrease in potential following reduction of mucosal Na to 2.4 mM was only 0.9 ± 1.1 mV, and that following the increase of serosal K to 111.5 was 2.3 ± 0.3 mV. Furthermore, when the ambient temperature was reduced to 5°C in two experiments (by means of circulation around the water-jacketed chamber of water from a constant temperature circulator), thus decreasing the PD to 0 and 4 mV, the changes in PD after reduction of mucosal Na to 2.4 mM were 0 and 0.5 mV, and the change after an increase in serosal K to 111.5 was 0 in both cases.

We next brought about a graded reduction in transepithelial PD by (1) progressive increases in the concentration of ouabain (added to the serosal solution only) from 10^{-5} to 10^{-3} M, (2) graded changes in the ambient temperature, and (3) progressive increases in the concentration of amiloride (added to the mucosal solution only, in concentrations ranging from 10^{-7} to 10^{-5} M).

Figure 2 shows the results plotted as ΔE , the change in potential after a solution change, against E , the PD obtained with Ringer on both sides just before the change. Note that essentially the same results were obtained with all inhibitors, and with either mucosal or serosal substitutions. Four further experiments with ouabain and two with amiloride were performed using SO_4^{2-} as the anion; equally strong correlation was found, with $r=0.98$ and 0.93 , respectively. Furthermore, if mucosal sodium was replaced by choline instead of potassium, identical results were obtained in two experiments with ouabain. None of these

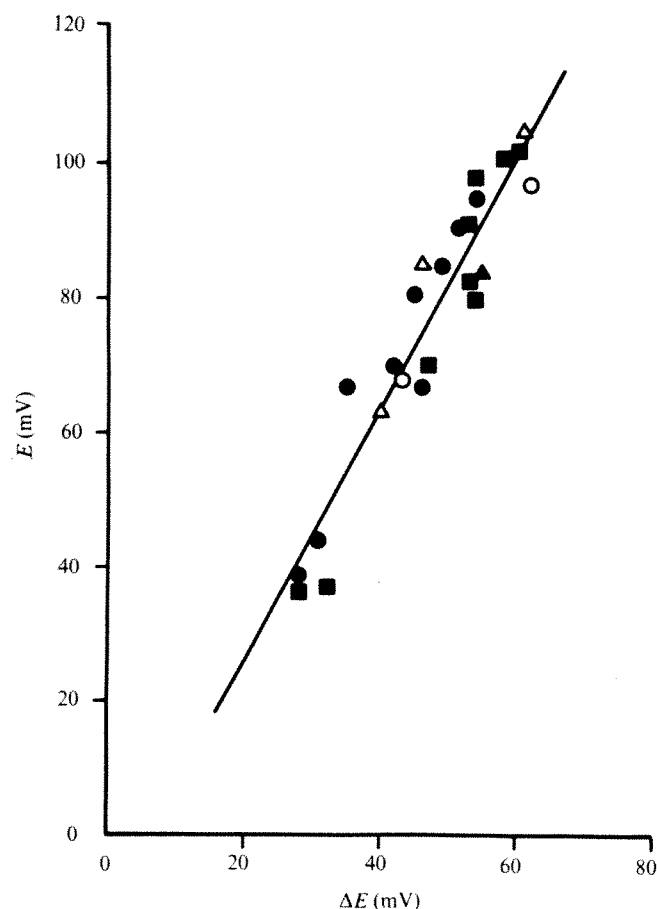


Fig. 3 Effect of stimulation of the transepithelial potential on the response to changes in serosal potassium. Data are plotted as in Fig. 2. The four rather low PD values are from two bladders whose PD decreased late in the experiment. In each bladder (shown by like symbols), serosal K was changed from 2.5 to 111.5 before and after the addition of vasopressin (25 mU ml^{-1}) to the serosal side. After the PD reached a new value, Ringer solution (with or without ADH) was replaced in the medium. $y = 1.96 (\pm 0.12) \times -15.5 (\pm 5.7) r = 0.96$.

Table 1 Transepithelial resistance

	Ouabain	Amiloride	Temperature change
Control	2305 ± 304	1493 ± 232	2270 ± 120
Experimental	2293 ± 322	2107 ± 231	3400 ± 209
n	5	4	5

Resistance (given in ohm cm^2) was measured either as the quotient of the open-circuit PD and the short-circuit current, or as $\Delta E/\Delta I$ when a 50 mV voltage step was applied for 20 s. These two techniques give identical results. In each case the experimental resistance shown is that measured at the highest concentration of ouabain or amiloride, or at the lowest temperature, usually 5° or 10°C , and all resistances are given as means \pm s.e.m.

procedures resulted in a decrease in transepithelial resistance (Table 1), thus excluding the possibility that the observations could be due to a progressive decrease in tissue resistance as the baseline PD falls with inhibition of the Na pump. Finally, in experiments in which the transepithelial PD was stimulated with anti-diuretic hormone (ADH), the results of serosal K-Na substitution were identical with those seen with inhibition (Fig. 3).

Since these results are not compatible with the diffusion potential hypothesis, one explanation might be that the permeability characteristics of the mucosal and serosal barriers are in some way determined by pump activity, so that when the pump changes, the permselectivity changes. This requires, according to the results in Figs 2 and 3, however, that at every level of pump activity both mucosal and serosal permselectivity change *pari passu* with the pump. Furthermore, the procedures chosen for pump inhibition and stimulation are quite different in their sites and mechanisms of action, and the fact that nevertheless, both mucosal and serosal characteristics were affected in a similar manner make it unlikely that the resting potential in this and presumably similar epithelia is due to Nernst-like permeability barriers.

The obvious alternative is that the PD is due to the operation of a charge-separating or electrogenic pump, and that the apparent permselectivities deduced from changes in ionic constituents of the media in control preparations depend on the effects of the various ions on the pump itself. Since its characteristics cannot be rigorously defined by these experiments, however, further speculation seems unwarranted.

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¹ Koefoed-Johnson, V., and Ussing, H. H., *Acta physiol. scand.*, **42**, 298–308 (1958).

² Gatzky, J. T., and Clarkson, T. W., *J. gen. Physiol.*, **48**, 647–671 (1965).

³ Leib, D. E., Hoshiko, T., and Lindley, B. D., *J. gen. Physiol.*, **48**, 527–540 (1965).

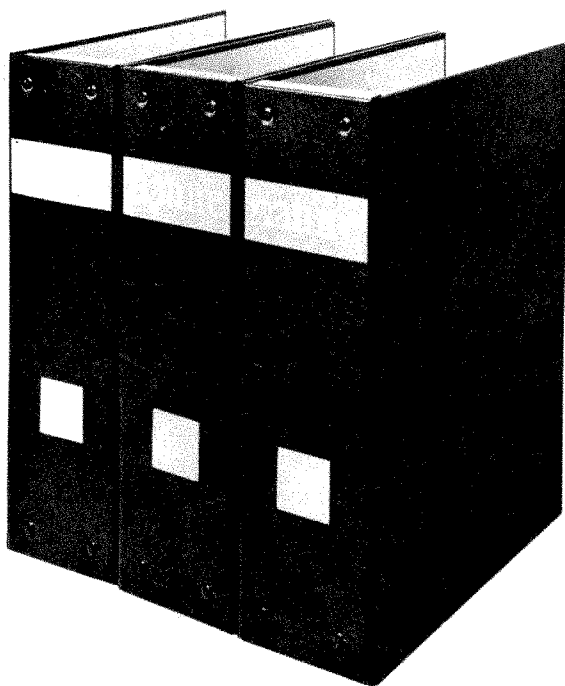
⁴ Frazier, H. S., and Leaf, A., *J. gen. Physiol.*, **46**, 491–503 (1963).

⁵ Pour-Hassani, H., and Finn, A. L., *Fedn. Proc.*, **33**, 216 (1974).

⁶ Snell, F. M., and Chowdhury, T. K., *Nature*, **207**, 45–46 (1965).

Transmission abolished on a cholinergic synapse after injection of acetylcholinesterase into the presynaptic neurone

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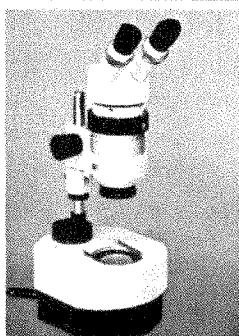
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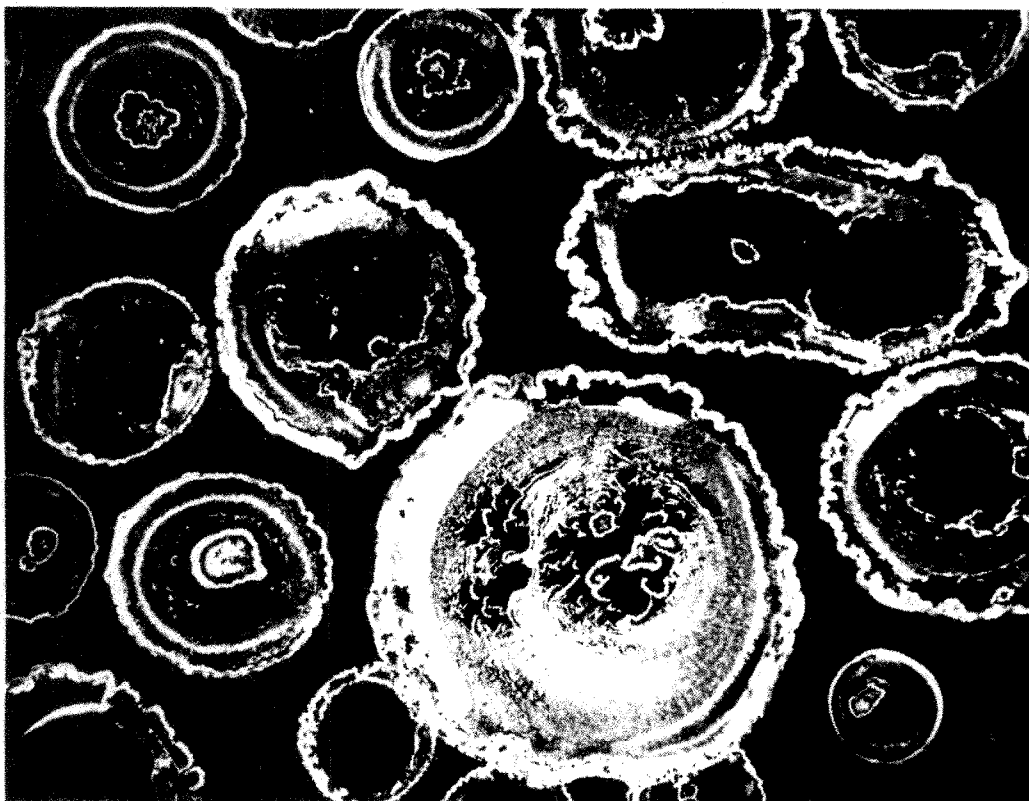
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synaptic vesicles had a high content of acetylcholine (ACh)¹⁻³ and were ideal carriers of 'packets' of ACh released at the synapse, a necessary requirement for the quantal theory of transmission⁴.

Two hypotheses try to explain how the transmitter leaves the cells. One considers that the vesicles, having migrated to strategic presynaptic sites, fuse with the presynaptic plasma membrane and discharge their contents directly into the synaptic cleft⁵. Some electron microscope pictures of the vertebrate neuromuscular junction were interpreted as confirming such liberation of ACh by exocytosis⁶. According to the second hypothesis the ACh of the synaptic vesicles is released into the surrounding axoplasm before crossing the synaptic membrane.

So far, problems of transmitter storage and liberation have been approached mainly by anatomical and biochemical methods. We thought that a physiological approach was possible, taking advantage of the observation that ACh inside the vesicles is protected against the hydrolysing effect of acetylcholinesterase (AChE)^{1,3}, but that ACh in the cytoplasm would be rapidly hydrolysed by AChE.

By artificially introducing AChE into the presynaptic neurone in sufficient quantity to reach the presynaptic terminal, we were able to provide conclusive evidence that synaptic activity in the presence of intracellularly injected AChE is no longer possible. From the evidence presented here we assume that for ACh to act as a transmitter, it must be present free in the axoplasm (eventually released from synaptic vesicles) where it can be broken down by AChE.

In the buccal ganglion of *Aplysia*, two easily identifiable cholinergic interneurons make inhibitory synaptic connections with a number of postsynaptic cells situated close to each other in the same ganglion^{7,8}. After suitable dissection and attaching of the buccal ganglion in an experimental chamber constantly perfused with seawater, one interneurone (injected interneurone) was penetrated with a micropipette filled with purified 5% solution of electric eel AChE in seawater. (Identical results were obtained using AChE from Sigma (type V) or Worthington (type ECHP).) The purity of the Sigma preparation at least can be considered as very high, as when injected in the rabbit only the specific immune serum anti-AChE was found⁹. The injection was performed by an air pressure system similar to that used in a previous study¹⁰. The quantity of solution injected was not known. But, experiments made towards the end of the present study using AChE dissolved in tritiated water enabled the calculation of the intracellularly injected quantity as being about 1% of the volume of the cell soma. The propagation of the enzyme to the presynaptic site was followed with both light and electron microscopy, using an improved version of Koelle's histochemical method¹¹.

The injecting micropipette was also a microelectrode. The postsynaptic potential (PSP) produced by direct stimulation of the interneurone was recorded in one chosen postsynaptic neurone impaled with a double barrelled KCl-filled microelectrode. In some experiments, the second interneurone afferent to the same postsynaptic cell was also impaled (test interneurone) and the synaptic activity monitored. The postsynaptic potentials caused by direct stimulation of the injected and test interneurons were compared throughout the experiment to eliminate possible changes in the properties of the postsynaptic neurone during the relatively long experiments, especially the effect of changes of the membrane potential and of the intracellular ionic concentrations on the amplitude of the synaptic response. During measurement of the PSPs the membrane potential of the postsynaptic neurone was adjusted to -80 mV at which level the IPSP was reversed to a depolarising potential of several mV in amplitude which was more convenient for the experimental analysis.

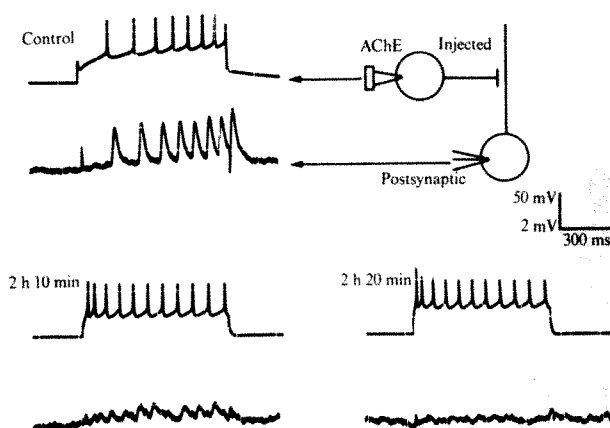


Fig. 1 Spike responses to direct stimulation of the AChE injected interneurone (upper traces) and the corresponding inverted IPSP in the postsynaptic cell at -80 mV membrane potential (lower trace). Control was taken 30 min after injection. At 2 h 20 min the depression of the PSPs was complete.

All experiments were performed at room temperature (20° – 22° C). After injection of AChE no significant change was observed in the respective amplitudes of the injected or test interneurone PSPs for about 2–2.5 h; then, in about 20 min or less, the PSP produced by stimulation of the injected interneurone decreased progressively and finally completely disappeared (Fig. 1). The amplitude of the test interneurone PSP remained within the normal range. The minimal time course of the depression of the PSP was identical whether the injected interneurone was previously firing at a high rate or whether its activity was completely abolished by hyperpolarisation.

In experiments in which presumably lesser amounts of AChE were injected, the injected neurone PSP decreased also but the changes in amplitude started after a longer time interval. Within 1 or 2 h an amplitude was reached which then remained relatively constant with respect to the test PSP or decreased slowly until the end of the experiment, that is for about 4 more hours.

It does not seem that the depression of synaptic transmission is caused by some modification in the size or con-

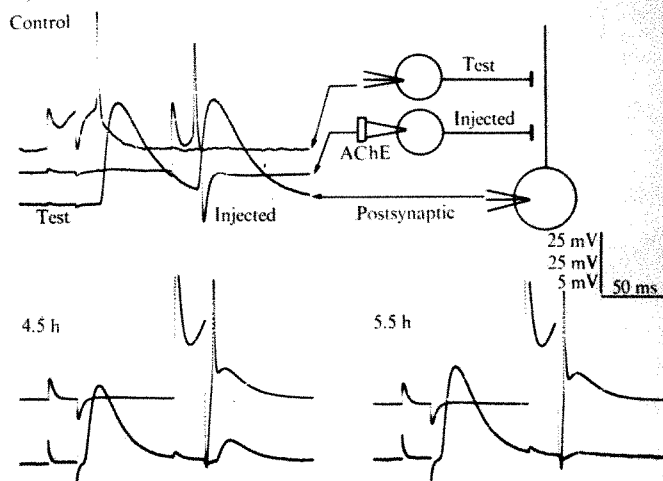


Fig. 2 Spike responses to direct stimulation of the injected and test interneurons and the corresponding inverted IPSPs in the postsynaptic cell at -80 mV. Traces of test spikes are omitted in the two lower recordings. Control was taken 30 min after AChE injection. The quantity of AChE was not sufficient to block the PSP due to the injected interneurone. The PSP corresponding to the test interneurone was not significantly changed.

duction of the spike, as the amplitude as well as the resting potential remained very much the same throughout the experiment (Fig. 2). Also the synaptic delay, in so far as it was detectable, remained constant. In addition the distance between the soma and the ending is short enough so that synaptic efficacy can be affected by modifying the somatic membrane potential by applied current (T. Shimahara and L. Tauc, unpublished) which leads one to suppose that membrane properties changing in the axonal part of the neurone would be detectable in the soma. Also the decrease of the PSP was continuous, without steps, indicating that there was no blocking of presynaptic spike on axonal branchings.

The injection of seawater alone or with addition of inactivated AChE in the same experimental conditions did not produce any change of synaptic efficacy in the injected cell. Contamination of our AChE preparation with proteases or any other enzymes seems highly unlikely. But, since no analysis was done, contamination causing the effect on synaptic activity observed here was ruled out by performing control experiments. Intracellular injection of mixtures of various proteases, especially trypsin and chymotrypsin, produced at higher concentrations marked modifications of the membrane potential and conductance, and of the spike of the injected cell. With concentrations too small to cause these changes in the properties of membranes no effect was detected on the level of the synapse.

Histochemical studies showed that AChE effectively propagates into the axon and in the axonal branchings in the neuropile (Fig. 3). The electron microscope (EM) pictures give additional evidence that AChE had reached the preterminal region of the injected axon. The black precipitate masks subcellular structures on the ending, but in some EM photographs, synaptic vesicles can be observed in spite of the abolition of transmission.

The distance from the soma of the studied terminal can

be estimated as <1 mm; the speed of propagation of the injected AChE was then about $200\text{--}300\text{ }\mu\text{m h}^{-1}$, comparable with the slow rate of progression of AChE in the vagus nerve¹².

Unless there exists a quite improbable action of sub-products of ACh hydrolysis, or an unknown effect of AChE on molecules other than ACh, our results indicate that before its liberation, ACh has to be present in the axoplasm where it is exposed to the hydrolysing action of AChE. ACh is in the axoplasm because it is synthesised there; and possibly it comes from the synaptic vesicles. Vesicular ACh does not seem to be released directly into the synaptic cleft, a conclusion that can be deduced from the result observed here that the minimal time of AChE effect on active and inactive synapses is very much the same. Indeed, if ACh stored in the vesicles was released directly into the synaptic cleft a contrary result would be expected, because the vesicular ACh is protected against AChE action, and thus would remain available for longer. This theory is strengthened by the presence of quite considerable ACh stores in *Aplysia* cholinergic synapses, demonstrated by using hemicholinium-3, which need intensive and prolonged stimulation to become exhausted¹³, and also by a rather slow loss of vesicular ACh observed *in vitro*¹³ and *in vivo* when axoplasmic ACh of *Torpedo* was drained by intensive stimulation¹⁴.

It is interesting to note that the presence of axoplasmic releasable ACh demonstrated here in a form which can be attacked by AChE could also be explained by the existence of two pools of ACh, free and bound, the free pool containing the releasable ACh, a hypothesis which has been proposed by Dunant *et al.*¹⁵.

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- ¹ Whittaker, V. P., Michaelson, I. A., and Kirkland, R. J. A., *Biochem. Pharmac.*, **12**, 300-302 (1963).
- ² De Robertis, E., Rodriguez de Lores Arnaiz, G., Salganicoff, L., Pellegrino de Iraldi, A., and Zieher, L. M., *J. Neurochem.*, **10**, 225-235 (1963).
- ³ Israel, M., and Gautron, J., in *Cellular Dynamics of the Neuron* (edit. by Barondes, S. H.), 137-152 (Academic Press, New York, 1969).
- ⁴ Katz, B., in *Nerve, muscle and Synapse* (McGraw Hill, New York, 1966).
- ⁵ De Robertis, E., *Int. Rev. Cytol.*, **8**, 61-96 (1959).
- ⁶ Couteaux, R., and Pécot-Dechavassine, M., *C. R. hebdom. Séanc. Acad. Sci. Paris*, **271**, 2346-2349 (1970); *Arch. Ital. Biol.*, **111**, 231-262 (1973).
- ⁷ Gardner, D., *Science*, **173**, 550-553 (1971).
- ⁸ Fiore, L., and Meunier, J. M., *J. Physiol. Paris*, **67**, 342A (1973).
- ⁹ Tsuji, S., Rieger, F., Peltre, G., Massoulie, J., and Benda, P., *J. Neurochem.*, **19**, 989-997 (1972).
- ¹⁰ Stinnakre, J., and Tauc, L., *Nature new Biol.*, **242**, 113-115 (1973).
- ¹¹ Tsuji, S., *C. R. hebdom. Séanc. Acad. Sci. Paris*, **267**, 801-803 (1968).
- ¹² Fritzell, M., Hasselgren, P. O., and Sjöstrand, J., *Expl Brain Res.*, **10**, 526-531 (1970).
- ¹³ Stinnakre, J., *J. Physiol. Paris*, **62**, 452-453 (1970).
- ¹⁴ Gautron, J., and Manaranche, R., in *Cholinergic transmission of the nerve impulse*, 215-221 (INSERM, Paris, 1973).
- ¹⁵ Dunant, Y., Gautron, J., Israel, M., Lesbats, B., and Manaranche, R., *J. Neurochem.*, **19**, 1987-2002 (1972).

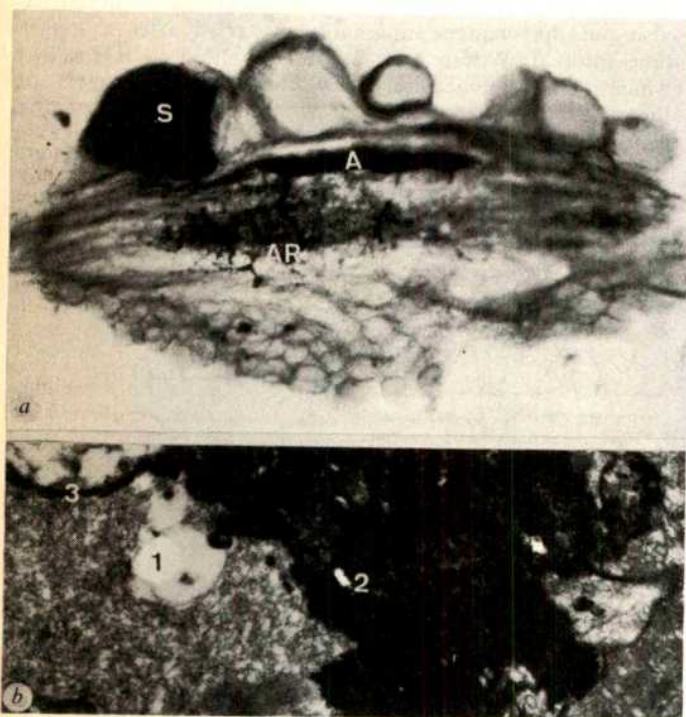


Fig. 3 a, Histochemical localisation of AChE injected into an interneurone of buccal ganglion of *Aplysia*. Thiocholine method. S, soma of BRI (right buccal ganglion, c.f. 7); A, axon; AR, axonal ramification. $\times 90$. b, Electron microscopic localisation of injected AChE detected in a preterminal region in the neuropile. Thiocholine method. $\times 23,000$. 1, nerve fibre without AChE injection; 2, preterminal region with injected ACh; 3, intercellular spaces with endogenous ACh activity.

Y chromosome effect on adult testis size

DURING the development of mammals with X and Y chromosomes the Y chromosome brings about the differentiation of the gonad primordia into testes¹. But apart from carrying the genetic determinants of maleness, little is known about its genetic constitution. In man, the gene for hairy tips to the ears shows Y linkage in at least some pedigrees². In mice there is evidence for a Y-linked histocompatibility locus^{3,4}, and for effects of the Y chromosome on the frequency of spermatozoa with abnormal heads⁵. Here we show that the size of the testes of adult mice is affected by factors on the Y chromosome.

Male mice of the CBA/FaCam strain are fully fertile, but have testes which are much smaller (90–110 mg) than those of males of most other strains^{6,7}. The differences remain significant when variation in bodyweight has been corrected for by regression analysis⁸. Small gonads have also been found in the J and Kw sublines of CBA^{7,9}. A comparison of CBA/FaCam mice with those of the A/Cam, SF/Cam and Peru strains showed that, after correction for variation in bodyweight, 87% of the observed variation was due to differences between the strains, 6% was due to litter effects and 7% to other environmental sources of variation. Reciprocal cross fostering of newborn mice between the CBA and Peru strains did not affect the size of the testes of adults of either genotype, showing that postnatal maternal effects were absent. A diallele analysis of simultaneous crosses between these three strains revealed significant general ($F_{6,321} = 383$) and specific ($F_{6,321} = 83$) combining abilities. There were significant differences between reciprocal crosses ($F_{6,321} = 48$). In all the crosses (see Table 1) F_1 males with CBA fathers had smaller testes than males from the reciprocal cross which had CBA mothers. This pattern of results is that expected for Y-linked factors but not for X-linked factors, or for direct maternal effects. Mice were bred from 18 further crosses between the CBA and SF strains. Table 2 summarises observations on the parental strains, reciprocal F_1 , F_2 and backcrosses, and on four F_3 and two F_4 crosses. All 11 stocks with Y chromosomes from the CBA strain had lower mean testis weights than the 11 stocks with a Y chromosome from the SF strain. Eleven A \times CBA hybrid generations showed the same pattern of results. Table 2 indicates that the average effect of a CBA Y chromosome was to depress mean testis weight by 31 mg. More sophisticated maximum likelihood analyses⁸, however, estimated the Y effect as 24 mg, which represented 41% of the CBA-SF strain difference of 58 mg. In the F_4 generation the two lines which differed in Y chromosomes differed in mean testis weight by 24 mg. All genetic models which omitted a Y effect were much poorer fits than ones which included such an effect. The remainder of the strain difference was due to several (more than two) autosomal factors and to interactions between sex-chromosomes and autosomes.

The testes of CBA mice grew more slowly, at least after birth, than those of mice of other genotypes. The lower weight of testes from CBA mice was detectable well before puberty, and seemed to be due to differences in the length of the semi-

niferous tubules rather than in their diameter, or in the quantity of interstitial tissue present. CBA mice are fully fertile (personal observation and ref. 5) and had morphologically normal sperm⁵. Thus they were very different from C57BL/10J mice⁷, even though adult males of both strains had small testes. The C57 phenotype, which only developed after puberty, was not due to Y-linked genes⁷. The post-pubertal fall in testis weight was secondary to deficiencies in the production of androgens by interstitial cells. Spermatogenesis and fertility were consequently affected in C57 mice, as was the development of sex differences in several target organs of androgenic steroids¹⁰. Unlike the C57 mice, the CBA mice showed no signs of androgen deficiency and had normal spermatogenesis⁹ and a clear sex difference in kidney weight. CBA males were found to have smaller seminal vesicles than those of SF mice, but the inheritance of this difference was free from Y-linked effects⁸,

Table 2 The mean testis weights of CBA, SF and hybrid mice arranged in descending order

Stock	Y chromosome	Mean (mg)	s.e.	Sample size
F_1 (C) (S)	SF	165	2.0	66
BC (S) (CS)	SF	158	3.9	25
F_3 (SC.CS) (SC.CS)	SF	155	4.6	14
F_2 (SC) (CS)	SF	151	2.9	37
F_4 High	SF	146	4.3	9
BC (C) (CS)	SF	144	1.5	70
F_3 (CS.SC) (SC.CS)	SF	142	2.2	33
Strain SF (S) (S)	SF	140	1.9	75
BC (SC) (S)	SF	140	3.6	38
BC (CS) (S)	SF	138	2.5	55
F_2 (CS) (CS)	SF	136	2.8	62
BC (S) (SC)	CBA	132	3.6	38
F_1 (S) (C)	CBA	128	2.0	76
F_3 (CS.SC) (CS.SC)	CBA	125	6.7	8
F_4 Low	CBA	122	3.1	13
F_2 (CS) (SC)	CBA	121	3.0	61
BC (C) (SC)	CBA	121	1.8	69
F_3 (SC.CS) (CS.SC)	CBA	118	3.4	31
F_2 (SC) (SC)	CBA	110	2.9	61
BC (CS) (C)	CBA	107	2.0	64
BC (SC) (C)	CBA	101	1.8	68
Strain CBA (C) (C)	CBA	92	1.0	68

All measurements were made on mice aged eight weeks and were corrected to a bodyweight of 21 g by regression analysis. The letters in parentheses refer to the genetic origin of the hybrids. The first set define the female parent used in the cross and the second set define the male parent. Thus F_1 (C) (S) was the F_1 bred by crossing CBA females with SF males, and the F_2 (SC) (CS) mice were bred by crossing F_1 (S) (C) females with males from the reciprocal F_1 (C) (S) cross. The F_4 high mice were [(SC.CS) (CS.SC)] [(CS.SC) (SC.CS)] and the F_4 low mice were [(CS.SC) (SC.CS)] [(SC.CS) (CS.SC)]. BC, Backcross.

and was not causally related to variation in testis weight. Genetic variation affecting the sensitivity of seminal vesicles to testosterone is known^{11,12}.

The synteny of the CBA factors and the Y-linked histocompatibility locus is intriguing as factors concerned with the small testes of C57 mice seem to be linked to the H-2 histocompatibility locus on chromosome 17 (ref. 13).

The Y effect on gonadal development is large enough to be experimentally useful, yet does not produce infertility. Thus CBA mice, and hybrids derived from them, are excellent material for investigation of the function of the Y chromosome and for studies of the regulation of gonadal development¹⁴.

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Table 1 Mean paired testis weights (mg) for reciprocal F_1 males bred by crossing CBA mice to three other strains

Other parent	Reciprocal F_1		Difference \pm s.e.	Sample size
	CBA mother	CBA father		
SF/Cam	167	135	$32 \pm 3^*$	47
Peru	144	104	41 ± 3	27
A/Cam	140	91	$49 \pm 4^\dagger$	23

All measurements were made on mice aged eight weeks, and were corrected to a bodyweight of 21 g by regression analysis.

* Data from Table 2 give a difference of 37 ± 3.9 ($N = 142$).

† Data collected on a different occasion showed a difference of 34 ± 3.5 ($N = 47$).

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¹ Welshons, W. J., and Russell, L. B., *Proc. natn. Acad. Sci., U.S.A.*, **45**, 560-566 (1959).

² Dronamraju, K. R., *Adv. Genet.*, **13**, 225-310 (1965).

³ Eichwald, E. J., and Silmsker, C. R., *Transpl. Bull.*, **5**, 148-149 (1955).

⁴ Poláková, M., and Vojtisková, M., *Folia biol., Praha*, **19**, 281-288 (1973).

⁵ Krzanowska, H., *Genet. Res.*, **13**, 17-24 (1969); *Proc. int. Symp. Genet. Spermatozoon*, 370-386, (edit. Beatty, R. A., and Gluecksohn-Waelsch, S., University of Edinburgh, 1972).

⁶ Shire, J. G. M., *Excerpta Medica Int. Congr. Ser.*, **184**, 328-332 (1969).

⁷ Shire, J. G. M., and Bartke, A., *J. Endocr.*, **55**, 163-171 (1972).

⁸ Hayward, P., *Thesis*, University of Cambridge, (1973).

⁹ Bartke, A., and Krzanowska, H., *J. Hered.*, **63**, 172-174 (1972).

¹⁰ Bartke, A., and Shire, J. G. M., *J. Endocr.*, **55**, 173-184 (1972).

¹¹ Chai, C. K., *Anat. Rec.*, **126**, 269-282 (1956).

¹² Bartke, A., *J. Endocr.*, **60**, 145-148 (1974).

¹³ Iványi, P., Gregorová, S., and Micková, M., *Folia biol., Praha*, **18**, 81-97 (1972).

¹⁴ Mittwoch, U., *Genetics of Sex Differentiation* (Academic Press, London, 1973).

Development and genetic analysis of *bithorax* phenocopies in *Drosophila*

PHENOCOPIES are developmental abnormalities which closely resemble known mutants, and can be induced experimentally¹. Phenocopies of a given mutant can be induced by various physicochemical agents, which share a unique and restricted effective period in development. In some instances it has been shown that the period of sensitivity to the agent coincides in turn with the effective period of the mutant itself^{2,4}. This is of special relevance because it places the phenomenon of the phenocopy in the same causal frame as that of gene action. Some of the morphogenetic mutants of *Drosophila* have been studied in clonal analysis and shown to act cell autonomously^{5,6}. We have investigated whether their phenocopies also result from events taking place at the cellular level.

In the present work phenocopies of mutants of the pseudoallelic *bithorax* system of *Drosophila* have been studied. Phenocopies of the mutant *bithorax*—one of the alleles—have been found following ether^{8,10} and temperature shocks¹¹ to embryos during the first 6 h of development. The genetic basis⁷ and developmental clonal parameters⁸ of these homeotic mutants are known. Our main conclusions are: (1) phenocopies can be induced before the syncytial nuclei reach the cortex; (2) the effect is produced in individual cells which propagate their new state by cell heredity; (3) the genetic constitution of the egg, with respect to some alleles of the *bithorax* system, has no effect on its sensitivity to ether.

Eggs were collected, following a prelaying period, for 0.5 h on a filter paper sprayed with a yeast suspension. At different times after oviposition the filter paper was transferred for 10 min to a 50 cm³ chamber with a saturated ether atmosphere. The adults were scored under the binocular microscope and subsequently mounted in Euparal for microscopic examination.

In wild-type flies the frequency of *bithorax* phenocopies varies with developmental age (Fig. 1). Phenocopies appear in embryos treated less than 30 min after oviposition (before the syncytial nuclei reach the cortex¹²), their frequency increases to a maximum in embryos treated at 2-2.5 h (syncytial blastoderm) and decreases to zero in eggs treated after 5 h (following gastrulation). It is interesting

to note that the embryonic mortality which follows ether treatment is not correlated with the frequency of *bithorax* phenocopies (Fig. 1).

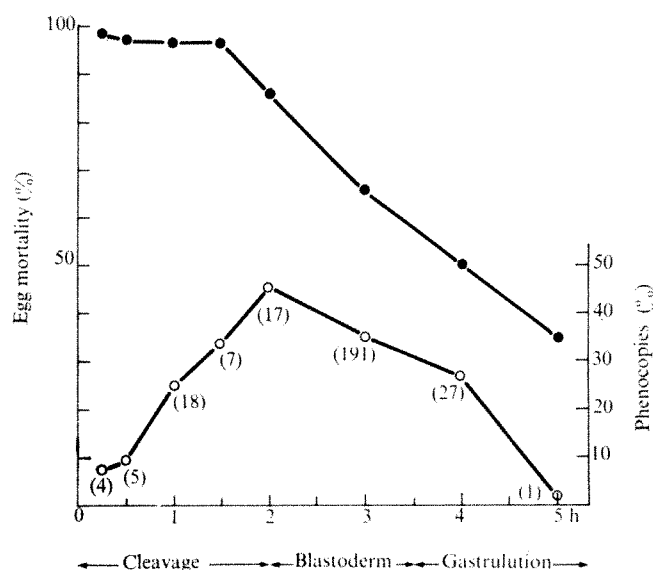


Fig. 1 Frequency of egg mortality (●) and of *bithorax* phenocopies (○) in wild-type individuals following ether treatment at different embryonic stages. The actual number of phenocopies is given in parentheses.

The phenotypes of the different pseudoallelic mutants of the *bithorax* system have been described in detail elsewhere⁷. *Bithorax* (*bx*) transforms the anterior and *post-bithorax* (*pbx*) the posterior metathorax, towards the corresponding regions of the mesothorax. Both regions are transformed in *Ultrabithorax* (*Ubx*) flies. A complete metathorax may appear instead of the normal mesothorax in *Contrabithorax* (*Cbx*) flies. Whereas the first two mutants are recessive and possibly correspond to structural loci, the last two are dominant and have been thought to represent operator mutations affecting the transcription of the entire *bithorax* system⁷.

In standard series of 3-h-old embryos, (experiments 1 and 2, Table 2) phenocopies of the different *bithorax* alleles appear with characteristic frequencies. A sample of 1,048 hemithoraces, corresponding to 524 phenocopy-carrying flies was studied. Phenocopies of *bx* in both dorsal and ventral (sternopleura) metathoracic regions appeared with a frequency of respectively 75% and 27% of the hemithoraces, and are usually the most frequent ones. Phenocopies of *pbx* are more difficult to identify, those with features of clear posterior wing, such as alula, appeared in 2% of the halteres. *Cbx* phenocopies—halter territories instead of wing structures in the mesothorax—were also found, but only in three cases (0.3%).

As previously observed⁸ phenocopies affecting both sides of the same thorax are more frequent than expected on the basis of independent events taking place on each side. This finding could indicate that the phenocopying agent produces some interference with the organisation of the embryo as a whole.

But, several observations suggest that phenocopies occur locally, affecting neighbouring cells in the embryo. Phenocopies are patchy, affecting different and discrete regions of the disk derivatives (see Fig. 2). In 728 hemithoraces, phenocopies embracing structures of both notum and wing represent 34% of the cases, those affecting only notum structures 11% and those affecting only wing structures 47%. In 54 cases (7%) we found independent spots in both notum and wing or in two separated regions of the wing.

Table 1 Frequency of *bithorax* phenocopies in different genotypes

Experiment <i>n</i>	Female	Cross	Male	Genotype	Adults <i>n</i>	<i>n</i>	Phenocopies %	ratio (bx)/TM1
1	<i>mwh jv</i>		+	<i>mwh jv/+</i>	704	191	27	
2	<i>mwh jv</i>		<i>M(3)i⁵⁵/TM1</i>	<i>mwh jv/M(3)i⁵⁵</i> <i>mwh jv/TM1</i>	2,112 2,169	438 586	21 27	0.8
3	<i>bx³e/TM1</i>		<i>sbd e</i>	<i>bx³e/sbd e</i> <i>TM1/sbd e</i>	302 269	100 99	33 37	0.9
4	<i>pbx e/TM1</i>		<i>sbd e</i>	<i>pbx e/sbd e</i> <i>TM1/sbd e</i>	148 127	39 28	26 22	1.2
5	<i>sbd bx³pbx e</i> <i>/TM1</i>		<i>Ubx¹/TM1</i>	<i>sbd bx pbx e/Ubx¹e</i> <i>sbd bx pbx e/TM1</i> <i>Ubx¹e/TM1</i>	325 508 530	0 148 127	0 29 24	
6	<i>Ubx¹e/TM1</i>		<i>sbd e</i>	<i>Ubx¹e/sbd e</i> <i>TM1/sbd e</i>	1,252 1,113	295 276	24 25	0.9
7	<i>Ubx¹³⁰e/TM1</i>		<i>sbd e</i>	<i>Ubx¹³⁰e/sbd e</i> <i>TM1/sbd e</i>	324 327	168 98	50 30	1.7
8	<i>Cbx e/TM1</i>		<i>sbd e</i>	<i>Cbx e/sbd e</i> <i>TM1/sbd e</i>	124 87	46 29	37 33	1.1

Also, the shape of the phenocopy patches resembles that of mitotic recombination clones of, for example, *bx³/bx³* homozygous cells in a heterozygous *bx³/+* haltere background⁸. Also, phenocopy patches correspond in size to one or more recombination clones of *bx³/bx³* cells initiated in first instar larvae. Finally, the borders separating transformed from nontransformed cells in phenocopies are clear cut, as in recombination clones. All these facts taken together suggest that the process which produces phenocopies acts on cells or groups of neighbouring cells which retain their abnormal determination during subsequent development.

This hypothesis was tested in the following experiment. Eggs of the genetic constitution, *mwh jv/M(3)i⁵⁵* were treated with ether at an age of 3 h and the early second instar larvae (72 h) irradiated with 1,000 r. X rays. Mitotic recombination induced by X rays will give rise to non-Minute cells, homozygous *mwh jv* (two-cell marker mutants), which will overgrow their neighbouring Minute cells and build large marked clones¹³. Among 474 hemithoraces (from 358 flies) with phenocopies we found 38 cases of *mwh jv* clones in the dorsal metathorax in either the haltere or in the transformed wing territories. These clones never included both haltere and phenocopied wing cells. In 22 cases the borders of the clones ran for some way along the separation between transformed and nontransformed cells (Fig. 2). As expected these clones did not cross the compartment borders of the phenocopied wing or those homologous borders in the haltere¹³. These results demonstrate that phenocopied cells maintain in their clones the effects of the phenocopying agent after its removal.

The possible role played by the genetic constitution of the egg upon the frequency of phenocopies was tested in crosses of females heterozygous for *bithorax* mutants and a balancer chromosome (TM1, *Mé*, *sbd*) with wild-type males. In the progeny, flies carrying the mutations and flies carrying the balancer chromosome (internal controls) can be distinguished. As shown in Table 1 flies heterozygous for the recessive alleles *bx³* and *pbx* (experiments 3 and 4) or the doubly heterozygous flies (experiment 5) show similar frequencies of phenocopies to internal controls (TM1) and wild-type flies (experiments 1 and 2). Flies heterozygous for the dominant alleles *Ubx¹* and *Cbx* (experiments 6 and 8) behave in a similar way. However, *Ubx¹³⁰* heterozygous flies (experiment 7) show a much higher frequency of phenocopies than control flies. The extent and types of the phenocopies in flies heterozygous for *bithorax* alleles are similar to those in control individuals, mentioned above, with three exceptions: (1) *Ubx¹³⁰* flies show, together with a higher frequency, a higher expressivity in the transformation than flies of the other genotypes, including *Ubx¹*; (2) *Ubx* flies show *bx* phenocopies in

the haltere (Table 1), but also transformation of part of the mesothoracic haltere territory back into wing (9 out of 92 hemithoraces); (3) in *bx³ pbx/Ubx¹* flies, which are phenotypically *bithorax-postbithorax*, we did not find any transformation of either mesothoracic or metathoracic wing back into haltere.

These results indicate that neither the maternal genotype nor the allelic constitution of the cells, wild-type or heterozygous with respect to *bithorax* (with the exception of *Ubx¹³⁰*), affect the sensitivity of the cells to phenocopies. The possibility of phenocopying *Cbx* wings in wild-type flies, mesothoracic haltere in *Cbx* flies back into wing, but not mesothoracic or metathoracic wings of *bx³ pbx/Ubx¹* flies back into haltere, suggests that wing and haltere developments are not reciprocally reversible.

The gene products of the *bithorax* system are assumed to be active in normal flies in the metathoracic segment, preventing mesothoracic development, and inactive in the mesothorax⁷. Thus, we can suppress, by the action of phenocopying agents, the function of the *bithorax* genes in either the metathorax (*bx* and *pbx* phenocopies) or in the mesothorax (suppression of the *Cbx* phenotype), but we cannot produce a haltere development in flies defective for the *bithorax* genes. If we assume that ether interferes with segmental positional signals in the cortex¹⁴, metathoracic properties would appear in an otherwise mesothoracic segment (leading to *Cbx* phenocopies) or *vice versa* (*bx* and *pbx* phenocopies). This hypothesis is consistent with the fact that phenocopies can be obtained even before the migration of the syncytial nuclei into the cortex (Fig. 1). Thus, it is possible that *bx* and *pbx* phenocopies result from the repression of the *bithorax* system in the metathorax. It is therefore interesting that in heterozygous flies only *Ubx¹³⁰* leads to a higher frequency of phenocopies relative to controls. *Ubx¹³⁰*, a rearranged chromosome with one break point in the *bithorax* locus, could represent an operator-deficient mutant and so heterozygous *Ubx¹³⁰* flies will lack one of the two possible repressor binding sites.

We have seen that *bithorax* phenocopies induced in the blastoderm cells are faithfully transmitted in the progeny of individual cells. But, mitotic recombination in heterozygous cells for structural genes, for example, *bx³/+* haltere cells, induced any time during development, lead to clones of *bx³/bx³* cells which show growth and differentiation properties of the anterior wing⁸. Thus, phenocopies of *bithorax* must consist of a stable inactivation of the entire *bithorax* system, irrespective of the allelic state of their structural genes. This inactivation is reminiscent of the process of determination and indicates that both may take place at the cellular level. We hope that the combined studies of genetic variables and of their phenocopies will throw new light on the underlying mechanism of cell

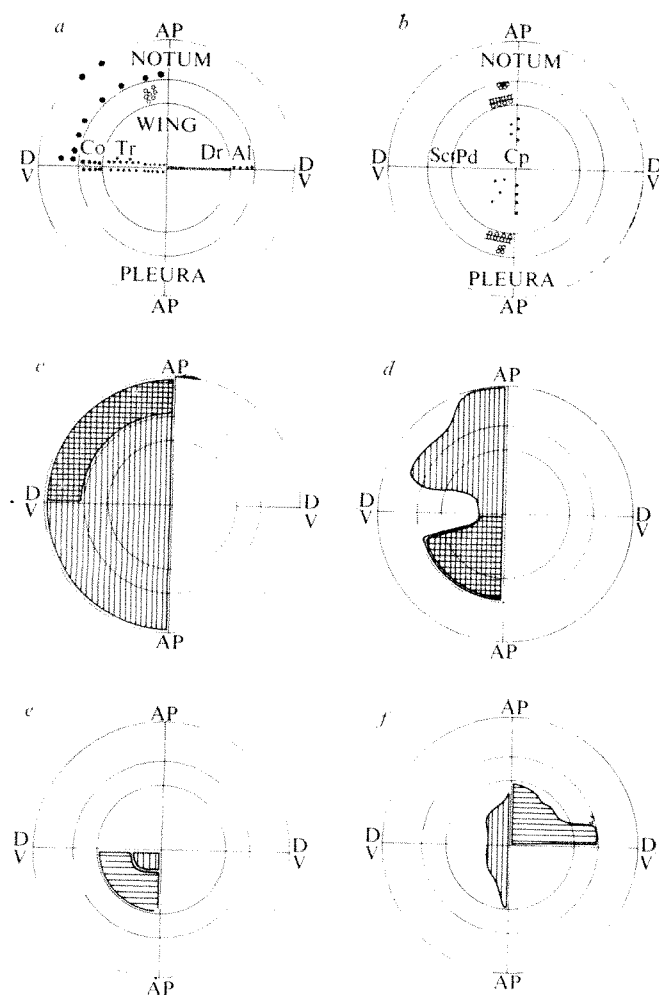


Fig. 2 *a-f*, Schematic representation of the adult dorsal mesothoracic (*a*) and metathoracic (*b*) disk derivatives with their region-characteristic landmarks (●, macrochaetes; ●, microchaetes; ○, sensillae). Regions in *a*, notum, pleura and wing with Co=Costa; Tr=Triple row; Dr=Double row; and Al=Alula. Regions in *b*, haltere with Sc=Scabellum; Pd=Pedicellum; and Cp=Capitellum. Quadrants correspond to developmental compartments (see ref. 13) defined by A/P: anterior-posterior, D/V: dorsal-ventral, notum wing and wing (or haltere) proximal-distal demarcation lines. *c-f*, Four examples of dorsal metathoracic disks showing the extent of 'bithorax' phenocopies, induced by either in 3-h-old embryos, and of *mwh* *ju* *M*⁺ (non-Minute) recombinant clones initiated 72 h later. Phenocopied regions (shaded) contain mesothoracic structures (as in *a*) and clones (hatched) either mesothoracic (as in *a*) or metathoracic structures (as in *b*).

determination.

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¹ Goldschmidt, R. B., *Z. Abst. Vererb.*, **69**, 38-131 (1935).

² Goldschmidt, R. B., *J. Madras Univ. B*, **27**, 12-24 (1957).

³ Landauer, N., *Am. Nat.*, **92**, 201-213 (1958).

⁴ Hadorn, E., *Developmental physiology and lethal factors* (Wiley, New York, 1961).

- ⁵ Stern, C., *Genetic mosaics and other essays* (Harvard University Press, 1968).
- ⁶ Garcia-Bellido, A., in *The biology of imaginal disks* (edit. by Ursprung, H., and Nothiger, R.) 61-91 (Springer, Berlin, 1972).
- ⁷ Lewis, E. B., in *Role of Chromosomes in development* (edit. by Locke, M.), 231-252 (Academic Press, New York, 1964).
- ⁸ Morata, G., thesis, Univ. Madrid (1973).
- ⁹ Gloor, H., *Rev. Suisse Zool.*, **54**, 637-712 (1947).
- ¹⁰ Waddington, C. H., *Evolution*, **10**, 1-13 (1956).
- ¹¹ Maas, A., *Wilhelm Roux' Arch.*, **143**, 515-572 (1949).
- ¹² Poulson, D. F., in *Biology of Drosophila* (edit. by Demerec, M.), 168-274 (Wiley, New York, 1950).
- ¹³ Garcia-Bellido, A., Ripoll, P., and Morata, G., *Nature new Biol.*, **245**, 251-254 (1973).
- ¹⁴ Herth, W., and Sander, K., *Wilhelm Roux' Arch.*, **172**, 1-27 (1973).

Plasma binding of vitamin B₆ compounds

PYRIDOXINE, the major dietary form of vitamin B₆, is rapidly converted in the body to pyridoxal phosphate (pyridoxal-P), the coenzyme form. Pyridoxine has been shown to be phosphorylated in liver and brain homogenates¹ and in red cells², and is subsequently oxidised to pyridoxal-P. In the red cell pyridoxal-P is dephosphorylated, and pyridoxal is the form that is then released into plasma². But the endogenous form of B₆ found in plasma is mainly pyridoxal-P. This paper reports our findings on the ability of plasma proteins to bind these two circulating forms—pyridoxal and pyridoxal-P, and their dietary precursor, pyridoxine. We found that there were marked differences in the extent to which each B₆ compound was bound. These findings can be interpreted to explain part of the mechanism which regulates the passage of B₆ compounds into and out of the red cell.

Heparinised or clotted blood samples were obtained from normal human subjects. When heparinised plasma was compared with serum, no differences in the elution pattern of vitamin B₆ compounds were observed. The proteins were fractionated by gel filtration at 4° C on a 2.2×90 cm acrylic column (Wright Scientific, Kenley, Surrey, UK) of Sephadex G200, eluted with 0.05 M phosphate buffer pH 7.4 containing 0.154 M NaCl. The flow rate was 12 ml h⁻¹ and 130×4 ml fractions were collected. The vitamin B₆ compounds used were crystalline pyridoxal and pyridoxal-P (Sigma) and ³H-labelled pyridoxine (Radiochemical Centre) with specific activity adjusted to 62 mCi mmol⁻¹ by the addition of unlabelled pyridoxine (Sigma). Pyridoxal, and pyridoxal-P after acid hydrolysis to pyridoxal, were measured by microbiological assay using *Lactobacillus casei*³. ³H-pyridoxal, derived from red cell conversion of ³H-pyridoxine, was distinguished from ³H-pyridoxine by *L. casei* activity of the former². Protein was measured by absorbancy at 280 nm.

The gel filtration pattern of the vitamin B₆ compounds in the absence of plasma was first established (see Fig. 1). In these experiments each compound (1,000 ng in 2 ml saline) was eluted separately through the column and fractions were selected for microbiological assay or ³H-counting. In each case the vitamin B₆ eluted in a single symmetrical peak with maximum concentration in fractions 106-108 (free position).

Experiments were then carried out in which 1,000 ng (0.02 ml) of each vitamin B₆ compound was incubated separately with plasma (2 ml) for 30 min at 37° C and then subjected to gel filtration. Fractions were selected for microbiological assay, ³H-counting and protein measurement. Each compound eluted differently as shown by the representative set of experiments illustrated in Fig. 1. Pyridoxine added to plasma eluted in exactly the same fractions as pyridoxine in saline, demonstrating an absence

of binding to protein (Fig. 1a). In contrast, pyridoxal-P eluted almost entirely with the third protein peak (Fig. 1b) which consisted mainly of albumin, transferrin and 3.5S α -glycoprotein¹. In the case of pyridoxal, a significant proportion was associated with the same protein peak, but most eluted in the free position (Fig. 1c). Exactly the same pattern of elution was demonstrated for pyridoxal contained in samples of plasma separated after whole blood had been incubated with 500 ng ml⁻¹ of ³H-pyridoxine (Fig. 2) or pyridoxal.

The binding of the different B₆ compounds to plasma protein will be discussed in relation to our earlier findings on vitamin B₆ metabolism in blood². These demonstrated that the uptake of pyridoxine by red cells, after an initial rapid phase, slowly proceeded to completion as conversion of pyridoxine to its active forms took place in the red cells. This uptake and conversion were independent of the presence of plasma since the kinetics were identical whether the red cells were suspended in plasma or saline. It is therefore not surprising that we found no binding of pyridoxine by plasma proteins (Fig. 1a).

In contrast, pyridoxal-P is totally bound to protein (Fig. 1b) and pyridoxal seems to be partially bound (Figs 1c and 2). In relating these findings to our earlier work it is valuable to consider some observations made by Dempsey and Christensen³ in a study of B₆ binding to purified bovine serum albumin. These authors came to the conclusion that albumin has two specific binding sites for pyridoxal-P with relatively high association constants, but that in addition, both pyridoxal-P and pyridoxal bind less strongly to additional sites. If this also applies to human albumin in plasma, and we found that pyridoxal-P was completely eluted in the albumin-containing peak (Fig. 1b), then the strong

binding of pyridoxal-P by albumin would account for the inability of pyridoxal-P to enter the red cell from plasma, in contrast to its substantial uptake by red cells suspended in saline².

In view of the findings of Dempsey and Christensen³ and of the elution pattern we observed (Figs 1c and 2), it is probable that the pyridoxal-binding protein of human plasma is also albumin. Although only partial binding of pyridoxal is apparent from our results, the poor separation between bound and free peaks (Figs 1c and 2) is suggestive of dissociation of pyridoxal from albumin during gel filtration, in accordance with their weak binding³. The binding of pyridoxal in the fresh whole plasma may therefore have been complete. This binding had already been suggested by our studies on red cell metabolism of B₆, for we found that whether pyridoxal was derived from red cell conversion of pyridoxine or was added directly to blood, the proportion

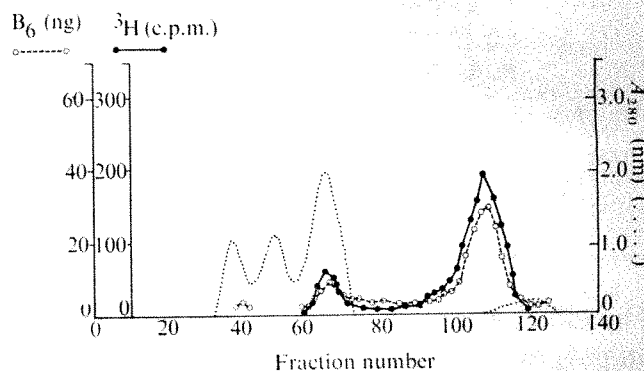


Fig. 2 Plasma protein binding of the pyridoxal originating from red cell conversion of pyridoxine added to whole blood *in vitro*. Heparinised blood was freshly obtained from a healthy subject and to 5.0 ml was added 2,500 ng ³H-pyridoxine (0.05 ml). The mixture was incubated for 2 h at 37° C in a shaking water bath kept in subdued light, after which the blood was centrifuged and the plasma removed. Gel filtration of 2.0 ml of the plasma (containing 520 ng ³H-pyridoxal as measured by microbiological assay and no residual ³H-pyridoxine) was carried out as described in the text for the other experiments, and the ³H-radioactivity (●—●), B₆ microbiological activity (○—○) and protein content (.....) of selected fractions were measured.

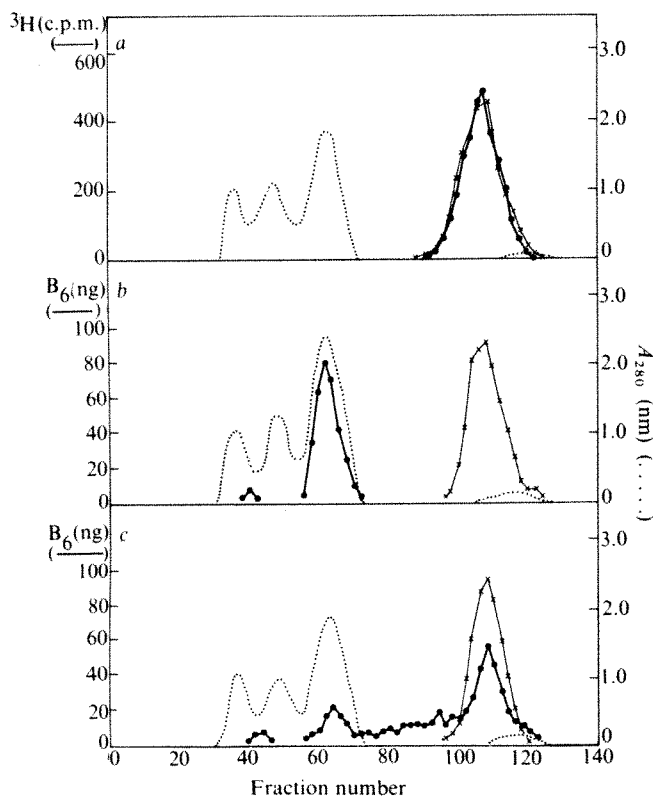


Fig. 1 Binding of a, pyridoxine; b, pyridoxal-P and c, pyridoxal to plasma protein. Gel filtration on Sephadex G200 of each compound added *in vitro* either to plasma (●—●) or to saline (×—×). Vitamin B₆ compounds detected either by ³H-radioactivity (pyridoxine) or microbiological assay (pyridoxal-P and pyridoxal). Protein determined by absorbancy at 280 nm (.....).

of pyridoxal in plasma was directly related to the amount of plasma present. In normal blood the proportion was approximately half that in the red cells, but if experimentally a constant volume of red cells was suspended in increasing saline dilutions of plasma, then the proportion of pyridoxal in the supernatant decreased; if plasma was totally replaced by saline or phosphate buffer, all the pyridoxal was accumulated in the red cells, confirming the work of Yamada and Tsuji⁶. It is probable therefore that the binding of pyridoxal to albumin in plasma plays an important part in the distribution of pyridoxal between the red cells and plasma.

Some additional mechanism must, however, be involved to explain the accumulation in red cells of pyridoxal when plasma is replaced by saline. Furthermore, the uptake of pyridoxal by red cells has been shown to be related to the volume of red cells, provided that the volume of plasma is kept constant (B. B. A., unpublished observations). These findings suggest that the distribution of pyridoxal between red cells and plasma may be controlled by competing binders in these compartments.

Further studies of the binding of vitamin B₆ compounds to plasma and to red cell components are being carried out with a variety of techniques in an attempt to confirm the identity of these binders and their significance.

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- ¹ Wada, H., Morisue, T., Nishimura, Y., Morino, Y., Sakamoto, Y., and Ichihara, K., *Proc. Japan Acad.*, **35**, 299-304 (1959).
- ² Anderson, B. B., Fulford-Jones, C. E., Child, J. A., Beard, M. E. J., and Bateman, C. J. T., *J. clin. Invest.*, **50**, 1901-1909 (1971).
- ³ Anderson, B. B., Peart, M. B., and Fulford-Jones, C. E., *J. clin. Path.*, **23**, 232-242 (1970).
- ⁴ Baumstark, J. S., *Archs Biochem. Biophys.*, **125**, 837-849 (1968).
- ⁵ Dempsey, W. B., and Christensen, H. N., *J. biol. Chem.*, **237**, 1113-1120 (1962).
- ⁶ Yamada, K., and Tsuji, M., *J. Vitam.*, **14**, 282-294 (1968).

The effect of environmental lighting on porphyrin metabolism in the rat

THE direct action of light on the skin in porphyria is well documented. There is a specific spectral response in that light of wavelengths between 400 nm or 500-600 nm causes lesions through photodynamic action of porphyrin in the skin¹⁻⁴. Light may however affect abnormal porphyrin metabolism apart from any action on the skin. We noticed that skin lesions were provoked in porphyric rats, whose coats were shaved, by continuous irradiation by white light fluorescent tubes. Some porphyric animals, in which the coat was not shaved off as a control, excreted more porphyrin when under continuous light than other control animals under continuous dark. This implicated light as an environmental factor effecting porphyrin metabolism. Porphyrin excretion as a measure of abnormality, however, gave inconclusive results. Instead we have used the activity of hepatic δ -aminolaevulinic acid synthetase (ALA-S), the rate controlling enzyme in the haem biosynthetic pathway⁵, as an index of porphyrin abnormality. Normally, ALA-S activity is low, but where porphyria is present clinically or has been induced chemically in experimental animals, activity is raised⁶. We used 1, 4-dihydro-2, 4, 6-trimethyl pyridine-3, 5-dicarboxylate (DDC) to induce porphyria; this chemical, like some other porphyrinogenic agents, such as griseofulvin, probably acts through interfering with a negative feedback inducing mechanism involving haem⁷.

The ALA-S assay was performed on whole liver homogenates⁸ from Sprague-Dawley rats weighing 200-300 g. We first found that normal rats taken straight from the animal house, after life-long exposure to natural cycles of night and day, had a mean value for hepatic ALA-S activity of 26.09 nmol ALA per g wet weight per h (s.d. \pm 7.68). The effect of exposure on six males and six females to continuous light for 11 d, or on six males and six females to continuous dark to 11 d, made no significant difference to ALA-S activity. This was not so, however, when porphyria was induced by DDC. Our final regime, which we shall call the light-dark-DDC regime, was as follows: days 1-8, food (FFG) (M) and water *ad libitum*; day 9, food and water *ad libitum* and DDC; day 10, food

Table 1 Effect of light on rat hepatic ALA-S activity

	Male		Female	
Light + DDC	94.18 \pm	51.16 (16) }	263.50 \pm	143.28 (16) }
Dark + DDC	63.78 \pm	33.82 (18) }	109.89 \pm	77.62 (17) }

Mean ALA-S activity expressed in nmol per g wet weight per h \pm 1 s.d. Numbers in brackets are the numbers of animals. * Difference between light and dark treated, by *t* test, $0.05 > P > 0.02$. † Difference between light and dark $P < 0.001$.

and water *ad libitum* and DDC; day 11, hepatic ALA-S assay. The rats were kept either in continuous light or dark for 11 d.

The light source was an array of eight parallel 20 W Atlas "white" fluorescent tubes, luminous flux approximately 1,000 lumens each, placed 25 cm above the cage floor containing six rats. The conditions of those animals kept in the dark were of photographic dark-room standard and the animals were only exposed to a few minutes of weak red light each day. The DDC was given by oesophageal tube as a 20% (w/v) suspension in arachis oil at a dose of 400 mg per kg body weight once daily on days 9 and 10.

In the light-dark-DDC regime, continuous light led to increased ALA-S activity compared with continuous darkness; this was more pronounced in the female than in the male (Table 1). Control experiments in which rats were given arachis oil, without DDC, showed no difference in ALA-S activity between light or dark treatment. A preliminary experiment of similar design using mice and griseofulvin as the porphyrinogenic drug, gave the same results as for DDC; increased ALA-S activity by light; but the remaining experiments we report are confined to the female rat and DDC.

To see if the stimulus for the above result might originate in the retina, we investigated the effect of orbitectomy. The difference in ALA-S activity previously found in intact animals was now abolished (Table 2a). Activity to hepatic ALA-S in the light-treated orbitectomised group, however, differed significantly from that in the light-treated unoperated female group (Table 1).

Oestrogens are known to be porphyrinogenic⁹, so we examined the effect of ovariectomy on rats in the light-dark-DDC regime. Again the difference between light and dark on hepatic ALA-S activity was abolished (Table 2b).

As the pineal gland has been implicated in the control of the reproductive system of the rat¹⁰, we next investigated the effect of pinealectomy. Sham operated controls had part

Table 2 Effect of various treatments on female rat hepatic ALA-S activity

a, Orbitectomy					
Light + DDC:	148.57	±	67.89 (12)	} *	
Dark + DDC:	150.15	±	80.08 (11)		
b, Ovariectomy					
Operated	{ Light + DDC:	80.96	±	67.89 (12) }	} †
	{ Dark + DDC:	126.32	±	37.59 (8) }	
Sham operated	{ Light + DDC:	279.01	±	155.49 (11) }	} ‡
	{ Dark + DDC:	153.57	±	100.89 (10) }	
c, Pinealectomy					
After 7 d	{ Light + DDC:	210.82	±	128.98 (12) }	} §
	{ Dark + DDC:	134.37	±	60.52 (12) }	
After 100 d	{ Light + DDC:	165.62	±	44.06 (8) }	} ¶
	{ Dark + DDC:	153.71	±	75.71 (8) }	
Sham operated	{ Light + DDC:	187.53	±	74.05 (10) }	}
	{ Dark + DDC:	85.04	±	52.32 (9) }	

Values as in Table 1.

* Between light and dark, no significant difference (n.s.); between light-treated orbitectomised and light-treated unoperated female in Table 1, $0.02 > P > 0.01$.

† Between light and dark n.s.; between light-treated ovariectomised and light-treated normal group, $P < 0.001$.

‡ Between light and dark treatment, $0.05 > P > 0.02$.

§ Between light and dark treatments, and between the 7 and 100 d post operative groups, n.s.

¶ Between light and dark treatment, n.s.

|| Between light and dark treatment, $0.005 > P > 0.001$.

of the calvarium and pia mater overlying the pineal gland removed but the gland was left intact and the wound sutured. In one group of pinealectomised rats, the light-dark-DDC regime was started 7 d later, in another group 100 d after operation. (In the latter group, body weight range was 300–400 g). The results in Table 2c show no significant difference between pinealectomised animals, whether kept in the light or the dark, or whether pinealectomy was 7 d or 100 d before the light-dark-DDC regime.

In rats, intact visual pathways are necessary for normal pineal activity and ovarian development^{8,9}, so light may act on haem biosynthesis in the liver *via* the retina, pineal and gonad. Preliminary results suggest that cycles of 8–12 h light and 16–12 h dark in the light-dark-DDC regime have much the same effect on ALA-S activity as continuous light compared with continuous dark, so that our results may be due to circadian fluctuations in some enzyme or other substance acting in the presence of oestrogen.

Coproporphyrin has been demonstrated in the nervous system of birds¹⁰, where it may play a part in the connection between photoperiodicity and reproductive function.

Continuous darkness or continuous light has been shown¹¹ to alter the activity of two hepatic enzymes in rats, hexobarbital oxidase and *p*-nitroanisole-O-demethylase. We now add evidence that abnormal hepatic metabolism may be under photic influences. The possible influence of environmental lighting on human hepatic porphyria should therefore be borne in mind, although there is so far no evidence for it.

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- ¹ Riemerschmid, G., and Quin, J. I., *Onderstepport J. vet. Sci. Anim. Ind.*, **17**, 89 (1941).
- ² Wiskemann, A., and Wulf, K., *Arch. klin. exp. Derm.*, **209**, 454 (1959).
- ³ Rimington, C., Magnus, I. A., Ryan, E. A., and Cripps, D. J., *Q. J. Med.*, **36**, 29 (1967).
- ⁴ Magnus, I. A., Roe, D. A., and Bhutani, L. K., *J. invest. Derm.*, **53**, 400 (1969).
- ⁵ Granick, S., *J. biol. Chem.*, **241**, 1359 (1966).
- ⁶ Granick, S., and Urata, G., *J. biol. Chem.*, **238**, 821 (1963).
- ⁷ De Matteis, F., *Biochem. J.*, **139**, 52P (1972).
- ⁸ Wurtman, R. J., Rother, W., Altschule, M. D., and Wurtman, J. J., *Acta endocr., Copenh.*, **36**, 617 (1961).
- ⁹ Moore, R. Y., Heller, A., Bhatnager, R. K., Wurtman, R. J., and Axelrod, J., *Archs Neurol. Chicago*, **18**, 208 (1968).
- ¹⁰ Klüber, H., *Science*, **99**, 482 (1944).
- ¹¹ Nair, V., and Casper, R., *Life Sci.*, **8**, 1291 (1969).

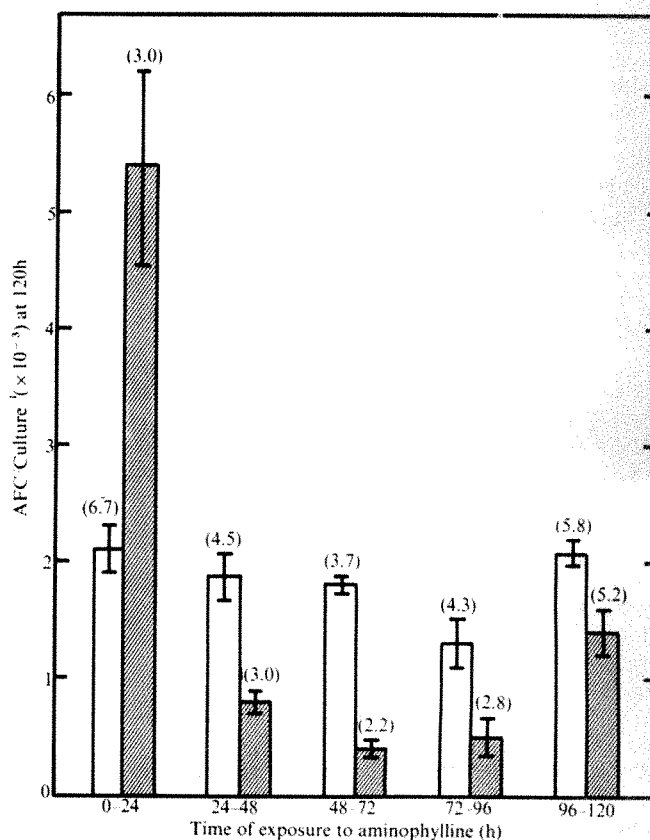


Fig. 1 Effects of aminophylline on the anti-sheep erythrocyte response *in vitro*. Spleen cells from normal CBA/J mice were cultured in Eagle's minimal essential medium containing 10% foetal calf serum, in the presence of 0.01% sheep erythrocytes (SRBC). Each culture initially contained 20×10^6 spleen cells, and was carried out in triplicate in the Diener-Marbrook culture system^{12,13}. At the end of the exposure to aminophylline, the cells were washed by centrifugation, and taken up in fresh medium for further culturing in the presence of antigen. Anti-sheep erythrocyte AFC were determined at 120 h by the method described elsewhere¹⁴. The control cells (no aminophylline) were in each case handled the same way as the experimental cultures. Numbers in parentheses indicate the viable cells, in millions, per culture at 120 h. There was no adjustment of cell numbers after exposure to aminophylline. Shaded columns: cells + medium + 0.01% SRBC + 1 mM aminophylline; unshaded columns: cells + medium + 0.01% SRBC.

mediator of immune induction. But the same agents, at higher than optimal levels, can inhibit the AFC response^{1,3}. Experiments have been done suggesting¹ that enhanced intracellular cyclic AMP is a mediator of immune paralysis, or tolerance. The apparently contradictory conclusions drawn from these various experiments may be resolved if one considers a common kinetic feature of cyclic AMP responses to various agents, in lymphoid⁶⁻⁸ and in non-lymphoid^{9,10} cells; namely, that the rise in cyclic AMP level is transitory, and the cyclic nucleotide level returns to control values after a few minutes or hours. A similar transient rise in cyclic GMP has been noted in phytohaemagglutinin-stimulated peripheral leukocytes, where this nucleotide seems to mediate the blastogenic response to the mitogen¹¹. Thus, the same agents which might enhance an AFC response if present during the increased cyclic AMP phase, could inhibit the response if present during the subsequent phase of declining intracellular cyclic AMP. To see if this pattern was in fact part of an immune response *in vitro*, we performed experiments with sheep erythrocytes as immunising antigen, and varied the time of exposure of the immunocompetent cells to the agents N⁶, 2'-O-dibutyl cyclic AMP or aminophylline. When present only during the first 12 or 24 h of exposure to antigen, these

Biphasic effect of cyclic AMP on an immune response

AGENTS which enhance intracellular adenosine 3', 5'-monophosphate (cyclic AMP) levels⁶ by inhibiting cyclic AMP phosphodiesterase activity can increase the number of antibody forming cells (AFC) in humoral immunity both *in vivo*^{1,2} and *in vitro*^{3,4}. Thus, cyclic AMP is a potential

Table 1 Stimulatory effect of dibutyryl cyclic AMP requires that antigen be present

Additives during first incubation (0-12h)	Viability after first incubation	Viable cells on day 4, $\times 10^{-6}$	Anti-SRBC AFC on day 4
SRBC only	98%	3.3	3,940 \pm 270
SRBC + 1mM dibutyryl cyclic AMP	73%	4.1	16,990 \pm 770
Medium only	91%	3.4	3,000 \pm 440
1 mM dibutyryl cyclic AMP	68%	4.1	3,100 \pm 200

Conditions of culturing and assay were as indicated in Fig. 1, except that the first incubation only was performed in 60×15 mm plastic Petri dishes. Each culture was adjusted to contain 14×10^6 viable spleen cells after the first incubation.

agents increased the number of AFC seen after 4 or 5 d of culture. But they inhibited the response if present during days 2 or 3. The effects of increasing intracellular cyclic AMP during the early period were twofold: (1) there was a delay in the arrival of cells at the antibody-secreting stage, and (2) the number of AFC at the optimal time was increased.

Anti-sheep erythrocyte responses were generated *in vitro*, using unprimed spleen cells from CBA/J mice. Cells and antigen were incubated with various agents for different times, the cells were washed, and the AFC response measured after further culturing with antigen alone. The biphasic effect of aminophylline is shown in Fig. 1. When present only for the first 24 h at 1 mM this agent enhanced the AFC response observed on day 5 of culture. But it strongly inhibited the response if it was present at the same concentration for 24 h periods between 24 and 96 h. Similar patterns were observed with dibutyryl cyclic AMP. Neither agent had a significant effect if present only on the last day of culturing. The stimulatory level of both agents was 1 mM.

The stimulatory effect of dibutyryl cyclic AMP was observed only when antigen was also present (Table 1). In this experiment, cells were exposed to dibutyryl cyclic AMP for the first 12 h, which produced a stimulation of the AFC response comparable to that observed after 24 h exposure to aminophylline or dibutyryl cyclic AMP. The stimulation was observed only if antigen was present simultaneously with the agent. In this experiment, as in the others described, the effects can be seen not to arise from differences in gross cell viability.

We next examined the kinetics of the AFC response following a 12 h pulse of either aminophylline or dibutyryl cyclic AMP. Cells were exposed to antigen and optimal levels of either dibutyryl cyclic AMP or aminophylline for 12 h, washed, and cultured further with antigen alone. The numbers of AFC were determined at various times. During the first 2 d of culture, fewer AFC were found in the treated cultures than in controls. At later times, the number of AFC increased rapidly to as much as six times control values (Fig. 2). Aminophylline and dibutyryl cyclic AMP gave identical results, suggesting that they function identically, by inhibition of cyclic AMP phosphodiesterase(s). This is the accepted mode of action of aminophylline, and as other data suggest, of dibutyryl cyclic AMP as well¹⁵.

Our data thus suggest that cyclic AMP plays a positive role in the first phase of an immune response, which is the commitment of an antigen-reactive cell to the expression of its specialised function. In the second phase, which begins around 24-36 h, proliferation occurs¹⁶. Since cyclic AMP seems to be antimitotic in lymphocytes as in other cultured cells^{17,18}, one should expect that inhibiting cyclic AMP phosphodiesterase activity at this time would inhibit the onset of proliferation. It has in fact been observed that the cyclic AMP levels in synchronised lymphocytes are highest in G1 and G2 phases, and at S phase and mitosis the levels are only about 20% of the G2 level¹⁷. This model is consistent with the day 2 and day 3 inhibitory effects that we observed. Other work suggests that the proliferative phase may depend

on elevated cyclic GMP levels^{11,18}, and that the two cyclic purine nucleotides vary inversely.

Watson *et al.*⁵ have demonstrated a time dependent, antigen independent inhibitory effect of dibutyryl cyclic AMP on immunocyte functioning. An interesting observation was that cyclic GMP reversed the cytotoxic effects of dibutyryl cyclic AMP. We also observed cytotoxic effects of incubating cells with dibutyryl cyclic AMP (Table 1) but

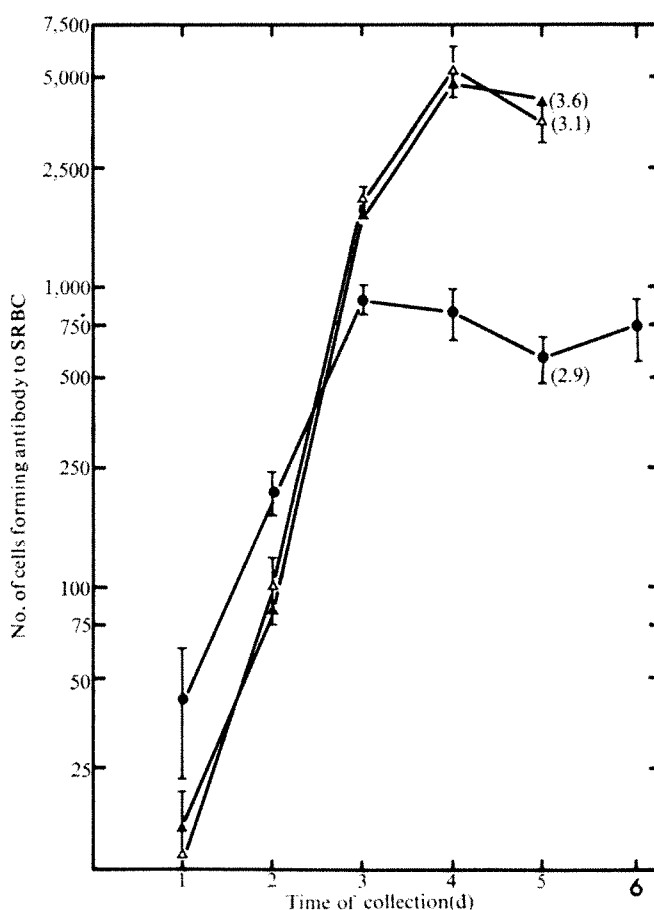


Fig. 2 Kinetic analysis of the effects of cyclic AMP phosphodiesterase inhibitors on the anti-sheep erythrocyte AFC response. Spleen cells were exposed to the agents indicated for the first 12 h in 60×15 mm Petri dishes. They were then transferred to the usual Diener-Marbrook culture flasks, and cultured in quadruplicate until the times indicated. Each culture was adjusted to contain 11×10^6 viable cells at the end of the 12 h period. Because of lower viability in treated cultures (Table 1), these represent 30-40% more starting cells than do the controls. The numbers in parentheses indicate the number of viable cells, in millions, per culture at day 5. Vertical bars give the standard errors of the means for the control and dibutyryl cyclic AMP treated cultures (the aminophylline treated values had similar standard errors to the latter) (●) untreated cells, given the same wash and incubation procedure; (Δ) cells treated with 1 mM dibutyryl cyclic AMP for 12 h first; (▲) cells treated with 1 mM aminophylline for the same period. Antigen was present throughout the culture period, including the first 12 h, in all flasks.

these were smaller than the effects reported by Watson *et al.*, perhaps because of differences in cells (C57BL/6 compared with CBA/J) or culture conditions. At any rate, they were not large enough to obscure our key finding, the antigen dependent stimulatory effect of cyclic AMP phosphodiesterase inhibitors at early times of immune induction. Preliminary experiments in our laboratory show that these agents act in a similar manner on a T cell independent antigen, the polymerised flagellin from *S. adelaide*. Our conclusions agree with those of Watson *et al.* inasmuch as the phase one (commitment) to phase two (proliferation) switch is inhibited by cyclic AMP, but add to them the important observation that an early enhancement of cyclic AMP increases the number of AFC observed at much later times. If the assumption that aminophylline and dibutyryl cyclic AMP elevate intracellular cyclic AMP levels is correct, the present work supports a model of primary immune induction in which a temporally limited rise in cyclic AMP is an early positive signal following antigen recognition. Immune paralysis might follow if the cells concerned were unable to reduce the pulse of cyclic AMP to levels consistent with cell cycling.

A preliminary report¹⁹ describes a similar pattern of effects of cyclic AMP enhancing agents in an *in vivo* immune response to sheep erythrocytes. In that system, an early rise in cyclic AMP appears to be followed by its return to normal levels by 1 h.

An inhibitory effect of exogenously added cyclic AMP on the anti-sheep erythrocyte response *in vitro* has been observed by others²⁰. We have not been able to affect the immune response in any way by adding up to 1 mM cyclic AMP itself to cell cultures. It is perhaps not surprising that at least under some conditions this nucleotide is without effect, since the half life of cyclic AMP in rat thymocyte cultures is less than 30 min, and no exogenously added cyclic AMP enters the cells²¹.

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- ¹ Braun, W., and Ishizuka, M., *J. Immun.*, **107**, 1036-1042 (1971).
- ² Uzunova, A. D., and Hanna, E. E., *Cell. Immun.*, **7**, 507-511 (1973).
- ³ Ishizuka, M., Braun, W., and Matsumoto, T., *J. Immun.*, **107**, 1027-1035 (1971).
- ⁴ Winchurch, R., and Actor, P., *J. Immun.*, **108**, 1305-1311 (1972).
- ⁵ Watson, J., Epstein, R., and Cohn, M., *Nature*, **246**, 405-409 (1973).
- ⁶ Smith, J. W., Steiner, A. I., Newberry, W. M., jun., and Parker, C. W., *J. clin. Invest.*, **50**, 432-441 (1971).
- ⁷ Parker, C. W., Smith, J. W., and Steiner, A. I., *Int. Arch. Allergy*, **41**, 40-46 (1971).
- ⁸ MacManus, J. P., Whitfield, J. F., and Youdale, T., *J. cell. comp. Physiol.*, **77**, 103-116 (1971).
- ⁹ Exon, J. H., Lewis, S. B., Ho, R. J., Robison, G. A., and Park, C. R., *Ann. N.Y. Acad. Sci.*, **185**, 85-100 (1971).
- ¹⁰ MacManus, J. P., Franks, D. J., Youdale, T., and Braceland, B. M., *Biochem. biophys. Res. Commun.*, **49**, 1201-1207 (1972).
- ¹¹ Hadden, J. W., Hadden, E. M., Haddox, M. K., and Goldberg, N. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3024-3027 (1972).
- ¹² Lee, K.-C., Langman, R. E., Paetkau, V. H., and Diener, E., *Eur. J. Immun.*, **3**, 306-309 (1973).
- ¹³ Diener, E., and Armstrong, W. D., *J. exp. Med.*, **129**, 591-603 (1969).

- ¹⁴ Cunningham, A. J., and Szenberg, A., *Immunology*, **14**, 599-600 (1968).
- ¹⁵ Heersche, J. N. M., Fedak, S. A., and Aurbach, G. D., *J. biol. Chem.*, **246**, 6770-6775 (1971).
- ¹⁶ Nakamura, I., Segal, S., Globerson, A., and Feldman, M., *Cell Immun.*, **4**, 351-366 (1972).
- ¹⁷ Millis, A. J. T., Forrest, G., and Pious, D. A., *Biochem. biophys. Res. Commun.*, **49**, 1645-1649 (1972).
- ¹⁸ McCrery, J. E., and Rigby, P. G., *Proc. Soc. exp. Biol. Med.*, **140**, 1456-1459 (1972).
- ¹⁹ Yamamoto, I., Shimamura, T., and Plescia, O. J., *Fedn Proc.*, **33**, 794 (1974).
- ²⁰ Bösing-Schneider, R., and Kolb, H., *Nature*, **244**, 224-225 (1973).
- ²¹ MacManus, J. P., Whitfield, J. F., and Braceland, B., *Biochem. biophys. Res. Commun.*, **42**, 503-509 (1971).

Plasma cell surface antigen on human blood lymphocytes

THERE is considerable evidence that lymphocytes bearing immunoglobulins on their surfaces are present in the peripheral blood of various animal species as well as man¹⁻³. Takahashi *et al.*⁴ indicated that neoplastic, as well as normal mouse plasma cells, contained specific surface alloantigens. These plasma cells do not, however, contain surface immunoglobulin determinants⁵. Harris *et al.*^{6,7} demonstrated that neoplastic plasma cells of the mouse could be used to prepare specific antisera against normal plasma cells. These anti-plasma cell sera (APS) were found to suppress specifically primary and secondary humoral immunity without apparently affecting cellular immunity. Recently, we established a long term culture of human plasma cells⁸ from a localised plasmacytoma tumour of a patient with multiple myeloma. The cultured cells morphologically resembled plasma cells, and they secreted an IgG immunoglobulin antigenically identical to the patient's myeloma protein. Using cells from this source, an anti-human plasma cell serum (HuAPS) has been prepared which recognises a surface antigen on human plasma cells.

Human plasma cells from the long term cell line⁸ were injected into rabbits in the same manner as in the prepara-

Table 1 Effect of APS on mitogenic response of human lymphocytes

<i>In vitro</i> assay	No. studied	c.p.m.	% reduction
Cells alone	8	1,298	
Cells and PHA	9	293,660	
Cells and PHA and APS	6	277,863	5
Cells and PWM	9	175,427	
Cells and PWM and APS	5	97,325	45

tion of rabbit anti-mouse plasma cell sera^{6,7}. The HuAPS was absorbed extensively with lymphoblast cell line RPMI 4098, which was chosen because it has been used for the preparation of anti-human lymphocyte globulin with potent T lymphocyte immunosuppressive activity⁹. Immunoabsorbents were used to purify the HuAPS further. The HuAPS was passed through an immunoabsorbent column prepared by conjugating human immunoglobulins to Sepharose 4B by cyanogen bromide activation.

Ten to twenty millilitres of venous blood was collected from each subject, all of whom were laboratory personnel. Ficoll-Hypaque density centrifugation, as modified from Boyum¹⁰, was used to prepare a relatively pure population of lymphocytes. In some cases, contaminating red cells were lysed by Tris-buffered ammonium chloride.

Human peripheral blood lymphocytes were cultured by the micro method of Hartzman *et al.*¹¹ using 2×10^5 lymphocytes per well in Falcon microtest plates. AB+ serum was used in a 25% concentration with HEPES-buffered RPMI 1640 media. Cells were either incubated

Table 2 Percentage of human blood lymphocytes with membrane antigens

Source of cells	IgG mean/range	IgM mean/range	IgA mean/range	Plasma cell Ag† mean/range
Normal human adults	14.8/6.1-29 (13)*	5.7/2-13 (8)	4.8/1-10 (8)	12 /7-18 (23)
Control <i>in vitro</i> culture	6.3/4-9 (5)	1.5/0-3 (4)	1.8/0-7 (5)	16.9/5-38 (9)
PHA <i>in vitro</i> culture	5.5/4-7 (4)	6.0/4-10 (4)	2.3/0-7 (4)	17.5/12-29 (7)
PWM <i>in vitro</i> culture	14.7/10-20 (5)	2.7/0-8 (5)	2 /0-7 (4)	39 /20-58 (9)

* Figures in parentheses indicate No. of individuals studied.

† Plasma cell surface antigen.

alone, with phytohaemagglutinin (Difco, PHA-P, No. 3110-56, 2 λ per culture); with pokeweed mitogen (Gibco, PWM, No. 536, 2 λ per culture), with PHA plus HuAPS, or with PWM plus HuAPS. The concentration of HuAPS was 2 λ per culture. This was observed to be noncytotoxic. After 4 d of growth, 1 μ Ci of 3 H-thymidine (specific activity 1.9 Ci mmol $^{-1}$) was added to each culture. Quintuplicate cultures from each donor were collected 4-6 h later using glass fibre filter paper precipitation. Filter disks were counted in a Packard Tri-carb liquid scintillation counter. The uptake of 3 H-thymidine was expressed as c.p.m. Table 1 shows that HuAPS had no effect on human peripheral blood lymphocyte response to PHA, a known T-lymphocyte mitogen^{12,13}. HuAPS, however, depressed the PWM response approximately 45%. This was significant for the following reasons. (a) PWM is a mitogen for both T and B lymphocytes. (b) Cells which appear morphologically to be plasma cells form after PWM stimulation. (c) Immunoglobulin synthesis is enhanced by PWM (refs 13, 14). Therefore, it seems likely that HuAPS in the study reported here was blocking a B lymphocyte response.

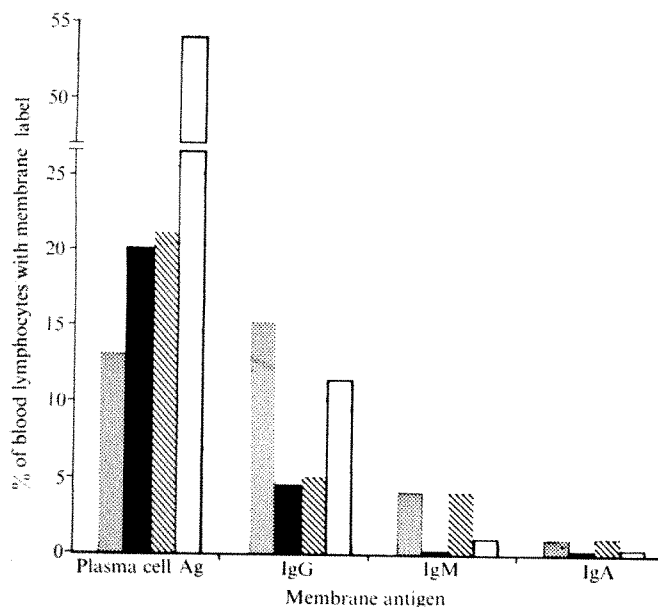


Fig. 1 The membrane staining characteristics of human peripheral blood lymphocytes and cultured lymphocytes from the same individual. Dotted columns, normal peripheral blood lymphocytes; solid columns, control *in vitro* culture; hatched columns, PHA *in vitro* culture; open columns, PWM *in vitro* culture.

Blood lymphocytes with membrane-bound immunoglobulins or with membrane-bound plasma cell antigen were detected by immunofluorescence¹. Cells with membrane-bound immunoglobulin were enumerated by using fluorescein-conjugated goat anti-human IgG, goat anti-human IgM and goat anti-human IgA (all from Cappel Laboratory). Before use, these conjugates were absorbed with cell line RPMI 4098 and with cultured plasma cells. These conju-

gated antisera were then diluted 1:2 and used to stain blood lymphocytes directly. Those used in double-labelling studies with the HuAPS were also absorbed with rabbit sera. For the enumeration of plasma cells, indirect fluorescent staining was used, with either fluorescein or rhodamine-conjugated goat anti-rabbit IgG (Cappel Laboratory). Both conjugated goat anti-rabbit preparations were purified by passage through an immunoabsorbent column conjugated with human serum, and by absorption with human peripheral blood lymphocytes.

To ensure that the HuAPS did not react with membrane-bound immunoglobulins, a blocking experiment was performed. The lymphocytes were incubated with unlabelled goat anti-human immunoglobulins before staining with rabbit anti-human plasma cell serum (HuAPS) and fluorescein-conjugated goat anti-rabbit IgG. In addition, double-labelling studies were performed in which the cells were treated sequentially with HuAPS, rhodamine-conjugated goat anti-rabbit IgG and fluorescein-conjugated goat anti-human IgG, IgM or IgA. Because of a possible peculiarity of the surface binding sites, the order of the procedure was reversed. Fluorescein-conjugated goat anti-human IgG, IgM or IgA was used first, followed by unlabelled HuAPS, and then by a rhodamine-conjugated goat anti-rabbit IgG.

The frequency of blood lymphocytes with membrane-bound immunoglobulins from normal individuals was similar to those reported previously^{2,15} (Table 2). It was also found that the number of lymphocytes with membrane-bound immunoglobulins in PHA or PWM-treated cultures either remained unchanged or decreased. Approximately 12% of peripheral blood lymphocytes had the plasma cell antigen. Ring staining and cap staining patterns were observed for both surface immunoglobulins and the plasma cell antigen. During the mitogenic response of human peripheral blood lymphocytes to PWM, there was an increase in the number of cells exhibiting the plasma cell surface antigen (Table 2). Figure 1 is a representation of an individual's typical membrane-staining distribution for immunoglobulins and plasma cell antigens on lymphocytes which were or were not exposed to mitogens.

The relative frequency of fluorescein-stained cells did not change when the cells were pretreated with unlabelled goat anti-human immunoglobulin. From the double-labelling studies, no cell was found with both the plasma cell surface antigen and the membrane-bound immunoglobulin. Therefore, I concluded that the plasma cell antigen did not cross react immunologically with surface immunoglobulin molecules. The blocking and double-labelling studies also support the concept that the plasma cell antigen is a distinct antigen on the surface of a significant number of peripheral blood lymphocytes; however, it is not known whether the antigen is expressed on nonlymphoid cells.

The observations of Takahashi *et al.*⁴ and Harris *et al.*^{6,7} led to the assumption that neoplastic human plasma cells could have antigens in common with normal human plasma cells. My study indicates that an anti-human plasma cell serum prepared against cultured human neoplastic plasma cells seems to have antibody activity against some normal human blood lymphocytes. (a) Stimulation with PWM, an agent which affects B and T lymphocytes^{13,14} was depressed

to the same extent that rabbit anti-mouse plasma cell sera modified the mouse system (unpublished observations), whereas, stimulation with PHA, a mitogen which affects primarily T lymphocytes^{12,13} was not affected. (b) Immunofluorescent examination showed that a population of blood lymphocytes had the plasma cell antigen, even though these cells do not resemble plasma cells morphologically. This is not surprising, however. Previous studies⁸ demonstrated that cultured plasma cells in spinner culture maintain the morphology of lymphoblasts. When transferred to Falcon tissue culture flasks, they assumed the typical configuration of plasma cells. Thus motion seemed to change the cultured plasma cell from a lymphoid appearance to a typical plasma cell morphology. Circulating plasma cells may not be recognised in multiple myeloma. Most patients examined had 90% or more plasma cells in their bone marrow. It is rare to find morphologically typical plasma cells in their peripheral blood, but metastatic plasma cell lesions are common.

As well as finding circulating blood lymphocytes with a plasma cell antigen, I have demonstrated that cells bearing the plasma cell antigen increased as a result of PWM stimulation. Since the number of immunoglobulin-bearing cells either decrease or remain the same in such cultures, it seems possible that the plasma cell surface antigen could develop as a consequence of stimulation of a certain population of B lymphocytes. This might suggest that the immunoglobulin-bearing cells are precursors of plasma cells. Future studies must examine the relationship between cytoplasmically stained immunoglobulin-containing cells and those cells bearing the plasma cell antigen on their surfaces. Another possibility is that plasma cell precursors migrate from the bone marrow into the peripheral blood. These blood-borne cells are then influenced to undergo further morphological changes and functional differentiation in the microenvironment of the peripheral lymphoid tissue and are consequently recognised as plasma cells.

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- ¹ Cooper, M. D., Lawton, A. R., and Bockman, D. E., *Lancet*, ii, 791 (1971).
- ² Papamichail, M., Brown, J. C., and Holbrow, E. J., *Lancet*, ii, 850 (1971).
- ³ Jondal, M., Holm, G., and Wigzell, H., *J. exp. Med.*, **136**, 207 (1972).
- ⁴ Takahashi, T., Old, L. J., and Boyse, E. A., *J. exp. Med.*, **132**, 1325 (1970).
- ⁵ Paraskevas, F., Lee, S., and Israels, L. G., *Nature*, **227**, 395 (1970).
- ⁶ Harris, N. S., McManus, D., Smolich, L., and Najarian, J. S., in *Proc. Sixth Leucocyte Culture Conf.* (edit. by Schwartz, M. R.), 375 (1972).
- ⁷ Harris, N. S., Jagarlamoodu, S. M., McKhann, C. F., and Najarian, J. S., *J. Immunol.*, **108**, 958 (1972).
- ⁸ Goldblum, R., Goldman, A. S., Rudloff, B., and Harris, N. S., in *Proc. Seventh Leucocyte Conf.* (edit. by Dauillard, F.), 15 (1973).
- ⁹ Najarian, J. S., Simmons, R. L., Gequrz, H., Moberg, A., Merkel, F., and Moore, G. E., *Ann. Surgery*, **4**, 617 (1969).
- ¹⁰ Boyum, A., *Scand. J. clin. lab. Invest. Suppl.*, **97**, 31 (1968).
- ¹¹ Hartzman, R. J., Segall, M., Bach, M. L., and Bach, F. H., *Transplantation*, **11**, 268 (1971).
- ¹² Abdou, N. I., and Abdou, N. L., *Science*, **175**, 446 (1972).
- ¹³ Greaves, M., and Janossy, G., *Transplant. Rev.*, **11**, 87 (1972).
- ¹⁴ Greaves, M., and Roitt, I. M., *Clin. exp. Immunol.*, **3**, 393 (1968).
- ¹⁵ Aiuti, F., Fontana, L., and Gatti, R. A., *Scand. J. Immunol.*, **2**, 9 (1973).

Linkage and rearrangement of genes encoding mouse immunoglobulin heavy chains

THE study of the genetic control of immunoglobulin synthesis has been greatly hampered by the difficulty of detecting individual genes encoding variable regions (*V* genes). The distinction by allotypy of several *V*-gene loci in the rabbit is useful, but genetic studies on this species have obvious disadvantages. The discovery in inbred mice of crossreactive idiotypes^{1,2} and of their polymorphic expression³⁻⁶ allows a new approach to the genetic analysis of mouse immunoglobulins. The results presented here suggest that idiotypes may serve to distinguish individual *V*-gene loci within the mouse heavy chain linkage group and to detect recombinations between them.

We have investigated linkage relationships between two mouse antibody idiotypes, termed ARS (ref. 3) and A5A (ref. 4), both of which are linked to the strain A/J *C_H* allotype Ig-1.e (refs 7-9). Idiotypic ARS is associated with antibodies to the *p*-azophenylarsonate hapten, produced in all A/J mice^{3,7} whereas idiotypic A5A is associated with antibodies to Group A streptococcal carbohydrate (A-CHO), produced in 94% of A/J mice^{4,8}. It is therefore proposed that strain A/J antibodies to these antigens are at least in part controlled by the *V_H* gene loci *ARS*+ and *A5A*+, respectively. Strain BALB/cJ (Ig-1.a) antibodies to both antigens lack these idiotypes^{7,8}. As was expected from the previously established linkage of each of the idiotypes to the same *C_H* allele^{7,9}, the results confirm that the two idiotypes are specified by linked genes.

We were fortunate to encounter a backcross mouse which was phenotypically suggestive of a recombination between A/J *V_H* genes and BALB/cJ *C_H* genes⁹. This permitted us to investigate whether or not *ARS*+ and *A5A*+ map at the same position within the A/J heavy chain linkage group. The backcross mouse, referred to as BB δ 7, was identified as the only A5A positive mouse among 16 Ig-1.a/a homozygous progeny from an (A/J \times BALB/cJ)F₁ \times BALB/cJ mating⁹. The association of the A5A idiotypic with the Ig-1.a allotype, suggesting a new linkage of *A5A*+ with Ig-1.a, has not been observed among more than 100 BALB/cJ mice, and has not been found again among 57 additional backcross segregants tested.

We ascertained the allotype and the presence or absence of two idiotypes in individual mice. Allotypes were determined

Table 1 Linked segregation of *A5A*+, *ARS*+ and Ig-1.e in (A/J \times BALB/cJ)F₁ \times BALB/cJ backcross mice

Mouse, Sex, No.	Ig-1	A5A*	ARS†
1. Litter			
♂1	a/a	—	—
♂2	a/e	++	+++
♂3	a/a	—	—
♂4	a/e	+++	+
♂5	a/e	+++	+
♀1	a/e	+	++
♀2	a/e	+++	++
♀3	a/e	+++	+
♀4	a/e	++	+
2. Litter			
♂1	a/a	—	—
♂2	a/e	++	++
♂3	a/a	—	—
♂4	a/a	—	—
♂5	a/a	—	—
♀1	a/a	—	—
♀2	a/e	+	++
♀3	a/e	+	++
♀4	a/a	—	—
♀5	a/e	+++	++

* — : <0.2 μ g A5A idiotypic ml⁻¹.

++ : 40–100 μ g idiotypic ml⁻¹.

+++ : 100–250 μ g idiotypic ml⁻¹.

++++ : >250 μ g idiotypic ml⁻¹.

† — : <50% inhibition by >5 μ l of antiserum

++ : >50% inhibition by 0.2 μ l of antiserum

+++ : >70% inhibition by 0.2 μ l of antiserum

++++ : >90% inhibition by 0.2 μ l of antiserum

by agar diffusion analysis using antisera prepared by cross immunisation between strains A/J and BALB/cJ (ref. 10), as previously described⁹. The determination of idiotypes ARS and A5A used indirect radioprecipitin inhibition tests, using rabbit anti-idiotypic antisera for ARS (ref. 3) and guinea pig anti-idiotypic antisera for A5A (ref. 8), respectively. Hyper-immune serum samples were tested for inhibitory capacity in at least three dilutions. Antibodies to A-CHO were determined by a modified Farr assay⁴ and the presence of antibodies to the *p*-azophenylarsonate group was determined by agar diffusion analysis⁸.

First we had to establish that A/J antibodies to phenylarsonate do not idiotypically crossreact with A/J antibodies to A-CHO and *vice versa*. Sixteen individual anti-phenylarsonate antisera were tested for their capacity to inhibit A5A idiotype binding and 19 individual anti-A-CHO antisera were tested for their capacity to inhibit ARS idiotype binding. Whereas each of these antisera was strongly inhibitory of its corresponding idiotype binding system, less than 10% inhibition was obtained in the heterologous idiotype binding systems, using up to 20 μ l of each undiluted antiserum. This shows the absence of idiotype cross reactivity between strain A/J antibodies to phenylarsonate and to A-CHO, respectively, and enabled us to determine both idiotypes in individual mice which were successively immunised with both antigens.

Table 2 Segregation of A5A+ in BB β 7 \times BALB/cJ progeny and its dissociation from ARS+

Mating, Sex, No.	Ig-1	A5A*	ARS*
BBβ7 \times BALB/cJ†			
1. Litter ♂1	a/a	—	—
♀2	a/a	+	—
♀1	a/a	—	+/-†
♀1	a/a	—	—
♀3	a/a	++	—
2. Litter ♂1	a/a	++	—
♂2	a/a	—	—
♂3	a/a	—	—
♂4	a/a	+	—
♀1	a/a	—	—
♀2	a/a	++	—
♀3	a/a	+++	—
♀4	a/a	—	—
BBβ1 \times BALB/cJ†			
♂1	a/a	—	n.t.
♂2	a/a	—	n.t.
♂3	a/a	—	n.t.
♂4	a/a	—	n.t.
♂5	a/a	—	—
♀1	a/a	—	—
♀2	a/a	—	n.t.
♀3	a/a	—	—
♀4	a/a	—	—
♀5	a/a	—	—

*See footnotes to Table 1.

†The BALB/cJ female was the same in both matings.

‡56% inhibition with 10 μ l of antiserum, doubtful typing.

In a second experiment, a number of (A/J \times BALB/cJ) F_1 \times BALB/cJ backcross mice, which will be referred to as ABB mice, were tested for allotype and for both idiotypes. ABB mice were bred in Cologne from A/J and BALB/cJ mice which were obtained from the Jackson Laboratory, United States. At 8–10 weeks of age the mice were bled and allotyped. Subsequently, the mice received one course of injections with Group A streptococci⁴. The anti-A-CHO antisera resulting from this immunisation were allotyped and then idiotyped in the A5A idiotype binding inhibition assay. After 8–10 weeks, the mice were immunised with KLH-*p*-azophenylarsonate as previously described³. The anti-phenylarsonate antisera resulting from this immunisation were allotyped and then sent to Chicago for idiotyping in the ARS idiotype binding inhibition assay. All experiments were done blind; the source and allotype

Table 3 Expression of ARS+ and A5A+ antibodies in various inbred strains

Strain	Ig-1	ARS	A5A
A/J	<i>e</i>	+	+
A/He*	<i>e</i>	+	+
AL/N	<i>d</i>	+	—
CAL-20†	<i>d</i>	+	—
RF/J	<i>c</i>	—	+

*These mice were immunised for A5A typing by M. Cramer, Basel Institute for Immunology.

†These mice were given to us by Drs M. Potter and E. Mushinsky, N.I.H.

of transferred serum samples were unknown to the investigator performing idiotype analysis.

Table 1 shows the results from allotyping and idiotyping 19 ABB mice. Among three successive allotype determinations on each mouse no discrepancy was observed, so that the allotypes can be taken as a true reflection of the C_H genotype for each mouse. All 11 *Ig-1.a/e* heterozygotes express ARS as well as A5A, whereas all eight *Ig-1.a/a* homozygotes express neither of the two idiotypes. This confirms the linkage between ARS+, A5A+ and *Ig-1.e* in the A/J heavy chain linkage group. The question remains as to whether ARS+ and A5A+ are controlled by a single gene or by different genes.

Therefore, in a third experiment, progeny of a BB β 7 \times BALB/cJ mating were allotyped and idiotyped in the same way as were the ABB mice. We included as a control another litter from the same BALB/cJ female sired by BB β 1, an *Ig-1.a/a* homozygous, but A5A negative (that is non-recombinant) brother of BB β 7 (ref. 9). The results are shown in Table 2. All of the 13 offspring from BB β 7 were *Ig-1.a/a* homozygous, confirming the *Ig-1.a/a* homozygosity of BB β 7. Six of the 13 mice clearly expressed idiotype A5A and thus had inherited the A5A+ - *Ig-1.a* haplotype of BB β 7. Seven of the 13 mice were A5A negative and had inherited the A5A- - *Ig-1.a* haplotype of BB β 7. In contrast, with one doubtful exception, all of the 13 mice were negative for the ARS idiotype, suggesting that the A5A+ - *Ig-1.a* haplotype does not contain the ARS+ gene. The data on the BB β 1 offspring confirm that the A5A+ - *Ig-1.a* haplotype was indeed inherited from BB β 7.

The results are consistent with the view that, in the heterozygous parent of BB β 7, A5A+ was separated by crossover from ARS+ as well as from *Ig-1.e*, and became linked by recombination to *Ig-1.a* of the BALB/cJ chromosome. In this case, ARS+ would map between A5A+ and *Ig-1* and the crossover had occurred between two distinct V_H gene loci. The alternative explanation is a mutation in the BALB/cJ V_H genes from A5A- to A5A+, without ARS- being affected. Such a mutation occurring in one cistron encoding both A5A- and ARS- V_H regions would be unlikely. Therefore, in the case of a mutational event, the data would be indicative of multiple V_H gene loci in strain BALB/cJ.

Though a mutational event is not ruled out, the case for two different germ line V_H gene loci for ARS+ and A5A+ is supported by the association of ARS+ and A5A+ antibodies with several distinct *Ig-1* genotypes (Table 3). Strain AL/N and its *Ig-1* congenic strain CAL-20 express ARS+ but do not express A5A+, whereas strains A/J and A/He express both idiotypes, and strain RF/J expresses A5A+ only¹¹. The separate as well as joint expression of these idiotype markers in different inbred strains, together with their dissociation in the BB β 7 progeny, strongly suggests that A5A and ARS map at different positions in the mouse heavy chain linkage group, and thus represent separate gene loci.

This seems to be the first demonstration of two distinct germ line V_H genes controlling distinct antibody specificities. The data suggest that the establishment of recombinant inbred strains from mice such as BB β 7, and their investigation with multiple idiotype markers, may reveal considerable information on the chromosomal arrangement of antibody genes.

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- ¹ Cohn, M., Notani, G., and Rice, S. A., *Immunochemistry*, **6**, 111-129 (1969).
- ² Potter, M., and Liebermann, R., *J. exp. Med.*, **132**, 737-754 (1970).
- ³ Kuettner, M. G., Wang, A. L., and Nisonoff, A., *J. exp. Med.*, **135**, 579-592 (1972).
- ⁴ Eichmann, K., *Eur. J. Immun.*, **2**, 301-307 (1972).
- ⁵ Sher, A., and Cohn, M., *Eur. J. Immun.*, **2**, 319-327 (1972).
- ⁶ Blomberg, B., Geckeler, W. R., and Weigert, M., *Science*, **177**, 178-180 (1972).
- ⁷ Pawlak, L. L., Mushinski, E. B., Nisonoff, A., and Potter, M., *J. exp. Med.*, **137**, 209-224 (1973).
- ⁸ Eichmann, K., *J. exp. Med.*, **137**, 603-621 (1973).
- ⁹ Eichmann, K., and Berek, C., *Euro. J. Immun.*, **3**, 599-601 (1973).
- ¹⁰ Herzenberg, L. A., McDevitt, H. O., and Herzenberg, L. A., *A. Rev. Genet.*, **2**, 209-238 (1968).
- ¹¹ Pawlak, L. L., and Nisonoff, A., *J. exp. Med.*, **137**, 855-869 (1973).

Interaction of paramyxovirus with erythrocyte membranes modified by concanavalin A

THE envelopes of paramyxoviruses have neuraminidase, haemagglutinating, haemolytic and fusion activities¹⁻⁶. The interrelation of these activities is poorly understood.

Burnet and Lind⁷ studied the interaction of Newcastle Disease virus with several species of mammalian and avian erythrocytes and found most of the cells susceptible to haemagglutination and haemolysis except horse erythrocytes, which agglutinated poorly and resisted haemolysis by the virus. Horse erythrocytes are also not susceptible to haemagglutination by Haemagglutinating Virus of Japan (HVJ: Sendai virus) (refs 8-10 and our own observations). It can be assumed that horse erythrocytes have no specific receptor for the viral haemagglutinin and/or haemolysin¹¹.

Yamakawa¹² reported that horse erythrocytes had no detectable amounts of N-acetyl-neuraminic acid either in the glycolipid fraction or in the residue extracted extensively with organic solvent. Instead, they were found to contain N-glycolyl-neuraminic acid exclusively. It is well known that erythrocytes from which the neuraminic acid residue is removed by the action of the so-called receptor destroying enzyme (neuraminidase) are completely resistant to agglutination and haemolysis by myxo and paramyxoviruses. These facts clearly indicate that the receptor for the viruses contains the neuraminic acid residue. N-glycolyl-neuraminic acid may not have a role as a virus receptor.

The initial interaction of virus with host cell membrane may be that between viral haemagglutinin and the receptor containing N-acetyl-neuraminic acid. It is not known whether this binding of the virus with host membrane is essential for the subsequent haemolysis or fusion. It would be of special interest, therefore, if HVJ proved to have haemolytic activity when the virus was artificially adsorbed on horse erythrocytes. In this report, we shall show that horse erythrocytes treated with concanavalin A (con A) are able to interact with HVJ and to be haemolysed.

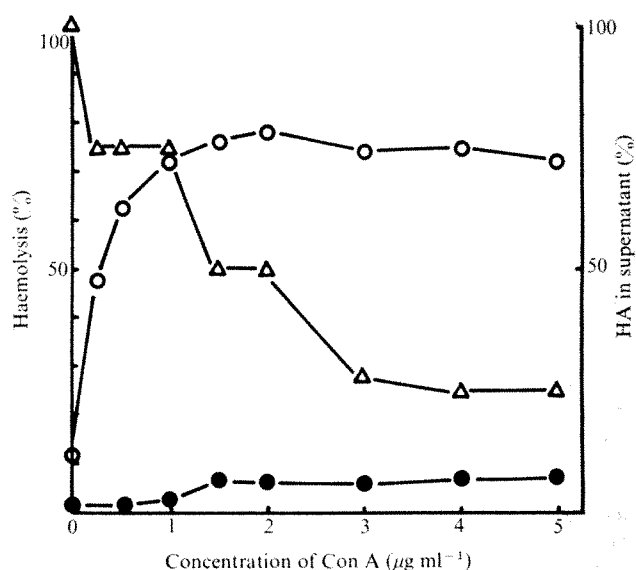


Fig. 1 Adsorption (Δ) and haemolytic activity (O) of HVJ to horse erythrocytes treated by various concentrations of con A. Aliquots of con A solution were mixed with equal volumes of 2% horse erythrocyte suspension in phosphate-buffered saline (pH 7.2). The concentration of con A added was expressed as final concentration. After the treatment with con A at 0° C for 4 h, whole mixtures were directly used without washing, since non-reacting free con A was not detected at the doses used through the present experiment. For the adsorption experiment, 0.1 ml of the virus suspension (4,000 HA per 0.5 ml) was mixed with 0.9 ml of intact and con A-treated horse erythrocytes. After incubating for 2 h at room temperature, HA titre remaining in the supernatant of centrifugation (1,500g, 5 min) was determined. For the haemolysis experiment, 0.5 ml of the same virus suspension (10,000 HA per 0.5 ml) was mixed with 0.5 ml of the same preparation of con A-treated cells as used in the adsorption experiment. After incubation of the mixtures for 2 h at 36° C, the optimum density of clear supernatant obtained by centrifugation (1,500g, 5 min followed by 80,000g, 15 min) was determined photometrically (at 541 nm). Control lysis of con A-treated horse erythrocytes without HVJ was also determined (—●—). Activities were expressed as a percentage of original HA titre or complete haemolysis obtained by hypotonic treatment.

HVJ was highly purified by differential centrifugation, sucrose density gradient centrifugation and finally by gel filtration through a Sepharose 2B column. Fractions in void volume were collected. The pooled fractions were finally concentrated in a reduced volume of phosphate-buffered saline by pelleting at 60,000g for 30 min. The viral sample was exposed to freezing-thawing two or three times to activate the haemolytic activity¹³. The treatment of horse erythrocytes with con A is described in Fig. 1. Horse erythrocytes were purchased as stored blood and used within 3 weeks. The haemagglutinating titre (HA) was routinely determined with chicken erythrocytes. The degree of adsorption of HVJ on con A-treated horse erythrocytes was estimated by titration of the HA remaining in the supernatant after the incubation mixture was centrifuged at low speed. An aliquot of the suspension of con A-treated horse erythrocyte (0.9 ml) was incubated with 0.1 ml of HVJ (4,000 HA per 0.5 ml) for 2 h at room temperature. Haemolytic activity of the virus was assayed as follows: an adequate dilution of HVJ and the same volume of con A-treated horse erythrocytes were mixed and incubated at 36° C for 2 h. After the incubation, nonhaemolysed cells were removed by sedimentation at 1,500g for 5 min. The supernatant thus obtained was further centrifuged at 80,000g for 15 min to remove viral particles. Haemolysis was measured by reading optical density at 541 nm of the final supernatant. For the inhibition experiment of virus-induced haemolysis by sugars, the mixture containing con A-treated horse erythrocytes

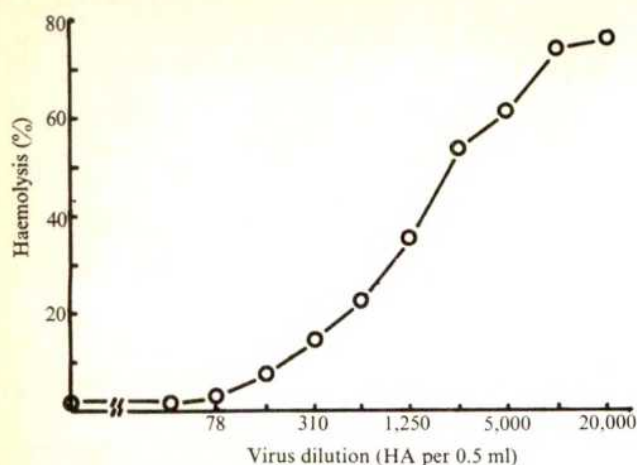


Fig. 2 Haemolysis of con A-treated horse erythrocytes by varying titres of HVJ. Viruses (32,000 HA per 0.5 ml) were diluted with the phosphate-buffered saline by twofold serial dilution of 0.5 ml. Horse erythrocytes were treated with con A at the final concentration of $1 \mu\text{g ml}^{-1}$. Suspensions of con A-treated horse erythrocytes (0.5 ml) were added to each dilution of the virus and haemolysis was assayed by the same procedure as in Fig. 1.

and a monosaccharide was preincubated at 4°C for 30 min. Adequate dilutions of HVJ were added to the mixture and further incubated at 36°C for 2 h. The haemolysis was assayed as described above. A plasma membrane fraction was prepared from intact and con A-treated horse erythrocytes by hypotonic haemolysis. The membrane pellets finally obtained were resuspended in a small volume of phosphate buffered-saline. For the electron microscopic observation, samples were negatively stained with phosphotungstate (pH 6.0).

The adsorption of HVJ on con A-treated horse erythrocytes and the subsequent haemolysis are shown in Fig. 1.

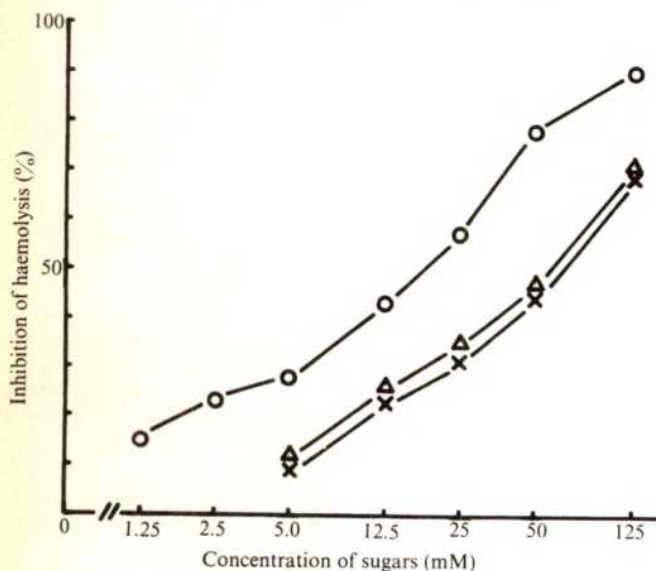


Fig. 3 Inhibitory effect of monosaccharides on haemolysis of con A-treated horse erythrocytes by HVJ. Horse erythrocytes were treated with con A at the final concentration of $1 \mu\text{g ml}^{-1}$ as described in the legend to Fig. 1. Con A-treated cells (0.5 ml) were mixed with 0.25 ml of solutions containing various amounts of (○), α -methyl-D-mannoside; (△), α -methyl-D-glucoside; (×), D-mannose. After incubating the mixtures at 4°C for 30 min, 0.25 ml of HVJ suspension (10,000 HA per 0.5 ml) was added. Haemolysis was assayed after incubating 2 h at 36°C . The degree of inhibition was expressed as a percentage of the haemolysis without sugars.

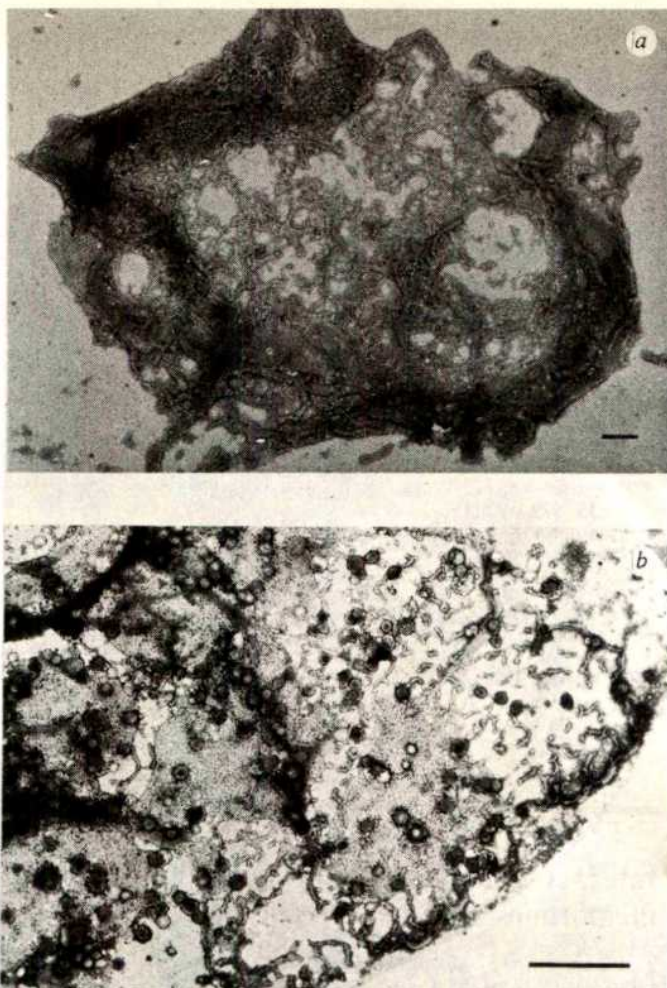


Fig. 4 Electron microscopic observation of adsorption of HVJ on plasma membrane derived from intact and con A-treated horse erythrocytes. Con A-treated horse erythrocytes (final concentration of con A: $5 \mu\text{g ml}^{-1}$) were prepared as described in the legend to Fig. 1. Plasma membranes were prepared by the following procedure. Ten millilitres of intact and con A-treated horse cells were diluted in 30 ml of distilled water and sedimented at $1,500g$ for 5 min. The pellets obtained were washed three times with phosphate-buffered saline and were resuspended in 0.1 ml of phosphate-buffered saline. The suspension was mixed with 0.1 ml of HVJ (5,000 HA per 0.5 ml) and the mixture was incubated for 30 min at room temperature. After washing three times with phosphate-buffered saline to remove non-adsorbed virus particles, the samples were stained with phosphotungstate (pH 6.0). a, Plasma membrane from an intact horse cell; b, a con A-treated horse cell. Scale: 1,000 nm.

Intact horse erythrocytes showed negligible interaction with HVJ and were not susceptible to viral haemolysis. With the increase in con A concentration, the HA titre remaining in the supernatant decreased, which indicated that more viruses were adsorbed on the cells. The susceptibility of cells to haemolysis increased in parallel with the increase of adsorption of the virus on the con A-treated cells. The degree of haemolysis reached a plateau at $1 \mu\text{g ml}^{-1}$ con A although higher concentrations of con A caused more HVJ to adsorb on to the cells. The haemolysis was also shown to be dependent on virus doses. The maximum haemolysis was obtained at a titre of 10,000 HA per 0.5 ml (Fig. 2). The control haemolysis of con A-treated horse erythrocytes was almost negligible without HVJ even at the highest concentration of con A (Fig. 1). These results suggest that the haemolysis may be due to the direct action of the HVJ virion on the erythrocyte membrane. Influenza viruses which had no haemolytic activity showed almost the same ability to

bind to con A-treated horse erythrocytes as HVJ, although the cells did not reveal detectable haemolysis. This fact may exclude the possibility that con A treatment makes the cells fragile and enhances nonspecific lysis due to the attachment of viral particles.

The effect of con A on horse erythrocytes described above was shown to be influenced by typical inhibitors of the plant agglutinin (Fig. 3). α -Methyl-D-mannoside showed the most potent inhibitory activity against the virus-induced haemolysis, while α -methyl-D-glucose and D-mannose showed much weaker inhibitory activity. It seems that the inhibition of haemolysis by a monosaccharide results from blocking the adsorption step of virus on to blood cells, since the degree of adsorption of the viruses was affected by those inhibitors (not shown in the figure). The pattern of inhibition by sugars shown here is almost the same as those reported for the aggregation of dextran by con A¹⁴. The reversibility of con A action on the blood cell membranes was also confirmed.

The adsorption of HVJ on con A-treated horse erythrocytes was examined under the electron microscope (Fig. 4). Plasma membranes of con A-treated horse cells could adsorb the viruses on the cell surface whereas those of the intact cells could not. The HVJ virion, once it had interacted, could not be removed even by washing extensively by phosphate-buffered saline. Haemolysis of con A-treated horse erythrocytes by the virus showed a similar characteristic to the haemolysis observed in the system of chicken erythrocytes and HVJ (manuscript in preparation).

The mechanism of the interaction between con A-treated horse erythrocytes and HVJ still remains to be clarified. HVJ may be bound to con A-treated horse erythrocytes through a con A bridge, since con A has polyvalent binding sites in its molecule¹⁵ and virions of HVJ have receptors for con A¹⁶. Alternatively, membranes of horse erythrocytes may acquire some new binding sites for the virus or just reveal buried receptors by combining with con A. This is plausible since con A modifies several membrane functions and structures¹⁷⁻¹⁹. Recent studies in which HVJ was fractionated to subcomponents suggested that the viral haemolysin might be on different molecules from the viral haemagglutinin and neuraminidase. These studies failed, however, to show whether the interaction of the virus with host membranes through the receptor for the haemagglutinin and/or neuraminidase is essential for the expression of haemolysis²⁰.

Our results suggest that haemolysis induced by HVJ does not require the binding of viruses to the specific receptors such as sites for haemagglutinin or neuraminidase on cell surface. Even when HVJ was adsorbed on erythrocytes by a different mechanism the cells were haemolysed

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¹ Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. exp. Med.*, **88**, 503-514 (1948).

² Kilham, L., *Proc. Soc. exp. Biol. Med.*, **71**, 63-66 (1949).

³ Fukai, K., and Suzuki, T., *Med. J. Osaka Univ.*, **6**, 1-15 (1955).

⁴ Okada, Y., Suzuki, T., and Hosaka, Y., *Med. J. Osaka Univ.*, **7**, 707-717 (1957).

⁵ Jhonson, C. F., and Scott, A. D., *Proc. Soc. exp. Biol. Med.*, **115**, 281-285 (1964).

⁶ Kohn, A., *Virology*, **26**, 228-245 (1965).

⁷ Burnet, F. M., and Lind, P. E., *Aust. J. exp. Biol. Med.*, **28**, 129-150 (1950).

⁸ Kuroya, M., Ishida, N., and Shiratori, T., *Virus*, **3**, 323-332 (1953).

⁹ Fukumi, H., and Nishikawa, F., *Jap. J. med. Sci. Biol.*, **7**, 354-363 (1954).

¹⁰ Jensen, K. E., Minuse, E., and Ackermann, W. W., *J. Immun.*, **75**, 71-77 (1955).

¹¹ Yamakawa, T., *J. Biochem.*, **43**, 867-874 (1956).

¹² Yamakawa, T., *Lipoide* (edit. by Schutte, C. E.), 87-111 (Springer, New York, 1966).

¹³ Hosaka, Y., *Biken's J.*, **1**, 70-89 (1958).

¹⁴ Goldstein, I. J., Hollerman, C. E., and Smith, E. E., *Biochemistry*, **4**, 876-883 (1965).

¹⁵ Kalb, A. J., and Lustig, A., *Biochim. biophys. Acta*, **168**, 366-367 (1968).

¹⁶ Okada, Y., and Kim, J., *Virology*, **50**, 507-515 (1972).

¹⁷ Nasipitz, C. K., and Richter, M., *Progr. Allergy*, **12**, 1-65 (1968).

¹⁸ Peter, J. H., and Hansen, P., *Eur. J. Biochem.*, **19**, 502-508 (1971).

¹⁹ Edelman, G. M., *Science*, **180**, 830-840 (1973).

²⁰ Hosaka, Y., and Shimizu, Y., *Virology*, **49**, 627-639 (1972).

Replication of *Escherichia coli* requires DNA polymerase I

For many years DNA polymerase I was the only known *E. coli* enzyme with the ability to synthesise DNA. It was, therefore, assumed by many to be the enzyme required for DNA replication. Doubts concerning this assumption were taken seriously only when DeLucia and Cairns¹ isolated a viable *E. coli* mutant with less than 1% of the normal polymerase I activity in *in vitro* tests. The conjecture that polymerase I might not be involved at all in replication but only functions in DNA repair processes was soon superseded by the observation that polymerase I is involved in the joining of Okazaki pieces^{2,3}. But the question whether DNA polymerase I is essential for replication or whether it is dispensable enzyme remained open. To investigate whether DNA polymerase I is essential for replication, we attempted the isolation of conditional lethal mutations in the gene coding for polymerase I.

Among many DNA polymerase I mutants of *E. coli* we have isolated one (BT 4113) which is conditional lethal at elevated temperature because of a temperature-sensitive DNA polymerase I. A bacterial culture was mutagenised by N-methyl-nitrosoguanidine and screened for *pol A* mutants using a new replica plating procedure in which microcolonies growing on membranes are tested after lysis *in situ* for their ability to synthesise DNA or to degrade DNA exonucleolytically. This method, which was originally developed for the large-scale isolation of *dna* temperature sensitive mutants will be described in detail elsewhere. Approximately

Table 1 Temperature dependence of polymerising and 5'-exonuclease activity

Strain	Polymerising activity (pmol TTP incorporated μ l ⁻¹)		Assay for 5'-Exonuclease activity (pmol ³² P released)	
	30°C	45°C	30°C	45°C
W 3110	44	140	0.20	0.33
BT 4109	18.3	1.79	0.27	0.31
BT 4113	9.3	0.72	0.16	0.05

DNA polymerase I was partially purified and assayed as described by Lehman and Chien⁴. Sucrose-gradient fractions were prepared and assayed for both polymerase and 5'-exonuclease activity. The polymerising activity was determined at pH 7.5 using calf thymus DNA as a template. The incubation was preceded by a 15-min preincubation under identical conditions. The 5'-exonuclease activity was determined by degradation of 5'-³²P-dTfA over 5 min as described by Lehman and Chien⁴. The activity measured is sensitive to DNA polymerase I antiserum (data not presented).

40,000 lysed colonies from this culture could be conveniently tested for their polymerase I activity at elevated temperature. Those lysed colonies unable either to synthesise DNA at 45° C in the presence of N-ethylmaleimide (NEM) or to degrade DNA exonucleolytically in the presence of NEM at 45° C were detected by autoradiography and the corresponding colonies were isolated from the replica. NEM inhibits many enzymes including DNA polymerase II and III but not polymerase I.

Among 40,000 bacterial colonies of *E. coli* W 3110 (ref. 1) we found 12 mutants with measurable defects in polymerase I activity, some having as little as 1% of wild-type polymerase activity. As expected¹, the mutants were sensitive to methylmethane-sulphonate. Some mutants had a temperature-sensitive DNA polymerase. The temperature dependence of polymerising and 5'-exonuclease activity for two strains is shown in Table 1. The polymerising activity of both strains was temperature sensitive. The 5' exonuclease activity seemed normal in BT 4109 and was temperature sensitive in BT 4113. When exposed to high temperature, bacteria of strain BT 4113 formed snakes and failed to form colonies. BT 4113 is the only conditional lethal mutant we have found.

DNA polymerase I is known to be involved in sealing of Okazaki pieces^{2,3}. According to sedimentation analysis of newly synthesised DNA the mutants tested varied in their ability to join Okazaki pieces, some (for example BT 4004) being almost as efficient as wild type, some (for example BT 4100 being grossly defective in sealing and some (for example BT 4113) being grossly defective in sealing only at elevated temperatures. In the latter strain there was almost no joining observed at 45° C even after 15 min, whereas joining at low temperature seemed nearly normal.

Transduction experiments with strain BT 4113 showed that both the conditional lethality and the temperature sensitivity in sealing are co-transducible with the *met E* marker at high frequency (about 10%). The transduction has been performed in both directions; BT 4113 has been transduced by P1 to become temperature resistant and *met E*⁻ and the *met E*⁻ strain JW 149 has been transduced to become *met E*⁺ and temperature sensitive.

We have not yet found a mutant to be temperature sensitive in the polymerising part only, and to be conditional lethal because of that mutation. The isolation and characterisation of BT 4113 and of a similar conditional lethal *pol A* strain by Conrad and Lehman⁴ show that *E. coli* bacteria require the 5'-exonucleolytic activity of DNA polymerase I for replication and that this function of polymerase I cannot be carried out by other enzymes in the cell.

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¹ DeLucia, P., and Cairns, J., *Nature*, **224**, 1164 (1969).

² Kuempel, P. L., and Veomett, G. E., *Biochem. biophys. Res. Commun.*, **41**, 973 (1970).

³ Okazaki, R., Arisawa, M., and Sugino, A., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2954 (1971).

⁴ Lehman, I. R., and Chien, J. R., *J. biol. Chem.*, **248**, 7717 (1973).

⁵ Conrad, E. B., and Lehman, I. R., *Proc. natn. Acad. Sci. U.S.A.* (in the press).

Affinity labelling the acceptor site of the peptidyl transferase centre of the *Escherichia coli* ribosome

We have identified two 50S proteins, L2 and L26-L27, in the peptidyl (P) site of the peptidyl transferase centre of *E. coli* ribosomes¹. BrAc-³H-Phe-tRNA^{Phe}, a peptidyl-tRNA affinity analogue, was shown to bind specifically to the P site and covalently react with only L2 and L26-L27. The ability of the same molecules of ribosome-bound BrAcPhe-tRNA^{Phe} to participate in dipeptide formation and covalent attachment to ribosomal proteins was the most compelling evidence for functional P site binding.

De Groot *et al.*² showed how peptidyl-tRNAs can be directed to either the P site or the aminoacyl (A) site. For example, purified Gly³-Phe-tRNA^{Phe} binds to the P site of 70S, salt-washed *E. coli* ribosomes in a cell-free system using poly (U) and either 10 mM or 30 mM Mg²⁺. This binding was not inhibited by tetracycline. The bound peptidyl-tRNA was very reactive towards puromycin. In contrast, when excess deacylated tRNA^{Phe} was added together with the peptidyl-tRNA, the distribution between the two sites was different. The greater part of the peptidyl-tRNA was now bound to the A site. This binding was inhibited by tetracycline, and puromycin reactivity of the bound peptidyl-tRNA was almost completely lost. It could be restored by adding translocation factor EF-G and GTP. Apparently, the

Table 1 Binding of BrAc-³H-Phe-tRNA

(a) Binding of BrAc-³H-Phe-tRNA to *E. coli* ribosomes (fmol)

Additions	Salt-washed ribosomes		Non-washed ribosomes	
	10 mM Mg ²⁺	30 mM Mg ²⁺	10 mM Mg ²⁺	30 mM Mg ²⁺
None	74	132	142	141
Tetracycline	123	121	134	133
400 pmol tRNA	47	113	98	124
400 pmol tRNA and tetracycline	34	73	45	91

(b) Puromycin reaction after binding of BrAc-³H-Phe-tRNA (fmol released)

Additions	Salt-washed ribosomes		Non-washed ribosomes	
	10 mM Mg ²⁺	30 mM Mg ²⁺	10 mM Mg ²⁺	30 mM Mg ²⁺
None	88	76	100	98
400 pmol tRNA	16	26	38	23
400 pmol tRNA + EF-G + GTP	—	91	65	98

tRNA^{E.coli}Phe enriched in phenylalanine acceptor activity was prepared by one fractionation of crude *E. coli* tRNA on a BD-cellulose column. This tRNA could accept about 50 pmol per A₂₆₀ of ³H-phenylalanine 60 Ci mmol⁻¹ and served also as uncharged tRNA in the specified reactions. *E. coli* ribosomes and BrAcPhe-tRNA were prepared as described previously¹¹. Salt-washed ribosomes were prepared according to Ertel *et al.*¹⁵ Binding assays were performed by the Millipore filter technique of Nirenberg and Leder¹⁶. A 20 µl binding reaction mixture contained 50 mM Tris-HCl (pH 7.6), 50 mM NH₄Cl, 3.0 A₂₆₀ of ribosomes, 20 µg of poly (U) plus magnesium acetate as indicated. Deacylated tRNA and tetracycline (0.5 mM) were added at 0° C before the addition of 240 fmol (11,000 c.p.m.) BrAc-³H-Phe-tRNA. The mixture was incubated for 30 min at 37° C. Puromycin reaction was determined as described by Leder and Bursztyn¹⁷. Where indicated EF-G (12 µg) and GTP (0.5 mM) were added after the initial incubation. The puromycin reaction in this case was measured after an additional 10 min incubation.

Table 2 Incorporation of ^3H -Phe into ribosomal proteins

	1.D	Run 1 2.D	Run 2 1.D
P site 10 mM Mg^{2+}			
Mg protein applied to gel	0.54	1.80	0.84
c.p.m. ^3H in protein L2	350 (0.076)	1,050 (0.073)	160 (0.27)
L16	75 (0.016)	246 (0.017)	180 (0.030)
L26-L27	2,900 (0.630)	9,840 (0.686)	1,130 (0.189)
P site 30 mM Mg^{2+}			
Mg protein applied to gel	—	—	1.20
c.p.m. ^3H in protein L2	—	—	60 (0.005)
L16	—	—	90 (0.007)
L26-L27	—	—	770 (0.064)
A site			
Mg protein applied to gel	0.45	1.50	0.98
c.p.m. ^3H in protein L2	130 (0.041)	420 (0.039)	170 (0.028)
L16	465 (0.145)	1,375 (0.129)	450 (0.075)
L26-L27	830 (0.260)	5,200 (0.488)	480 (0.080)

The amount of radioactivity found associated with certain ribosomal proteins was determined from the analysis of one-dimensional and two-dimensional polyacrylamide gels as described previously^{1,14}. After each ^3H c.p.m., in parentheses, is shown the corrected covalent c.p.m. ^3H incorporated per equivalent of tRNA bound per mg of protein analysed on the gel. In run 1, P site conditions actually represent 88% P site, A site represent 75% A site as determined by puromycin reactivity. In run 2, the corresponding figures are 71% P site and 75% A site.

deacylated tRNA^{Phe} could compete effectively with the peptidyl-tRNA for binding to the P site, but much less so for binding to the A site. This effect was much more pronounced at 30 mM Mg^{2+} . Watanabe and Tanaka reached similar conclusions³. AcPhe-tRNA could be directed specifically to the A site at 15 mM Mg^{2+} if the ribosomes had been preincubated with excess unfractionated deacylated tRNA.

We have used these methods to direct BrAc- ^3H -Phe-tRNA to either the P site or A site by using tRNA^{Phe} *E. coli*, and by varying the Mg^{2+} concentration. Table 1 shows that BrAc- ^3H -Phe-tRNA behaves similarly in every way to Gly₃-Phe-tRNA and AcPhe-tRNA in a poly(U)-directed cell-free system. This is true for saltwashed and unwashed ribosome preparations. BrAc- ^3H -Phe-tRNA in 10 mM Mg^{2+} and the absence of deacylated tRNA, bound almost exclusively to the P site. It was not inhibited by tetracycline. Puromycin reactivity of the bound tRNA was generally 70% or higher. In the presence of deacylated tRNA and 30 mM Mg^{2+} , however, binding seemed to be mostly to the A site. This binding was inhibited by tetracycline. Puromycin reactivity was reduced by a factor of 3 to 4. It could be restored fully by adding EF-G and GTP.

Covalent labelling reactions were done under similar conditions to those used previously¹. Unwashed ribosomes were used to facilitate comparison with earlier results and because their overall binding was generally higher than saltwashed ribosomes. Two different systems were chosen. (1) P site binding in the presence of 10 mM or 30 mM Mg^{2+} and the absence of deacylated tRNA. (2) A site binding in the presence of 30 mM Mg^{2+} and deacylated tRNA.

Subunit separation and stripping of ribosomal proteins were done as described previously¹. Most of the covalent ^3H -Phe incorporation occurred with 50S proteins. The small extent of reaction with the 30S particles will be discussed elsewhere. One-dimensional and two-dimensional acrylamide gel electrophoreses were performed to identify as unambiguously as possible any covalently labelled 50S proteins. Typical gel patterns are given in Figs 1 and 2.

Only three 50S proteins contained significant covalently incorporated ^3H -Phe. These were L2, L26-L27 and L16. Table 2 shows that the pattern of radioactive incorporation into the three proteins was markedly affected by the conditions used. The good agreement between parallel one-dimensional and two-dimensional analyses on the same sample encourages quantitative comparisons. The P site

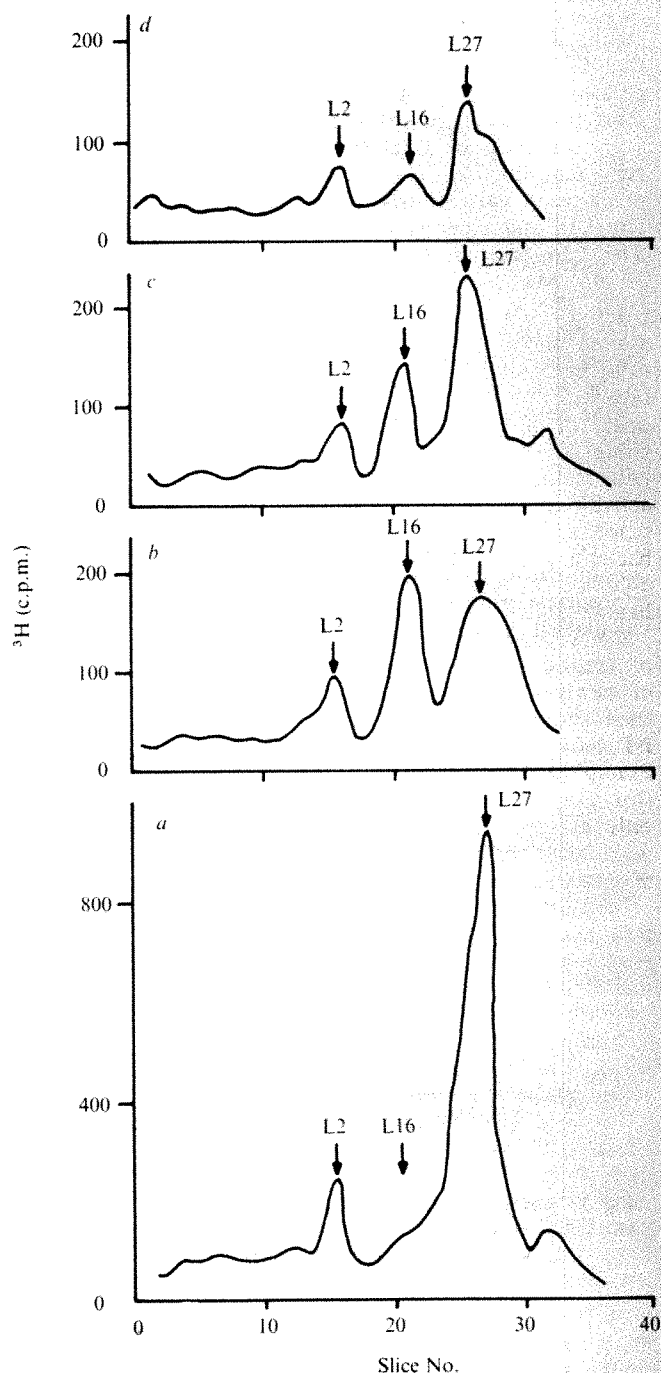


Fig. 1 BrAc- ^3H -Phe-tRNA-reacted 70S ribosomes were separated into subunits and the ribosomal proteins were analysed by one-dimensional gel electrophoresis as described earlier¹⁴. Labelling patterns from one-dimensional gels are shown for 50S proteins from: (a) P site reaction mixture; (b) A site reaction mixture; (c) A site reaction mixture in the presence of 0.5 mM tetracycline; (d) A site reaction mixture in the presence of 1 mM puromycin. Total protein put on the gel was 0.54 mg in (a), 0.45 in (b), 0.48 in (c), and 0.30 mg in (d). Labelling reactions contained 20 mg of ribosomes with 24 pmol (1.1×10^6 c.p.m.) of BrAc- ^3H -Phe-tRNA and 2 mg of poly (U) in the presence of 10 mM Mg^{2+} for P site binding, and 30 mM Mg^{2+} and 1 mg of uncharged tRNA for A site binding. The concentrations of Tris buffer and NH_4Cl were the same as given in Table 1. After incubation for 30 min at 37°C, 20 μl aliquots were taken and checked for binding and puromycin reactivity in the presence and absence of EF-G and GTP. Where indicated either tetracycline (0.5 mM) or puromycin (1 mM) were added to the A site reaction mixtures before the addition of BrAc- ^3H -Phe-tRNA. It was found that 8,520 c.p.m. of BrAc- ^3H -Phe-tRNA were bound to a sample of 0.2 mg ribosomes from the P site reaction mixture as measured by Millipore binding assay¹⁸. 7,450 c.p.m. (88%) of this sample could react with puromycin. The binding to an equivalent amount of ribosomes from the A site reaction mixture was 7,130 c.p.m. of which only 1,760 c.p.m. (25%) were puromycin reactive.

results are quite comparable to previous results¹, except that L2 is less reactive with this particular ribosome preparation. Similar variations have been seen before. For the same preparation of ribosomes and BrAcPhe-tRNA, however, the ratio of L2:L26-L27 in a P site experiment is very reproducible. The A site conditions with the same preparation gave a striking increase in reaction with protein L16 and a decrease in reaction with L2 and L26-L27. The L16 reaction is not simply due to the presence of 30 mM Mg²⁺ as shown in Table 2. In the absence of deacylated tRNA, even with 30 mM Mg²⁺, the binding of BrAcPhe-tRNA is mostly to the P site (Table 1). Only when excess deacylated tRNA is added does BrAcPhe-tRNA move to the A site with a concomitant rise in the L16 reaction.

One reason why the pattern of BrAcPhe-tRNA reactivity shown in Table 2 is complex is that P site and A site conditions still lead to binding to both sites. If puromycin reactivity is used as a measure of true P site binding, P site conditions still allow 12–29% A site binding while A site conditions allow 25% P site binding. These cross-contaminating binding modes can be corrected for by the appropriate simple algebraic manipulation. This permits calculation of the relative efficiencies of reaction of a pure

inhibition of the binding of the aminoacyl end of BrAcPhe-tRNA to the A site would explain the diminished L16 reaction.

Our assignment of L16 as an A site protein may clarify other recent affinity labelling studies. Several lines of evidence^{6–9} indicate that chloramphenicol and puromycin compete for the same binding site on the 50S particle, the A site. Sonnenberg *et al.*¹⁰ observed a covalent reaction of the chloramphenicol analogue, bromamphenicol, with two proteins, L2 and L27. Pongs *et al.*¹¹ found that another analogue, moniodoamphenicol, reacted covalently with a single 50S protein, L16. Reconstitution experiments demonstrated that L16 was required for chloramphenicol binding¹². One explanation for this discrepancy is the known occurrence of two ribosomal binding sites for chloramphenicol¹³. The iodoamphenicol results^{11,12}, however, are consistent with our identification of L16 as exclusively an A site protein. L2 L26-L27 can react with BrAc-³H-Phe-tRNA bound at both the A and P sites. This is consistent with the bromamphenicol results¹⁰. It appears that proteins L16, L2, L26-L27 are all located at the peptidyl transferase centre and are probably quite close to each other.

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- ¹ Oen, H., Pellegrini, M., Eilat, D., and Cantor, C. R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2799 (1973).
- ² de Groot, N., Panet, A., and Lapidot, Y., *Eur. J. Biochem.*, **23**, 523 (1971).
- ³ Wantanabe, S., and Tanaka, K., *Biochem. Biophys. Res. Commun.*, **45**, 728 (1971).
- ⁴ Zagorska, L., Dondon, J., Lelong, J. C., Gros, F., Grunberg-Manago, M., *Biochemie.*, **52**, 63 (1971).
- ⁵ Lessard, J. L., and Pestka, S., *J. biol. Chem.*, **247**, 6901 (1972).
- ⁶ Celma, M. L., Monro, R. E., and Vazquez, D., *FEBS Lett.*, **13**, 247 (1971).
- ⁷ Pestka, S., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 709 (1969).
- ⁸ Pestka, S., *Arch. Biochem. Biophys.*, **136**, 80 (1970).
- ⁹ Lessard, J. L., and Pestka, S., *J. biol. Chem.*, **247**, 6909 (1972).
- ¹⁰ Sonnenberg, N., Wilchek, M., and Zamir, A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1423 (1973).
- ¹¹ Pongs, O., Bald, R., and Erdmann, V. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2229 (1973).
- ¹² Nierhaus, D., and Nierhaus, K. H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2224 (1973).
- ¹³ Pestka, S., *J. biol. Chem.*, **247**, 4669 (1972).
- ¹⁴ Pellegrini, M., Oen, H., and Cantor, C. R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 337 (1972).
- ¹⁵ Ertel, R., Brot, N., Redfield, B., Allende, J. E., and Weissbach, H., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 861 (1968).
- ¹⁶ Nirenberg, M., and Leder, P., *Science*, **145**, 1399 (1964).
- ¹⁷ Leder, P., and Bursztyn, H., *Biochem. Biophys. Res. Commun.*, **25**, 233 (1966).

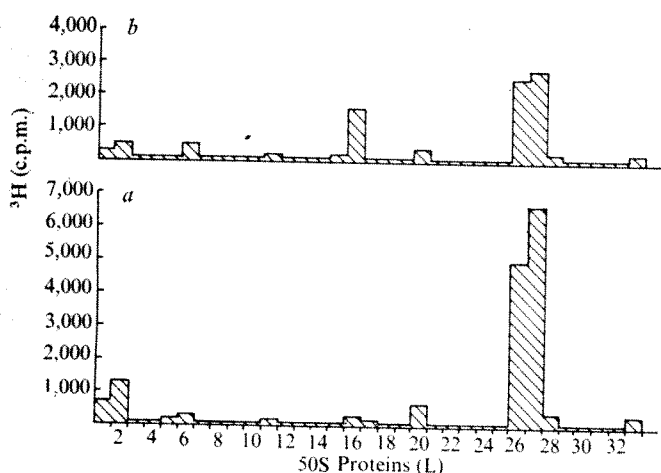


Fig. 2 50S protein samples from reaction mixtures (a) and (b) (see Fig. 1), were analysed by two-dimensional gel electrophoresis as described previously¹.

A or P site bound species with each of the three reactive 50S proteins. Such calculations show that virtually all the small L16 reactivity seen under P site conditions can be accounted for by contaminating A site-bound material. Therefore, L16 is definitely an A site protein. It is vastly more reactive when BrAcPhe-tRNA is bound in this site than in the P site.

L26-L27 and L2 appear to be P site proteins. The reactive sites of L26-L27 and L2 may be reached from both A and P site bound BrAcPhe-tRNA, but the higher efficiency from the latter suggests that they are much closer to the P site or the reaction is sterically more favourable.

Figure 1 shows the effect of 0.5 mM tetracycline and 1 mM puromycin on the pattern of covalent labelling under A site conditions. Tetracycline, a known inhibitor of A site binding⁴ diminished the reaction of L16 by a factor of 2. A much smaller effect was seen with L2 and L26-L27. Puromycin which releases any P site-bound peptidyl-tRNA reduced the reaction with L16, L26-L27 and L2. The marked reduction of L16 reaction needs to be explained since 1 mM puromycin is not likely to release any A site-bound peptidyl-tRNA. Lessard and Pestka⁵ have shown that puromycin competition appreciably reduced the binding of aminoacyl oligonucleotides to ribosomes. A similar

Identification of a population of mouse leukocytes using wheat germ agglutinin

A GROUP of proteins occurring commonly in plant extracts have the property of agglutinating blood cells by intercellular linkage of surface carbohydrate groups^{1,2}. These proteins, usually called lectins, are often able to distinguish cell types by preferentially agglutinating cells which express a particular membrane glycoprotein³. We report here that one lectin, wheat germ agglutinin (WGA)⁴, is able to distinguish a group of mouse leukocytes carrying neither immunoglobulin nor T-cell surface markers.

Cells teased from spleens of normal BALB/c mice were washed with Eagle's minimum essential medium containing 10% foetal bovine serum. Suspensions containing 10^7 cells ml^{-1} were treated with increasing concentrations of fluorescein-conjugated WGA and the number of labelled cells counted immediately using a Leitz Ortholux ultraviolet microscope with incident illumination. The results are shown in Fig 1. A consistent proportion of spleen cells was found to be strongly labelled at WGA concentrations of between 20 and $100 \mu\text{g ml}^{-1}$. Staining was eliminated either by previous addition of 0.025 M N-acetyl glucosamine (NAG) to the cell suspension, or by subsequent washing of the cells with Eagle's medium containing 0.05 M NAG. In contrast, washing with 0.1 M NAG had no effect upon spleen cells previously labelled with another fluorescein-conjugated lectin, concanavalin A.

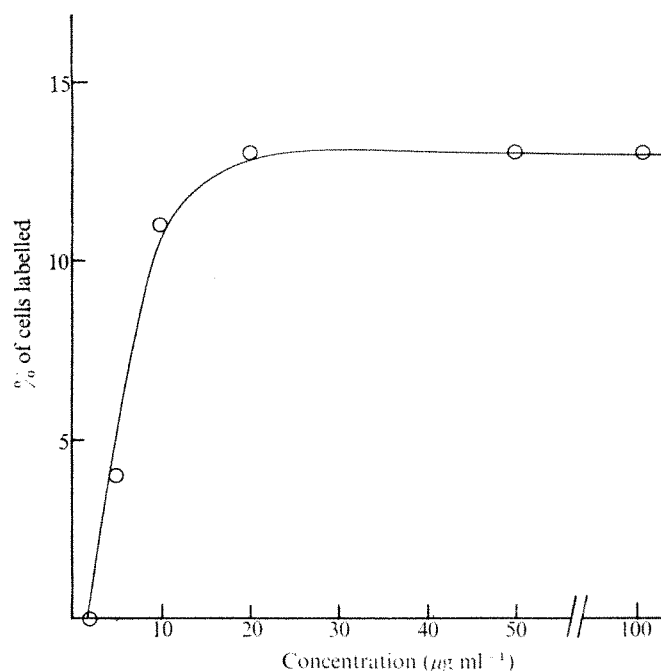


Fig. 1 Proportion of mouse spleen cells labelled with increasing concentrations of fluorescein-conjugated WGA.

Table 1 shows the proportions of cells from other mouse lymphatic tissues having receptors for WGA.

The morphology of this cell population was investigated by electron microscopy. Mouse spleen cells were incubated for 5 min at 4°C with a $250 \mu\text{g ml}^{-1}$ concentration of WGA/horseradish peroxidase conjugate⁵ in phosphate buffered saline. The cells were then washed, fixed and treated with diaminobenzidine and osmium tetroxide before embedding and sectioning. Cells with heavy surface labelling were characterised as monocytes and polymorphonuclears, many with peroxidase-positive cytoplasmic granules. Lymphocytes and plasma cells showed very weak or no staining. Further details of these experiments will be published elsewhere.

To see whether cells with abundant WGA receptors carried immunoglobulin or T-cell markers the following experiment was performed. Aliquots of 2×10^6 cells were treated with either 100 μl of a 1:4 dilution of rabbit anti-mouse immunoglobulin serum or with 100 μl of rabbit anti-mouse brain serum, sequentially absorbed with erythrocytes, liver and B-spleen (spleen from irradiated animals repopulated with bone marrow cells) to render it specific for T lymphocytes⁶. All cells were subsequently

Table 1 Percentage of leukocytes in mouse tissues labelled with WGA ($50 \mu\text{g ml}^{-1}$)

Spleen	Lymph node	Thymus	Blood	Bone marrow
12	<0.5	<0.5	7	72

stained with rhodamine B-conjugated goat anti-rabbit immunoglobulin serum. The cells were then washed thrice and labelled with 100 μl of a $50 \mu\text{g ml}^{-1}$ solution of fluorescein-conjugated WGA. Of the 12% of cells in each sample labelled with wheat germ agglutinin, none showed rhodamine fluorescence indicative of either immunoglobulin or T-cell surface markers. Conversely, T cells were not stained with WGA. Although WGA did not stain immunoglobulin-bearing cells under these conditions, at lectin concentrations greater than $100 \mu\text{g ml}^{-1}$, both immunoglobulin-bearing cells and erythrocytes showed weak fluorescence.

This is consistent with the findings of Schnebli and Dukor⁷ that mouse B cells are agglutinated more strongly than cortisone-resistant thymocytes at low concentrations of WGA. Wheat germ agglutinin has also been shown to agglutinate certain malignant cells⁸. Our results show that mouse leukocytes in lymphoid organs and blood which have abundant binding sites for WGA are essentially of myeloid morphology and do not carry the surface markers of B or T lymphocytes.

We thank Dr A. K. Allen for his gift of purified WGA, Dr C. Maino for a number of other lectins used in this study and Miss M. Gyöngyösy for rabbit anti-mouse brain serum. This work was supported by the Medical Research Council.

Note added in proof: In contrast with resting mouse lymphocytes, lymph node cells transformed with phytohaemagglutinin or concanavalin A were found to bind WGA to a moderate degree.

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¹ Mäkelä, O., *Annls Med. exp. Biol. Fenn.*, **35**, Suppl. 11 (1957).

² Lis, H., and Sharon, N., *A. Rev. Biochem.*, **42**, 541-574 (1973).

³ Blumberg, S., Hildesheim, J., Yariv, J., and Wilson, K. J., *Biochem. biophys. Acta.*, **264**, 171-176 (1972).

⁴ Allen, A. K., Neuberger, A., and Sharon, N., *Biochem. J.*, **131**, 155-162 (1973).

⁵ Huet, C., and Garrido, J., *Expl Cell Res.*, **75**, 523-527 (1972).

⁶ Gyöngyösy, M. I. C., and Playfair, J. H. L., *Cell. Immun.*, **7**, 118-123 (1973).

⁷ Schnebli, H. P., and Dukor, P., *Eur. J. Immun.*, **2**, 607-609 (1972).

⁸ Burger, M. M., and Goldberg, A. R., *Proc. natn. Acad. Sci. U.S.A.*, **57**, 359-366 (1967).

Adjuvant activity in delayed hypersensitivity of the peptidic part of bacterial peptidoglycans

It is generally accepted that the presence of killed mycobacteria in complete Freund's adjuvant is necessary to induce a typical delayed hypersensitivity to soluble antigens¹ and attempts have been made to isolate the active component. The preparations obtained initially were of a peptidogly-

Intact peptidoglycan from

- ¹ Nelson, D. S., and Boyden, S. V., *Int. Arch. Allergy appl. Immun.*, **25**, 279-303 (1964).
- ² White, R. G., *Br. Med. Bull.*, **23**, 39-45 (1967).
- ³ Adam, A., Ciorbaru, R., Petit, J. F., and Lederer, E., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 851-854 (1972).
- ⁴ Hiu, I. J., *Nature new Biol.*, **238**, 241-242 (1972).
- ⁵ Migliore-Samour, D., and Jolles, P., *FEBS Lett.*, **25**, 301-304 (1972).
- ⁶ Nauciel, C., Fleck, J., Martin, J. P., and Mock, M., *C.r. hebdom. Séanc. Acad. Sci. Paris*, **276**, 3499-3500 (1973).
- ⁷ Nauciel, C., Fleck, J., Martin, J. P., Mock, M., and Nguyen-Huy, H., *Eur. J. Immun.* (in the press).
- ⁸ Nauciel, C., Fleck, J., Mock, M., and Martin, J. P., *C.r. hebdom. Séanc. Acad. Sci. Paris*, **277**, 2841-2844 (1973).
- ⁹ Migliore-Samour, D., and Jolles, P., *FEBS Lett.*, **35**, 317-321 (1973).
- ¹⁰ Adam, A., Ciorbaru, R., Ellouz, F., Petit, J. F., and Lederer, E., *Biochem. biophys. Res. Commun.*, **56**, 561-567 (1974).
- ¹¹ Leskowitz, S., Jones, V. E., and Zak, S. J., *J. exp. Med.*, **123**, 229-237 (1966).
- ¹² Nauciel, C., and Raynaud, M., *Eur. J. Immun.*, **1**, 257-262 (1971).
- ¹³ Alkan, S. S., Nitecki, D. E., and Goodman, J. W., *J. Immun.*, **107**, 353-358 (1971).
- ¹⁴ Becker, M. J., Levin, H., and Sela, M., *Eur. J. Immun.*, **3**, 131-135 (1973).
- ¹⁵ Schleifer, K. H., and Kandler, O., *Arch. Mikrobiol.*, **57**, 335-364 (1967).
- ¹⁶ Martin, J. P., Fleck, J., Mock, M., and Ghuyssen, J. M., *Eur. J. Biochem.*, **38**, 301-306 (1973).
- ¹⁷ Fleck, J., Martin, J. P., Monteil, H., and Mock, M., *C.r. hebdom. Séanc. Acad. Sci. Paris*, **274**, 1856-1859 (1972).
- ¹⁸ Fleck, J., Mock, M., Minck, R., and Ghuyssen, J. M., *Biochim. biophys. Acta*, **233**, 489-503 (1971).
- ¹⁹ Bricas, E., Ghuyssen, J. M., and Dezelee, P., *Biochemistry*, **6**, 2598-2607 (1967).
- ²⁰ Schleifer, K. H., and Kandler, O., *Bact. Rev.*, **36**, 407-477 (1972).

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book reviews

Quality quantified

Social Stratification in Science. By Jonathan R. Cole and Stephen Cole. Pp. xiv+283. (University of Chicago: Chicago and London, 1973). £5.65.

SINCE 1967 the Coles have been addressing themselves to a set of interesting problems concerning the internal social organisation of science, using as principal tool the *Science Citation Index*. The number of citations received is taken to be a measure of the quality of scientific work. Those who disdainfully dismiss this way of measuring quality should read the discussion of its reliability and validity in chapter 2 of the book. Despite the admitted drawbacks—notably the fact that important discoveries may become accepted to the extent that the original papers stop being cited—it emerges from the scrutiny as a reasonable indicator.

The Coles' major concern is the degree to which the reward system of science operates by universalistic criteria. How far do inequalities in the rewards received reflect quality of work, rather than particular attributes like sex or race or being related to the right people? Is the homage which conventional rhetoric pays to universalistic ideals better matched in practice by science than by other social institutions? The answer is reassuring. Science—which here means mostly American physics—emerges in a favourable light. For receipt of honorary awards, for visibility and for appointments in prestigious university departments, quality of research is found to be a key determinant.

Women are not much discriminated against—once they are over the Ph.D. hurdle. The same limitation effectively removes from the field of study the question of discrimination against blacks, because they receive so few Ph.Ds (less than one per cent of those given in science). The restricted scope of the Coles' study is exposed by their revealing use of the expression "social origins" to refer to the rank of the scientist's doctoral department (page 117). It is only for those who have got inside the system that its inequalities can be seen to be equitably distributed.

Is there a serious departure from universalism in what R. K. Merton has called "the Matthew effect" ("For unto every one that hath shall be given"—Gospel according to St Matthew)? The Coles test the consequences of such an

assumption for the diffusion of knowledge and conclude that the effect is rarely important. Controlling for assessed quality at time 2, the assessed quality of papers at time 1 is only slightly influenced by the reputation already achieved by their authors.

The Coles also test what they call "the Ortega hypothesis", after an assertion by Ortega y Gasset that the many scientists who are of no more than mediocre quality nevertheless contribute in a major way to scientific progress. The work that physicists use in their best papers, as indicated by the references they cite, is itself found to be produced largely by elite physicists. This does not support the hypothesis and leads to the suggestion, which may antagonise some readers, that the number of active physicists could be cut substantially without crippling progress.

Science as studied by the Coles is defined in a narrow way. Only the publication of research papers counts; even review articles are excluded. Teaching students or administering research establishments or advising governments is "unproductive"; so is improving the productivity of industry. The "quality" of a company or government agency is determined by its commitment to basic research (page 43). Social responsibility in science is outside this impregnable circle of definitions—so far outside that the idea that "rather than society influencing science, science influences society" is described as one that "some of the more polemical historians of science have gone so far as to argue" (page 14).

The most immediate danger arising from work like that described in this book is that statistical measures adequate for fair-sized samples may be misapplied to individual cases. Within a few pages of a warning about this (page 31), the authors themselves are teetering on the brink. In general, however, they remain clearly aware of the limitations of their approach and they use their tools with care as well as ingenuity. They have done a useful exercise in the art of the possible. Like it or not, citation counts will doubtless continue to be used as well as misused, and the Coles' work itself is likely to be plentifully cited. The smokescreen of sociological jargon in their book is refreshingly thin and the style is lucid, considering the high density of correlation and regression coefficients.

F. R. JEVONS

Ignorance of interferon

Interferon: Theory and Applications. By V. D. Solov'ev and T. A. Bektemirov. Translated from Russian by Basil Haigh. Pp. xvii+304. (Plenum: London and New York, 1974.) \$30.

THERE have been several international meetings on interferon during the past few years, but unfortunately Russian workers have not been able to attend any of them—often having to withdraw at the last moment. Since the Soviet Union has also had few visitors from the West, this has led to the isolation of the Russian workers and the effects of this isolation are only too apparent in this present book.

The book claims, in its foreword, "to give a systematic account of the existing information on interferon obtained both from the extensive literature, and from the authors' own observations made over a period of several years..." The book falls sadly short in its first aim—that is to give a systematic account of the existing information on interferon. First of all, it is out of date. This is obvious as soon as one looks at the extensive list of references given at the end of the volume. The first hundred and ninety refer to Russian papers published up to 1967, the next four hundred to papers by non-Russian authors (only three being later than 1967), while the final hundred are made up of more recent Russian publications and Western papers, but only twenty odd are dated 1970 or later. This is presumably because the Russian text was published in 1970, but it is a pity that the text was not updated before translation. The book shows its age in other ways too—the account of interferon purification is nearly ten years old and data presented on the molecular weight and amino acid composition of interferon is quite incorrect. The translational-inhibiting protein theory of Marcus and Salb is restated although it was abandoned years ago. Isaacs's theory that interferon induction is due to 'foreign' nucleic acid is described, although Isaacs himself later withdrew it. Nagano's inhibitory factor is described as an interferon, despite Fantes's (1966) careful analysis of the results, showing that the inhibitory factor is almost certainly a polysaccharide. There is no account of the interferon standardisation meeting in London in 1969, nor of the development of

reference research standards for interferon.

The other aim of the book is to describe Russian work hitherto unpublished in English. This falls into two main areas—measurement of interferon production by different viruses in a variety of cells and the use of interferon in man. The interferon production experiments are presented at great length and quite uncritically, and their total effect is merely to demonstrate that many factors are responsible for the control of interferon formation. The work on the role of interferon in infection and its use in clinical trials in the Soviet Union is more interesting, and is the only justification for translating the book into English. I am much less competent to assess this data, which seems to me to be interpreted rather optimistically.

In summary, this is not a book to purchase or to recommend for library purchase, unless one has an interest in learning of Russian work on the role of interferon in natural infections.

D. C. BURKE

Technology of insect flight

Insects in Flight. By Werner Nachtigall. Translated by Harold Oldroyd, Roger H. Abbott, and Marguerite Biederman-Thorson. Pp. 150; 32 plates. (Allen and Unwin: London, May 1974.) £5.50.

MUCH that is new has been learned about insect flight in the past fifteen years, and Werner Nachtigall has been one of the most productive workers in this field. He has published a long series of papers in German which provide a detailed analysis of wing movements in the blowfly in free flight, by the use of highly sophisticated experimental methods. A few years ago he conceived the idea of writing a book about the flight of insects which would illustrate the thrills and frustrations of research, and form a tribute to the beauty and ingenuity of the technology of insect flight. The book has now been translated into English and appears in well printed and beautifully illustrated form.

The author writes for students and teachers with interests in technology and engineering, for those engaged in piloting gliders or powered aircraft, and indeed for the general reader. He is wholly successful in putting across the biophysics of the flying insect—though he expects his readers to concentrate! He describes the aerodynamics of flight in terms of instantaneous linear forces and avoids the more demanding aerodynamical approach: bound vortices and the generation of the aerodynamic cross force are passed over and Reynolds numbers appear only in a single caption. The story is varied and enlivened

by literary excerpts; by excursions into locust plagues; the rôle of flight in the life of the honey bee and other insects; insect migration; fuel, temperature and sensors in flight; the technological rivalry between sonar hunting bats and acoustically equipped moths; the contrasts and comparisons of insect and human flight—and much more besides.

The author has a gift for vivid depiction: "to a fly, a cine film must look like a lantern lecture, with long dark pauses in between the slides". The book is admirably translated but in at least one place the popular error in translating 'Schmetterling' leads to the description of the hawk-moth *Celerio* as a 'nocturnal butterfly'.

V. B. WIGGLESWORTH

Exploring evolution

Evolution in the Microbial World. (24th Symposium of the Society for General Microbiology, held at Imperial College London, April 1974.) Edited by M. J. Carlike and J. J. Skehel. Pp. x+430. (Cambridge University: London, April 1974.) £7.50; \$22.50.

THIS volume, in the words of the editors, is believed to represent the first account devoted to the topic of microbial evolution. Such an embracing title would be expected to cover a very wide range of topics indeed and, although there is a great deal of information in this book which bears on the evolution of microbes, it would be useful to indicate what the book does not contain. It does not include, and does not claim to cover, aspects of comparative microbial anatomy nor a discussion of phylogeny based on such evidence. In fact, no indication is given of the wide range of structural diversity found in microbes. Similarly, there is little reference to the possible route of evolution of major groups of microorganisms.

Instead, the emphasis of the text is placed, not upon the apparent path of evolution, but on those special properties of microorganisms which make them such favourable experimental material for exploring the mechanisms of evolution. The sequence of chapters is essentially hierarchical, starting with problems of taxonomy and phylogeny and leading to essays dealing with mutation, recombination and selection. Successive chapters deal with the evolution of molecules, with organisms, and with energy-yielding processes as well as with the evolution of a biological association (symbiosis).

The first chapter (by P. H. A. Sneath) is a lucid summary of current strategies for constructing the phylogenetic relationships of microbes and includes a full discussion of taxonomic principles. The following four chapters deal

with aspects of the evolutionary mechanism. Drake, for example, discusses the role of mutation in evolution and the measurement of mutation rates. Richmond and Wiedeman discuss plasmids in relation to bacterial evolution. Although the significance of plasmid-borne resistance factors is stressed, the basic theme of the essay is recombination mechanisms involving plasmids and their possible similarities to recombination in phages. The wider role of recombination in regulating breeding systems and the formation of new species is considered by Esser. Kubitschek summarises some of the material concerning selection pressures on bacteria grown in the environment of a chemostat.

One of the most powerful analytical techniques yet applied to the study of phylogenetic relationships is the technique of doublet analysis, by estimating the frequency of nearest-neighbour base sequences in DNA. Subak-Sharpe *et al.* give a full account of their studies on the reliability of the technique as applied to the DNA of bacteria and viruses. Experimental approaches to the problems of the evolution of enzymes and the way in which specificity of action is related to enzyme activity are discussed by Hartley. A similar theme is reiterated by Clarke, who summarises her work on the nature of adaptation of bacteria exposed to novel substrates in terms of the evolution of the requisite enzymes needed to utilise the substrate.

Three chapters are devoted to the evolution of energy-yielding processes: photosynthesis (Stanier), sulphur metabolism (Peck) and nitrogen fixation (Postgate). These essays do not merely summarise the salient features of the relevant biochemistry but also discuss the possible origins and evolution of such mechanisms in prokaryotes and eukaryotes. The remaining chapters are devoted to patterns of organisation and association among microbes. As examples of the evolution of organisms two divergent levels of organisation are considered: viruses (Joklik, Skehel) and the haemoflagellate *Trypanosoma* (Baker). The nature of symbiosis and mutualism are discussed by Lewis. Finally, Ponnamburama and Gabel discuss the possible condition of the prebiotic milieu in relation to the formation of complex molecules and the emergence of life.

As a summary of current thinking on problems of evolution among microbes this volume contains much to interest the reader. More importantly, the essays included succeed in demonstrating the feasibility of using microorganisms to unravel some basic aspects of the evolutionary mechanism.

I. D. J. BURDETT

Mouths of waders

Feeding and the Feeding Apparatus in Waders: A Study of Anatomy and Adaptations in the Charadrii. By P. J. K. Burton. Pp. 150. (British Museum (Natural History): London, 1974.) £3.50.

DURING recent decades workers may have been somewhat deterred from studying the Charadriiformes by the documentation provided in "Witherby" and by the detailed knowledge possessed by amateur ornithologists but there is now evidence of a widespread resurgence of interest. This present book, a quite admirable discussion of functional morphology, considers charadrioid bill structure and oral musculature from a variety of viewpoints.

The information on overt feeding behaviour which is contained in the opening sections includes many original observations alongside a useful panoramic survey culled from the world literature. I was particularly interested in the statements about *Pluvialis squatarola* as they contrast with the data obtained by Dr G. A. Parker and myself. The remainder of the book deals in detail with the degrees of rhynchokinesis, and the variations in bill, tongue and hyoid structure, which are associated with different methods of feeding. In particular Dr Burton emphasises that highly rhynchokinetic upper jaws have probably evolved independently in at least seven lines of Charadriodea. The interdependence of modifications for a particular method of feeding is certainly widely known but this account provides many further examples.

Written with great clarity, the book will be a valuable addition to specialist libraries and encourage a continuing synthesis of morphology and ethology. One can only express passing regret that, although the work was initially supported by the Scientific Research in Schools Committee of the Royal Society, the author gave up his teaching post to complete it. Surely this was not the intention behind the committee? Britain needs able and enthusiastic teachers.

RONALD PEARSON

Seabed skeletons

Recent Sedimentary Carbonates. Part 1: Marine Carbonates. By J. D. Milliman. Pp. xv + 375 + 39 plates. (Springer-Verlag: Berlin and New York, 1974.) DM66; \$27.10.

This book is an excellent attempt to synthesise present knowledge about calcium carbonate in the marine environment, its composition, sedimentation and diagenesis. It is apparent that Milliman has used his experience

gained in researches over a broad spectrum of the field of Recent sediments and also a wealth of data from the latest literature to put together this comprehensive textbook, which will appeal to final year undergraduates, research students and established carbonate sedimentologists.

The book is divided into four parts: introduction, carbonate components, marine carbonate sedimentation and carbonate diagenesis. Part 1 includes a valuable introduction to the techniques employed in the analysis of such parameters as texture, petrography, mineralogy and elemental and stable isotope composition. This practical aspect is supplemented by the two appendices, which, although very brief, do establish a starting point for the identification, in thin section and under reflected light, of the major carbonate components of Recent marine sediments. The major portion (part 2) of the book synthesises an extensive literature on the ecology, calcification, petrography and composition of various skeletal and non-skeletal carbonate components. This section includes many quantitative data on topics including growth rates, mineralogies, minor trace element and stable isotope distributions which will serve as a useful reference for marine sedimentologists. The third part of the book deals with the distribution of marine carbonates. Milliman does not concentrate on a few shallow tropical sea environments, which is sensible, for this particular setting is treated in considerable depth in other recent books, but instead he gives equal attention to the shallow seas, shelf waters and the deep sea. The final part traces the diagenetic alteration of carbonates within the marine environment through degradation, cementation and dolomitisation.

The style is clear and the presentation straightforward. One of the most useful and original features of the book is the plentiful tabulation of quantitative and descriptive data from a wide variety of sources. In this way such information as chemical composition of skeletons, distribution of major planktonic foraminifera species, and depositional environments and chemical properties of modern marine dolomites are succinctly expressed for easy comparison and assimilation. The photographs are arranged in plates which on the whole are clear and appropriate, though for ease of reading I would have preferred photographs interspersed with the text. Several photographs are too small to serve much value and the general standard of plates is not the excellent quality one has come to expect from Springer-Verlag geological publications.

It is inevitable that this book will be compared with the recent comprehensive textbook on carbonate sediments and their diagenesis by Bathurst.

Though the themes of the two books are similar the contents overlap to a relatively small degree (as an indication only 375 references are common to the two books which have a total of over 2,000 references). This partly results from the different experiences and approaches of the two authors and partly from the inclusion in Milliman's book of many references to works published during the past three years.

T. P. SCOFFIN

Explaining solid state

Electronic Properties of Crystalline Solids: An Introduction to Fundamentals. By Richard H. Bube. Pp. xiii + 524. (Academic: New York and London, January 1974.) \$35; £16.80.

THE solid state physics contained in this book is, at first sight, fairly traditional, both as regards subject matter and treatment. On closer examination, however, it becomes apparent that Dr Bube has made a useful and significant addition to the range of text books now available to third year undergraduate and first year postgraduate students specialising in solid state physics. The author clearly intended to explain the main elements of the subject within a sound mathematical framework. He wisely resisted the temptation to discuss many aspects superficially and instead concentrated on taking a few examples of solid state phenomenon and treating them in some depth. Reviewers who judge a book by what has been left out may, therefore, find the choice of material not to their liking; I, for example, would like to have seen a little more on metallic alloys. But these are matters for personal judgment and should not be allowed to detract from a well written and beautifully produced textbook.

There are twelve chapters in the book which cover the three principal themes of solid state physics. The first three chapters focus attention on wave theory and the application of quantum mechanics to simple systems. The examples here were carefully chosen with an eye to later chapters. The next three chapters develop the band theory of solids taking as a starting point the free electron (Hartree) model. The reader will, if he works through these chapters and the excellent range of problems associated with each of them, gain a good understanding of basic band theory. I was particularly pleased to see a discussion of 'bands' versus 'bonds' and that the concept of electronegativity is at last appearing in solid state physics textbooks. The role of localised energy levels leading to the characteristic behaviour of semiconductors is described, a shade too briefly perhaps, in chapter 9. The remainder of the

book deals with the motion and excitation of carriers by external fields and includes a particularly helpful discussion of photoelectron effects. The book is well indexed, remarkably free from typographical errors and will, I feel sure, be a useful textbook for a variety of students (and their seniors) for the next few years.

J. E. ENDERBY

Lichens

The Lichens. Edited by Vernon Ahmadjian and Mason E. Hale. Pp. xiv+697. (Academic: New York and London, January 1974.) \$35; £16.80.

ABOUT one fifth to one quarter of all described species of fungi are lichenised, but very few books have been written about them. Indeed, this is the first large and comprehensive work of advanced scholarship in the English language about lichens since the classic monograph of A. L. Smith in 1921. Furthermore, since so few botanists specialise in these plants, there is unlikely to be another book of this type for some time to come. It is, therefore, something of a milestone in the development of the subject.

There are 23 authors contributing 19 chapters and 3 appendices, covering a wide range of topics including morphology, taxonomy, reproduction, physiology, ecology, secondary metabolic products and symbiont interactions. Inevitably, there are some gaps. In the preface, the editors say that chemotaxonomy is one of the main present day areas of research in lichens, yet the book has no chapter devoted to this richly controversial topic: indeed, it gets but a passing mention in one appendix, and is not cited at all in the index.

Unevenness in quality is to be expected in all multiauthor works, and this is no exception. Although the editors have been very thorough and painstaking—so that errors and unclear passages are rare—it is a pity that they did not persuade authors to be more interpretive in their approach. Too many contributors are content to give meticulous accounts of published observations without following up with an incisive section which says, in effect, "... now what all this means is ... so that the problems which now require solution are ...".

Hence, the memorable parts of this book are the handful of chapters in which the authors develop a critical, personal synthesis of their views. Poelt's critique of the systematic value of morphological characters is particularly good. The account by Tuominen and Jaakola of the accumulation of mineral elements and radionuclides is

not only a valuable review of widely scattered literature, but it also sets a refreshingly high standard of rigour in discussing physiological topics. Brodo's excellent chapter on substrate ecology is profoundly thoughtful: at long last, here is a lichen ecologist who can write, quite simply, "I think the terms 'nitrophilous' and 'calciphilous' imply a knowledge of the requirements of lichens that we do not yet have".

For some students, the book will be hard going for it lacks an introductory chapter to set the scene. More acutely, it needs a concluding chapter to draw together the diverse strands of the individual contributions. Here would have been a chance for the editors to paint the broad canvas of the nature of the interactions between the symbionts; or to integrate the different themes of the chapters on substrate ecology, resistance to extreme environments, and mineral accumulation; or point out the gaps which, in the preface, they say exist.

The price puts this book beyond the reach of almost all students and academics, but I think it is essential for all botanical libraries. Although it does not achieve the same classic status as A. L. Smith's 1921 monograph, it nevertheless contains some important, substantial and valuable contributions about lichens which are not available elsewhere. Academic Press may be able to blame inflation for the price, but they cannot blame anyone but themselves for a subject index which is so skimpy, has so very many omissions and is so poorly organised as to be nearly worthless.

D. C. SMITH

Blue-eyed Negroes

Light-Eyed Negroes and the Klein-Waardenburg Syndrome. By Jenni Soussi Tsafirir. Pp. viii+153. 20 plates. (Macmillan: London and Basingstoke, March 1974.) £7.50.

THIS little book describes and discusses 18 non-Caucasian families ascertained by eye colour, where one or more members had Waardenburg's syndrome, and 10 families where one or more members had unilateral or bilateral blue or light green eyes, or heterochromia iridis.

Much of the book is devoted to discussion of Waardenburg's syndrome and, though the clinical descriptions of manifestations of the disorder are excellent, there is much repetition and nothing new is added to knowledge or understanding of an autosomal dominant trait which is relatively common and has been reported in many races. The families where there was only hypochromia of iris, which resulted in blue or greenish eye colours, or

where there was heterochromia inherited as a dominant trait are of much greater interest, and this is the best account so far given of these phenomena in Africans.

I feel that all the information in the book could have been presented in a short paper, or possibly in two papers, one on Waardenburg's syndrome in Africans and one on the other group of subjects. As it stands the book reads as if it was a condensation of an excellent thesis, although such an origin is not mentioned. Presumably the very high cost of this book is in part due to the nine pages of excellent colour photographs.

ALAN C. STEVENSON

African medicine

La Pharmacopée Sénégalaise Traditionnelle: Plantes Médicinales et Toxiques. By J. Kerharo, with J. G. Adam. Pp. 1011. (Editions Vigot Frères: Paris, 1974.) 370 francs.

PROFESSOR Kerharo is well known for his previous studies on the indigenous medicine and medicinal plants of the Ivory Coast and Upper Volta. During the past 15 years he has carried out similar, very detailed investigations in Senegal and his findings are now made available in this monumental work. The first part (about 75 pages) fills in the historical, (phyto) geographical, and ethnic background. It also discusses the religious and magical concepts underlying the various indigenous systems of medicine as well as the more positive aspects like diagnosis, pharmaceutical operations, and how medicines are dispensed and administered.

The second and main part of the book (about 700 pages) comprises detailed, individual monographs on about 555 plants. These monographs are divided into sections dealing with synonyms, vernacular names, distinguishing botanical features, habitat, folk-medicinal uses, chemistry and pharmacology. The chemical and pharmacological sections are extended and up to date accounts which summarise data from more than 2,200 references (closing date, May 1973). They are a particularly important feature of the book and extend its usefulness and value far beyond the confines of Senegal, since much of the flora of that country occurs in other parts of West Africa and elsewhere.

The third part (about 200 pages) comprises the bibliography and the various indices. Professor Kerharo's book provides a welcome complement to Watt and Breyer-Brandwijk's *Medicinal and Poisonous Plants of Southern and Eastern Africa*.

N. G. BISSET

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APPOINTMENTS VACANT
**UNIVERSITY OF ALBERTA
DEPARTMENT OF ENTOMOLOGY**

Applications are invited for the position of ASSISTANT PROFESSOR, effective April 1, 1975. Qualifications are Ph.D. with postdoctoral experience in insect physiology and interest in aspects of this field applicable to agricultural or forest entomology. Duties include teaching courses in general or applied entomology and insect physiology, the development and direction of a research program and supervision of graduate students in insect physiology and in application of some aspect of physiology to agricultural or forest entomology. Maximum starting salary \$14,043.

Please send full curriculum vitae and names of 3 referees by October 31, 1974 to: Dr George E. Ball, Chairman, Department of Entomology, 260 Agriculture Building, University of Alberta, Edmonton, Alberta T6G 2E3. (297)

**THE UNIVERSITY OF
NEWCASTLE UPON TYNE
CHAIR OF GEOGRAPHY**

Applications are invited for the post of Professor of Geography. The vacancy arises as a result of the resignation from September 30, 1974, of Professor J. W. House, to take up the Halford Mackinder Professorship of Geography at the University of Oxford. Salary in accordance with the Professorial Scale (£5,973 by £96 to £6,069 by £195 to £6,849 per annum).

Further particulars may be obtained from the Registrar, the University of Newcastle upon Tyne, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, should be submitted not later than September 14, 1974. (495)

**LINCOLN UNIVERSITY
COLLEGE OF AGRICULTURE
New Zealand
CHAIR IN AGRICULTURAL
MICROBIOLOGY**

The Council of Lincoln College, a University College of Agriculture located in Canterbury, New Zealand, invites applications for appointment to the Chair in the Agricultural Microbiology at this University College.

The successful applicant will be Head of the Department of Microbiology, which is responsible for a major teaching programme from Diploma to Post Graduate level, and which has active research and advisory interests. The present establishment includes 1 Reader, 2 Senior Lecturers, 1 Lecturer, 1 Demonstrator and 3 Technicians.

Applicants should hold an advanced university degree in an appropriate field and it would be expected that they would have had experience in teaching and/or research.

The successful applicant will be appointed to the position within the existing range of professorial salaries, the commencing salary being in accordance with qualifications and experience within the range of \$NZ15,111 to \$NZ19,233 per annum.

Expenses of appointment reimbursed up to specified limits. The appointee will be eligible to join the New Zealand Government Superannuation fund, or if eligible, he may elect to continue for a limited period existing F.S.S.U. policies.

Apply in writing for Conditions of Appointment obtainable from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, Lincoln College Canterbury, New Zealand. Applications close on **October 15, 1974.** (582)

Ecological and Biological Aspects of Pollution Control

£7,000-£8,000+

This is a key appointment in the Central Unit on Environmental Pollution which is the nucleus of the Government's anti-pollution activities. It is responsible for identifying and investigating problem areas, often on an international scale, for formulating appropriate policies and for co-ordinating the work of Government Departments in this field.

As a senior member of this group, you will provide biological and ecological advice over a wide range of these activities. Your particular responsibility will be the marine, freshwater and terrestrial fields, where you will initiate and control detailed studies on specific problems. You will also represent the Department of the Environment on Government and international committees and working parties.

You must hold a degree or equivalent in a biological science. While a background in marine or freshwater ecology would be of particular interest, other fields could also be relevant, and experience of pollution problems including national and international liaison duties would be an advantage.

Starting salary for this London-based appointment will be within the range quoted above.

Further details and an application form (to be returned by 2nd September 1974) may be obtained by writing to the Civil Service Commission, Alencon Link, Basingstoke, Hants RG21 1JB or by telephoning BASINGSTOKE 29222 ext 500 or LONDON 01-839 1992 (24 hour answering service). Please quote S/8710/1.

Department of the Environment

(611)

DORSET AREA HEALTH AUTHORITY EAST DORSET HEALTH DISTRICT

A vacancy exists for a

BASIC GRADE BIOCHEMIST

In the Group Biochemistry Service. Applicants should have a 1st or 2nd Class Hons. Degree in Chemistry or Biochemistry. Experience in Clinical Biochemistry would be an advantage.

Terms and Conditions as laid down in Whitley Council Regulation PTA/A.

Application forms from Employment Officer, District Administrative Offices, Royal Victoria Hospital, Shelley Road, Boscombe, BOURNEMOUTH, Dorset BH1 4HX giving two referees.

Enquiries about Post to Dr. J. H. Johnstone, Consultant Biochemist, Department of Biochemistry, Royal Victoria Hospital, Boscombe, BOURNEMOUTH, Dorset BH1 4JG.

*Closing date: August 31, 1974.

(612)

**Tropical Products Institute
London**

Meat Technologist

■ Laboratory investigations of meat quality ■ Applied technology in handling, preserving and processing ■ Abattoir improvement in tropics ■ Liaison with tropical agriculturists ■ Some overseas travel.

□ Degree or equivalent in Food/Agricultural Science or other appropriate subject □ 5 years' R & D in meat or allied technologies □ Age under 30 □ Appointment as Higher Scientific Officer (£2800–over £3700) □ Ref: SA/29/JD.

□ Application forms (for return by 2 September 1974) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

**Tropical Products Institute
Culham, Berks**

Cereal Technologist

■ R & D on composite flour technology, milling of tropical cereals, and preparation of food products of enhanced nutritional value from indigenous crops of less developed countries ■ Advise on cereal processes and products ■ Some overseas work.

□ Degree or equivalent in appropriate scientific/technical subject □ R & D experience in milling or bakery an advantage □ Age under 30 □ Appointment as Higher Scientific Officer (over £2550 to around £3500) or Scientific Officer (over £1700–£2800), according to age, qualifications and experience □ Ref: SA/30/JD.

□ Application forms (for return by 2 September 1974), from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.



(620)



Experimental Biologist

A vacancy exists for an honours graduate in pharmacology, physiology or a related discipline to work on experimental aspects of thrombus formation. Experience in micromanipulative techniques is essential and an interest in innovation is desirable.

The position offers pleasant working conditions, excellent fringe benefits and opportunities for advancement. Salary scale £2,350 to £3,400.

Please apply in writing to Miss J. M. Dunbar, Inveresk Research International, Inveresk Gate, Musselburgh EH21 7UB, Midlothian, quoting reference 0441. (637)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING (University of Reading)

A Scientific Officer or Higher Scientific Officer is required to join a group in the Bacteriology Department investigating the intestinal microflora of the neonate with particular reference to the antibacterial activity of milk. Experience with immunological techniques will be an advantage.

Candidates should have a degree, H.N.C. or equivalent qualification. Appointment will be in the grade of Scientific Officer (£1,592 to £2,675) or Higher Scientific Officer (£2,461 to £3,371), with a starting point dependent on qualifications and experience. At least five years' relevant post-qualifying experience is required for appointment in the higher grade. The post is pensionable.

Apply on forms obtainable from the Secretary, National Institute for Research in Dairying, Shinfield, Reading RG2 9AT, quoting reference 74/17. (561)

UNIVERSITY OF EDINBURGH DEPARTMENT OF ASTRONOMY RESEARCH ASSISTANT IN INFRARED ASTRONOMY

Applications are invited for a post of Postdoctoral Research Assistant in observational infrared photometry from October 1, 1974. The appointment is for a period of one year, but extension will be considered.

The Research Assistant will continue the development of an existing infrared photometer, and its use in Tenerife and elsewhere for observations of H II regions and of extragalactic infrared sources.

Salary will be in the range £2,118 to £2,931 per annum with superannuation under F.S.S.U.

Applications, including curriculum vitae and the names of two referees, should be sent by August 24, 1974 to Dr M. J. Smyth, Department of Astronomy, Royal Observatory, Edinburgh EH9 3HJ from whom further information about the post can be obtained. Please quote reference number 5041. (591)

UNIVERSITY COLLEGE GALWAY

Professorship of Experimental Physics

Applications are invited for the above statutory whole-time post. Salary scale £5,427 x 141 (8) to £6,555, plus Family Allowances. Non-contributory Pension Scheme.

The closing date for receipt of applications is **September 16, 1974**. Prior to application, further information should be obtained from the Registrar of the College. (562)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF ZOOLOGY

Applications are invited for the post of Lecturer in the Department of Zoology. Preference will be given to candidates with an interest in terrestrial ecology.

Initial salary within the range £2,118 to £2,412 per annum, plus threshold payment, on a scale rising to £4,896 per annum, according to qualifications and experience.

Applications, stating age, academic qualifications and experience, together with the names of three referees should be received not later than August 23, 1974, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote ref. RV/N/276163. (607)

SEFTON AREA HEALTH AUTHORITY SOUTHERN DISTRICT PRINCIPAL BIOCHEMIST

with duties primarily at the new Fazakerley District General Hospital. There is a District Biochemistry service (serving about 2,500 beds), operated in conjunction with Walton Hospital (where there is a Principal Biochemist in post). Equipment includes some A.A.11 analysers, a Vickers M300, Wallace Autogamma counter and E.D.P. equipment.

Further particulars may be obtained from the Consultant Chemical Pathologist (Dr I. J. L. Goldberg) 051-525 5980 Ext. 310, with whom arrangements to visit the laboratories may be made.

Applications stating age, experience, qualifications and the names and addresses of three referees to be forwarded to Mr K. A. P. Gill, District Administrator, Sefton A.H.A.—Southern District, Walton Hospital, Rice Lane, Liverpool L9 1AE not later than September 3, 1974. (609)

Technical Field Officer

We require a Technical Field Officer, preferably aged between 20-25 years, (Male or Female), who has recently graduated with a science degree. His/Her main responsibilities will be to assist in the design, running and evaluation of field trials on current animal health products and the development of new products. There will be some involvement in the area of technical sales support.

This post offers a chance to obtain experience in commercial field research and development within a large international organisation.

The successful candidate will be provided with a company car, there is a non-contributory pension scheme in operation, together with assisted B.U.P. membership and other fringe benefits.

Please apply for an application form to:-

Mr K. G. Usher,
Head of Administration,
Elanco Products Ltd.,
Broadway House,
The Broadway,
Wimbledon,
London SW19 1RR.
Telephone No: 01-542 6600.
(594)



ROYAL POSTGRADUATE MEDICAL SCHOOL CHIEF TECHNICIAN

(H.N.C. or equivalent qualifications) required for Department of Clinical Pharmacology. Experience with modern analytical instruments, e.g. gas chromatographs and mass spectrometers desirable. The appointee will be expected to be fully involved with research projects and be responsible for the day-to-day running of the department.

Salary in range £2,907 to £3,516 per annum (plus threshold allowance).

Applications to the Secretary, R.P.M.S., Hamersmith Hospital, Du Cane Road, London W12 0HS, quoting Ref. No. 20/104N. (646)

Professor in Biology

ODENSE UNIVERSITY
DENMARK

Applications are invited for the above position which is to be filled as soon as possible after November 1, 1974.

Candidates should have research interests in experimental biology covering the areas of both ecology and physiology. Research at the Institute of Biology includes, among others, the fields of ecology, analytical/environmental chemistry and biochemical adaptation-physiology with special reference to the aquatic environment.

The professorship carries teaching responsibilities in experimental and environmental biology at an advanced level as well as involvement in the Institute's introductory biology courses.

The salary is regulated by law and cannot be negotiated, at present it is D. Kr. 174,164.01. Applications demonstrating research and teaching abilities commensurate with the position of professor together with reprints of relevant publications should be sent in 5 copies to the Administration, Odense University, Niels Bohrs Alle, DK 5000 Odense, Denmark no later than September 25. Inquiries can be sent to the Institute of Biology. (621)

Graduate in Biology

A vacancy exists for a graduate interested in cell biology to work in a small research team concerned primarily with wound healing. This involves the search for drugs which are beneficial to the healing process and more fundamental studies aimed at understanding the mechanism of cell movement and the control of cell division.

Modern equipment, including a scanning electron microscope is available, and a

high degree of practical ability and ingenuity is essential.

Pharmaceuticals Division is situated in rural North Cheshire and offers a good range of reasonably priced housing.

Applications in writing, requesting an application form, should be addressed to:

M. F. Losse, Personnel Officer,
ICI Pharmaceuticals Division,
Mereside, Alderley Park,
Macclesfield, Cheshire.



Pharmaceuticals
Division

(624)

UNIVERSITY OF STRATHCLYDE DEPARTMENT OF BIOCHEMISTRY

Research Assistant

Applications are invited for a post of Research Assistant in the M.R.C. Group for Biochemistry of Reproduction. Applicants who should preferably be at postdoctoral level or have equivalent experience, should have a good background in Biochemistry or cognate subject with interests in production in protein fractionation and isolation. Previous experience in reproductive studies would be an advantage but is not essential.

Appointment would be for the period October 1, 1974 to September 30, 1976.

Salary scale £2,118 to £2,412 with placing according to age and experience. F.S.S.U. benefits.

Applications (quoting R29/74) to Professor P. J. Heald, Department of Biochemistry, University of Strathclyde, Royal College, 204 George Street, Glasgow, G1 1XW from whom further information may be obtained.

(627)

DEPARTMENT OF EMPLOYMENT

Assistant Scientific Officers

The Department of Employment has vacancies for Assistant Scientific Officers with the Employment Medical Advisory Service, Baynards House, 1 Chepstow Place, Westbourne Grove, London W2 in the Central Reference Laboratory.

The duties are varied and mainly concerned with the examination of biological samples from industrial workers as an index of exposure to potentially toxic hazards. An aptitude for working with, or an interest in, modern laboratory equipment would be an advantage.

A knowledge of physio-chemical methods of analyses would be desirable.

QUALIFICATIONS

The minimum qualifications required are passes in 4 distinct subjects in GCE or equivalent. The passes must include English or English language, and a Science or Mathematical subject. Other acceptable qualifications are an ONC (candidates must have successfully completed the 01 year) the Intermediate examination of the Institute of Medical Laboratory Technology and Science Laboratory Technician's Ordinary Certificate. Alternative qualifications will be considered.

AGE

Candidates for all posts must be over 16 and normally under 26 years of age.

SALARY

£1,115 to £2,127 per annum according to age, inclusive of £228 inner London weighting, which is currently under review.

HOLIDAYS

Four weeks, rising eventually to six weeks.

HOW TO APPLY

Application forms from Mr D. C. Werran, Department of Employment (Est B3b), 12 St James's Square, London SW1Y 4LL.

Closing date for receipt of applications: September 6, 1974. (613)

NEW ZEALAND MINISTRY OF AGRICULTURE AND FISHERIES

SCIENTIST (PASTURE AGRONOMY)

INVERMAY AGRICULTURAL RESEARCH CENTRE,
MOSGIEL, SOUTH ISLAND, NEW ZEALAND

Duties: Study the effects of various cutting and grazing treatments on sward productivity and composition.

Investigate competitive mechanisms within establishing and permanent swards.

Qualifications Required: Ph.D., M.Agr.Sc. or M.Sc.

Salary: This will depend on qualifications and experience.

Passages and Incidental Expenses: The provision of assistance with fares and incidental expenses will be considered.

Application forms and general information are available from the New Zealand High Commission, New Zealand House, Haymarket, London SW1 Y 4TQ, with whom applications will close on September 13, 1974.

Please quote reference 4294 when making enquiries. (690)

UNIVERSITY OF EXETER DEPARTMENT OF CHEMISTRY S.R.C. POSTDOCTORAL RESEARCH ASSISTANTSHIP

SYNTHESIS OF MATERIALS FOR PHOTOELECTRIC ENERGY CONVERSION

Applications are invited from chemists with a good grounding in organic or inorganic or organo-metallic synthesis, to work on the preparation of potential photoelectric transducers. Some straight forward measurement of electrical properties will also be involved. Salary on scale £2,118 to £2,417 per annum, together with current threshold payments according to age and experience. Appointment for one year extendable to a second.

Applications naming two referees to Drs K. Kite, G. Read and D. R. Rossinsky, The University, Exeter EX4 4QJ, as soon as possible. Please quote reference 1/62/7077. (617)

UNIVERSITY OF HAMBURG RESEARCH ASSISTANT IN MEDICAL GENETICS

required for clinical and laboratory research and work in a genetics clinic. Salary in the range of £5,000 per annum net. Applicants should contact Dr Eberhard Passarge, Division of Cytogenetics and Clinical Genetics, Department of Human Genetics, Martini-strasse 52, 2000 Hamburg 20, W. Germany. Tel. (040) 468-3120. (623)

WEST BERKSHIRE HEALTH DISTRICT MEDICAL PHYSICS TECHNICIAN

for the modern Isotope Laboratory at the Royal Berkshire Hospital, Reading, for interesting work in the chemistry and physics of medical isotope techniques. Post will be on Grade V or Grade IV (£1,308 to £1,677 or £1,530 to £1,953). Day release for further training possible. (Normally O.N.C. or 2 'A' levels required).

Reading is a pleasant University town offering easy access to London, Oxford, Windsor, Henley and attractive surrounding countryside.

Written applications with relevant details and naming 2 referees to the Hospital Secretary, Royal Berkshire Hospital, London Road, Reading, Berkshire. (642)

"METALS ABSTRACTS"

The international abstracting service for metallurgy offers permanent positions as SENIOR EDITORIAL ASSISTANTS. The work consists of editing, indexing, and checking abstracts for publication. A science degree, preferably in metallurgy, physics, or chemistry, is necessary and a working knowledge of a foreign language would be an advantage.

Applications, stating age, education, qualifications, and experience, to Dr T. Graff, Metals Abstracts, The Metals Society, 1 Carlton House Terrace, London SW1Y 5DB. (633)

SALOP AREA HEALTH AUTHORITY

Robert Jones and Agnes Hunt
Orthopaedic Hospital

OSWESTRY, SHROPSHIRE SY10 7AG

BIOCHEMIST

Graduate research assistant required to take part in investigations into the biology and biochemistry of muscle in relation to human musculo-skeletal disorders.

Salary in the range of £1,680 to £2,112 per annum.

Applications, with the names of two referees, should be addressed to:

• Dr M. Worsfold
Principal Research Biologist
Charles Salt Research Centre
Robert Jones and Agnes Hunt Orthopaedic
Hospital
Oswestry, Shropshire. (641)

KING'S HEALTH DISTRICT (TEACHING)

LAMBETH/SOUTHWARK/LEWISHAM AREA
HEALTH AUTHORITY (T)

GRADUATE RESEARCH ASSISTANT IN CYTOGENETICS

Applications are invited for the above post in the genetic sub-section of Haematology. Experience desirable. Salary according to N.H.S. Biochemist Scale.

Application forms obtainable from the Personnel Office, King's College Hospital, Denmark Hill, S.E.5 Tel.: 01-274 6222 Ext. 2726 (Miss Millwood). should be completed and returned by August 22, 1974. (616)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF GEOLOGY JUNIOR RESEARCH OFFICER

A vacancy exists for a Junior Research Officer in the above Department who will work under the direction of Professor T. S. Westoll, F.R.S., on Vertebrate Palaeontology with special emphasis on lower vertebrates.

The post is expected to be tenable for three years from October 1, 1974. It is particularly suitable for a recent graduate in Geology with good Zoological background, or in Zoology with some Geological background. A successful candidate may register for a higher degree, and though preference will be given to recent graduates other qualified persons are not excluded.

Salary will be at an appropriate point on the scale £1,569 by £81 to £1,731 by £87 to £1,818 according to age, qualifications and experience.

Applications (3 copies) giving a full curriculum vitae and the names of three referees, should be lodged with the Registrar, The University, Newcastle upon Tyne NE1 7RU not later than September 2, 1974. Please quote reference N. (618)

HUMAN PHYSIOLOGIST

required for research on inhaled drugs, asthma and exercise. Project could lead to higher degree. Salary according to qualifications and experience.

Applications, giving full details, to: Secretary, (N) Department of Child Health, Hammersmith Hospital Du Cane Road, London W12 0HS. (619)

TECHNICIAN (Grade 3)

required to assist in Biological research including projects on the diagnosis and immunology of cancer. Background of chemistry, biochemistry or pharmacology useful. Opportunities for further education up to H.N.C. Salary in range £1,650 to £1,920 plus £228 London Weighting plus threshold. Application forms from Personnel Officer (Technical Staff F.E.2), University College London, Gower Street, London WC1E 6BT. (622)

CHARING CROSS HOSPITAL MEDICAL SCHOOL (UNIVERSITY OF LONDON)

The Animal Unit requires a RESEARCH ASSISTANT to collaborate with the Veterinarian in charge in the establishment of a quality control laboratory. The post would be suitable for a graduate or similarly qualified person with a background in microbiology or a relevant biological science. Starting salary not less than £1,350 depending upon experience.

For further information please contact: Head of Animal Unit, 55 Aspenlea Road, London W6 9HH. Telephone 01-385 7709. (628)

UNIVERSITY COLLEGE OF NORTH WALES Bangor

DEPARTMENT OF APPLIED ZOOLOGY

Applications are invited for the post of LECTURER IN APPLIED ZOOLOGY. The appointment will take effect from October 1, 1974 or as soon as possible thereafter.

Candidates should have an interest in either the epidemiological or experimental aspects of animal parasitology or in crop damage by ectoparasites (or insects).

Salary will be on the scale £2,118 to £4,896, according to age, qualifications and experience.

Further particulars may be obtained from the Secretary and Registrar, and applications (two copies) giving details of age, qualifications and experience, together with the names and addresses of three referees should be sent to reach the Secretary and Registrar, University College of North Wales, Bangor LL57 2DG, by August 27, 1974. (631)

CSIRO

AUSTRALIA

DIVISION OF BUILDING RESEARCH HIGHETT, VIC.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field:

RESIDENTIAL ENVIRONMENT

General: The Division has a total staff in excess of 300 including some 150 professional scientists. It undertakes research for the benefit of the Australian community into the theory, techniques and economics of building and community design, construction, and materials development and is seeking an understanding of the inter-action between the built environment and people. Programmes for studying the built environment are being developed in the context of both urban communities and remote settlements. The appointee will be located at Highett, Victoria.

Duties: To conduct research into the effects of the built environment, the relevant aspects of the natural environment and related factors of community living on residents or urban and remote settlements in Australia. This will involve the development of research techniques capable of providing guidance to physical and social planners and others who determine the nature of human settlements.

Qualifications: A Ph.D. degree or equivalent research experience, and demonstrable research ability. Applicants could be qualified in the relevant branches of psychology, sociology or geography, but graduates in architecture, planning or other disciplines, who consider that they have an appropriate background should not be deterred from applying. Experience in multi-disciplinary research teams involving social, physical and biological sciences would be an advantage.

Salary: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a.

Tenure: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 390/530 should reach:

**The Personnel Officer,
Australian Scientific Liaison Office,
64-78 Kingsway,
LONDON WC2B 6BD.**

by the 30th August, 1974

Applications in U.S.A. and Canada should be sent to:

**The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.**

(625)

CSIRO**AUSTRALIA**

DIVISION OF FISHERIES AND OCEANOGRAPHY CRONULLA, N.S.W.

RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organization has a broad charter for research into primary and secondary industry areas. The Organization has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field:

FISHERIES BIOLOGY

General: The Division has a staff of about 50 scientists investigating the fisheries resources and the physical, chemical and biological features of the oceans. Headquarters of the Division is located at Cronulla, Sydney, and the Division has laboratories in Perth and near Brisbane. The appointee will be located at Cronulla.

Duties: To participate in investigations of marine fish resources around Australia. The appointee will be expected to develop and conduct his/her own programme. Initially attention will be directed to pelagic resources.

Qualifications: A Ph.D. degree or equivalent qualifications in one of the ecological sciences with training in Mathematics and Statistics, together with demonstrable research ability.

Salary: Appointment will be made within the salary range of Research Scientist: \$A9,698 to \$A12,194 p.a.

Tenure: An indefinite or 3 year fixed term appointment may be negotiated. An indefinite appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees and quoting Reference Number 320/520 should reach:

**The Personnel Officer,
Australian Scientific Liaison Office,
64-78 Kingsway,
LONDON WC2B 6BD**

by the 30th August, 1974

Applications in U.S.A. and Canada should be sent to:

**The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.**

(626)

ROYAL POSTGRADUATE MEDICAL SCHOOL TECHNICIAN (A.I.M.L.T.)

required for interesting post in the Postgraduate Teaching Laboratories. The department deals with a wide range of work in all the four major disciplines of Clinical Pathology, and offers good facilities for further studies.

Applicants qualified in Histology (or Bacteriology) preferred. Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS, quoting Ref. No. 9/102N. (629)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING (UNIVERSITY OF READING) INFORMATION OFFICER

An information scientist, preferably with a degree in microbiology or biochemistry, is required to take part in the new information service of the Institute.

Appointment will be in the Scientific Officer Class, with a starting salary dependent on qualifications and experience. Present scales Scientific Officer £1,592 to £2,675; Higher Scientific Officer £2,461 to £3,371. At least five years' relevant post-qualifying experience is required for appointment in the higher grade. The post is pensionable.

Apply on forms obtainable from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT. Quote reference 74/20. (630)

NATIONAL INSTITUTE FOR BIOLOGICAL STANDARDS AND CONTROL

TECHNICIAN

DIVISION OF VIRAL PRODUCTS

We require someone with H.N.D., H.N.C. or equivalent for work concerned with the control and development of viral vaccines and involving the manipulation of viruses under sterile conditions in various types of cell cultures. Other procedures such as radio-isotope labelling and radioimmuno assays are also employed. A knowledge of basic microbiological techniques would be an advantage plus an ability to work without direct supervision.

Salary on scale £1,683 to £2,577 including London Weighting.

The Institute is situated in pleasant surroundings close to Hampstead Underground Station. There is an active sports and social club plus superannuation benefits. Please apply giving brief details (an application form will be sent to you) to R. S. Dunn, Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead, N.W.3. Tel. 435 2232. Please quote Ref. 0027. (649)

THE UNIVERSITY OF SHEFFIELD RESEARCH ASSISTANT

ACADEMIC DIVISION OF MEDICINE

Applications are invited for a RESEARCH ASSISTANT to join a group working with Professor D. S. Munro on human thyroid disorders. The work will include the development of new assay methods for circulating thyroid stimulating factors in health and disease. A graduate with a degree in Chemistry or in a biological science is sought. Salary up to £1,929 according to qualifications and experience. A successful candidate wishing to read for the degree of Ph.D. would be offered a Research Studentship, £695 a year and fees. Further particulars from The Registrar and Secretary to whom applications (one copy only) should be sent by August 27, 1974. Please quote reference R124/G. (632)

BIOCHEMISTRY TECHNICIAN

required to join group investigating enzyme kinetics of muscle proteins. Applicants should have degree or H.N.C. in Biochemistry or Chemistry. Previous laboratory experience in the use of biochemical and radioisotope techniques preferred. Salary on the scale £1,173 to £2,073 plus £126 London Weighting according to age and experience.

Applications to John Cribbin, M.R.C. C.E.11 Biophysics Unit, 26-29 Drury Lane, W.C.2. (643)

UNIVERSITY OF LEEDS DEPARTMENT OF PLANT SCIENCES

Applications are invited from graduates in Botany, Biochemistry or other suitable branch of Biology, for two posts of RESEARCH ASSISTANT in the Department of Plant Sciences.

- A three year project to work on the control of nucleic acid synthesis in the cell cycle of fungi. This project will involve nucleic acid fractionation, DNA/RNA hybridisation studies and some microscopy.
- A one year project to work on biochemical aspects of fungal disease in plants. This project will involve working with animals to raise antisera.

Salaries on the scale £1,449 to £1,818 p.a. according to experience (at present under review).

Applicants should write directly to Dr J. A. Callow, Department of Plant Sciences, quoting 2 referees, as soon as possible. (614)

Histology Technician

required for Pharmacological and Toxicological Laboratory. Experience essential in the preparation and processing of animal tissues. Good working conditions. Pension and Assurance Scheme. Application forms from: The Secretary, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London N1 2HB.

(640)

UNIVERSITY OF EXETER

DEPARTMENT OF PHYSICS

POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a Postdoctoral Research Assistantship in theoretical physics supported by the Science Research Council. The research work, with Dr. T. W. Preist, will involve the use of Faddeev methods to study rearrangement collisions at thermal energies and should be of interest to applicants with experience in either Atomic, High-Energy or Nuclear physics.

The appointment is for two years from October 1, 1974 (starting salary £2,118 plus F.S.S.U.). Applications giving details of experience and qualifications and including the names of two referees should be sent, by August 23, 1974 to Miss S. Chinn, Department of Physics, University of Exeter, Stocker Road, Exeter EX4 4QL (Tel. Exeter 77911 ext. 625) from whom further information may be obtained.

Ref. 1/12/7079.

(668)

THE QUEEN'S UNIVERSITY OF BELFAST

Department of Biochemistry

Postdoctoral Research Assistant

Applications are invited for an S.R.C. Postdoctoral Research Assistantship for work in collaboration with Dr R. J. H. Davies on the PHOTO-CHEMISTRY AND PHOTOBIOLOGY OF PURINES. The project will involve an assessment of the photo-reactivity of purines in nucleic acids, polynucleotides and simpler model systems. The primary aim of this research is the identification of purine photoproducts and an evaluation of their biological consequences. Previous experience in the isolation and chemical characterization of natural products would be an advantage though not essential.

The appointment is for two years from October 1, 1974, or an agreed date thereafter, at a salary of up to £2,247 by £165 p.a. (with F.S.S.U.) depending on age and experience. Applications, together with the names of two referees, should be sent to the Personnel Officer, Queen's University, Belfast BT7 1NN, from whom further particulars are available.

(663)

SYDNEY HOSPITAL

New South Wales

Australia

**Staff Specialist
in Intensive Care**

Applications closing September 15, 1974, are invited from suitably qualified medical practitioners for the position of Staff Specialist In Charge of the Intensive Care Unit at Sydney Hospital.

Teaching at both undergraduate and postgraduate levels is an integral part of the position.

Salary and conditions are those set out in the Hospital Specialist (State) Award (range \$A15,203 to \$A21,006). The Award is presently under review. The limited right of private practice is available under the terms laid down by the Health Commission of New South Wales in circular 72/78. The terms allow earnings up to 16 per cent of base rate salary.

Under certain circumstances The Board may give consideration to two part time appointments to this post. Further enquiries may be directed to The Director of Anaesthesia.

Sydney Hospital is a 460 bed teaching hospital of the University of Sydney. It conducts a wide range of medical, surgical and special surgical services and is accredited for post graduate training in general and special medicine and surgery.

The Director of Anaesthesia will be visiting the United Kingdom in August/September. Applications in writing, stating age, training, experience and the names of three referees should be directed as follows:-

1st copy to Dr F. R. Berry, C/- Bank of New South Wales, 9-15 Sackville Street, London.

2nd copy by airmail to The General Medical Superintendent, Sydney Hospital, Box 1614, GPO, Sydney, 2001, New South Wales. (610)

nature**EDITORIAL SECRETARY**

We are looking for someone who will be involved in both the editorial and production processes and has:

- A good basic knowledge of science
- An eye for detail
- Shorthand and typing

Although not essential, a knowledge of production work would be an advantage.

Write, giving personal details, to:

- The Editor, *Nature*, Macmillan Journals Ltd,
4 Little Essex Street, London WC2R 3LF.

STATE OF KUWAIT



Kuwait University—Academic Posts for 1974-1975

Applications are invited for teaching posts in Marine Ichthyology, Marine Fisheries & Mariculture, Oceanography, Immunology, and Marine Parasitology. Contracts start on February 1, 1975 for two years, renewable for a further period of four years if convenient to both applicant and University.

Applicants should be:

- (a) Holders of an academic Post at present in an accredited University or research center.
- (b) Ph.D. holders

Curriculum vitae forms are obtainable from Kuwait Embassies in Washington D.C. (Cultural Division, 4301 Connecticut Avenue, N.W., Site 158, Washington, D.C. 20008) and London, (Cultural Attache office, (Al-Jahra House, 3 Stratford Place, London W1N 9AE) or from Kuwait University, Kuwait. Completed forms, together with copies of the candidate's publications, must be received by Kuwait University, Kuwait, not later than September 30, 1974.

Those who applied before can renew their applications by writing to the University.

Salaries are within the range of Kuwaiti Dinars 3,900 to 6,660 per annum, tax free (K.D.=£2.90)

Candidate is also entitled to the following privileges:

1. Annual return air tickets to his country, would be provided to him, his wife and three of his children not exceeding the age of twenty.
2. Free furnished accommodation with water and electric supplies.

(648)

NEW ZEALAND

Department of Scientific & Industrial Research

Applications are invited for the position of Scientist (Soil Physics) with the Soil Bureau, DSIR, Lower Hutt, where there is a vacancy for a senior soil physicist in the soil physics section.

Salary: Up to NZ\$10,232 p.a. dependent on qualifications and experience.

Duties: Work of the section includes systematic studies of the physical properties of soil in relation to soil classification and effects of modification of soils by man, and will expand into the field of soil evaluation for irrigation and effluent disposal.

Qualifications: PhD. or good Honours degree in soil physics, with experience in field operation support and irrigation planning desirable.

Passages: Fares for appointee and his wife and family, will be paid.

Incidental expenses: Up to NZ\$120 for a single man and NZ\$800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London SW1Y 4TQ, with whom applications will close on 13 September 1974. Please quote reference P/T 113 when enquiring. (666)

ROYAL POSTGRADUATE MEDICAL SCHOOL TECHNICAL OFFICER

required for M.R.C. Team involved in assaying new metabolic hormones and assessing their role in health and disease. This post is suitable for a new graduate in Biochemistry or Physiology and could offer opportunities for a higher degree. Salary according to age and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, DuCane Road, London W12 0HS quoting ref. no. 2/149. (662)

UNIVERSITY OF READING TECHNICIAN (Grade 3)

required in Department of Microbiology to assist a group engaged in research on the genetics and biochemistry of bacteria and to prepare materials for practical classes. Salary in the range £1,650 to £1,920 p.a. according to qualifications and experience. Apply quoting Ref: TN68, stating qualifications and experience and giving the names of two referees to Senior Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. (670)

UNIVERSITY OF NOTTINGHAM Department of Electrical and Electronic Engineering

Applications are invited for appointment as LECTURER in this Department. The salary scales range from £2,118 to £4,896, but the appointment will be made initially within the first three points of the scale. The appointment can be made from October, 1974, or from a later date to be arranged.

Applicants should preferably have had industrial, research or lecturing experience in communications, control or electronics. The person appointed will be expected to pursue research in addition to their teaching duties, for which facilities are available.

The appointment will be subject to the terms and conditions of the Grading and Salary Scheme, a copy of which is available on request. The successful applicant will be required to join the Federated Superannuation System for Universities, under the terms of which there is an annual premium of 15% of the salary. The University will pay two-thirds of this premium and the member one-third. The University reserves the right to appoint to the post a person who has not submitted an application.

Further particulars and forms of application, returnable not later than August 31, 1974, are obtainable from the Staff Appointments Officer, University of Nottingham, University Park, Nottingham NG7 2RD Job No. 389. (667)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY DEPARTMENT OF METALLURGY

Applications are invited from Honours graduates in Metallurgy, Materials Science, Engineering or Pure Science for the post of

RESEARCH ASSISTANT

which becomes vacant on October 1, 1974. Preference will be given to candidates who have had some research or industrial experience. The post is a full-time research appointment with the opportunity to work for a higher degree of the University. A wide range of research topics is available in chemical, physical or engineering metallurgy and it will be possible to match the nature of the research to the interests of the person appointed. The appointment is for one year in the first instance with the possibility of annual re-appointment.

Salary £1,764 to £1,839 with F.S.S.U.

Applicants should send a brief statement of their academic qualifications, experience and research interests to Professor K. M. Entwistle, U.M.I.S.T., P.O. Box 88, Sackville Street, Manchester M60 1QD. (656)

UNIVERSITY OF KHARTOUM SUDAN

Applications are invited for the following posts in the Faculty of Medicine:—

1. PROFESSOR/SENIOR LECTURER OR LECTURER (4 posts) in ANATOMY. Applicants must be medically qualified and must hold a Ph.D. or equivalent or a postgraduate diploma in Anatomy. Candidates will be assessed for the respective status according to research and relevant teaching experience in Anatomy (Regional and Applied) plus a sound knowledge of Neuro-Anatomy and/or Embryology and/or Histology.
2. SENIOR LECTURER/LECTURER (2 posts) IN BIOCHEMISTRY.

Salary scales: Senior Lecturer £52,400 to £52,800 p.a., Lecturer £51,500 to £52,300 p.a. (£1 sterling=£50.82). The British Government may supplement salaries in range £1,300 to £1,600 p.a. (sterling) for married appointees or £800 to £900 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. These rates of supplementation are currently under review. Family passages; various allowances; superannuation scheme; annual overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than September 4, 1974 to the Personnel Secretary, University of Khartoum, P.O. Box 321, Khartoum, Sudan. Applicant resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (650)

Senior Biochemist

A leading British pharmaceutical Research Organisation has a vacancy for a **Senior Biochemist** to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines. Persons with appropriate experience are invited to apply in writing for an Application Form from The Secretary, Biorex Laboratories Limited, Biorex House, Canonbury Villas, London N1 2HB. (655)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

University of Reading

HEAD OF DEPARTMENT of PROCESS ENGINEERING

A vacancy will occur on the retirement of Mr. H. S. Hall, O.B.E. for a suitably qualified person to direct and organise the Institute's research and development programme on the processing of milk and milk products.

Candidates should have a degree in engineering or an allied science and have had relevant experience in research and in the industrial processes concerned with milk or food products.

The post, which is pensionable, will be graded as Senior Principal Scientific Officer (salary £6,300 by 3 increments to £7,280). Further particulars and forms may be obtained from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT and applications should be returned to him by October 1, 1974. Quote reference 74/26. (671)

UNIVERSITY COLLEGE LONDON

Department of Biophysics

ELECTRON-MICROSCOPIST

RESEARCH ASSISTANT required to work with Professor R. Miledi on problems in Neurobiology, which forms part of a Long Term Project supported by the M.R.C.

Preference given to applicants with experience of freeze-etching or analytical electron-microscopy. Salary within Lecturer scale plus London Allowance.

Applications with curriculum vitae and names and addresses of two referees to Mrs. K. Garnons-Williams, (N) Biophysics Dept., University College London, Gower St. London WC1E 6BT. (680)

Manchester Area Health Authority

(Teaching) South District

WITHINGTON HOSPITAL,
MANCHESTER M20 8LR.

GRADUATE required with a good honours degree in biochemistry. The appointee will take part in research into thrombosis and development work in the National Reference Laboratory for Anticoagulant Control Reagents at Withington Hospital.

Further information can be obtained from Dr. L. Poller, Consultant Haematologist, Withington Hospital.

Applications with the names of two referees to the Hospital Secretary, quoting ref. B 22. (52135). (657)

Countryside Commission

The Countryside Commission maintains close liaison with organisations throughout England and Wales which share its concern with the conservation of the countryside and with the recreational opportunities it can offer. There are specialist posts, at the Commission's new Headquarters in Cheltenham, for experts who have the ability to originate ideas and to communicate them effectively both orally and in writing.

AGRICULTURALIST

to give advice on agricultural matters arising from submitted schemes, promote research and experiments into the integration of landscape conservation and recreation with farming, and liaise with M.A.F.F., N.F.C. and Country Landowners Association. (Reference SB/1/HG).

Candidates (normally aged under 32) must hold a 1st/2nd class honours degree in agriculture or other appropriate scientific subject and have at least 4 years' postgraduate experience. Practical involvement in farming will be an advantage.

ECOLOGIST

to give ecological advice on schemes under consideration, instigate and supervise research and experiments into environmental capacity for recreation, promote action to conserve wild-life in country parks, and liaise with Nature Conservancy Council. (Reference SB/2/HG).

Candidates (normally aged under 32) must hold a 1st/2nd class honours degree in botany, zoology, biology or other appropriate scientific subject and have at least 4 years' postgraduate experience, including conservation work or qualification in ecology or conservation.

Starting salary in the range £3,250 to around £4,550, according to qualifications and experience. Prospects of promotion. Non-contributory pension scheme.

For full details and application form (to be returned by September 5, 1974) write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB or telephone Basingstoke 29222 ext 500 or London 01-839 1992 (24 hour answering service). Please quote appropriate reference. (669)

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for the following posts in the Faculty of Science:—

- PROFESSOR IN DEPARTMENT OF BOTANY.** Applicants should be Botanists with several years' post-doctoral teaching and research experience, and should be familiar with the organisation and supervision of postgraduate research with particular reference to tropical plants and the use of modern laboratory equipment. Appointee will be expected to administer and plan the development of the University Botanical Gardens and Herbarium as well as the Department of Botany.
- SENIOR LECTURER AND LECTURER IN THE DEPARTMENT OF GEOLOGY.** For Senior Lectureship, appointee will be expected to lead research in either Geophysics, Geochemistry with a bias for Mineral Exploration, or Mineralogy with a bias for X-Ray Diffraction. The Geophysicist should have a bias in Exploration and should be conversant with most geophysical methods. He will be expected to supplement Petrological and/or Mineral Exploration Research in the Department with geophysical data. The Mineralogist should have long experience with X-Ray Crystallography and should be particularly interested in X-Ray Diffraction. For Lectureship, appointee should have special interest or experience in one of the following areas: Engineering Geology with strong background of rock mechanics and/or hydrogeology; Mineralogy with special bias in optical and X-ray crystallography and with experience of X-ray diffraction and power camera work; Igneous Petrology; Experimental Geology.

Salary scales: Professor N6,600 p.a. Senior Lecturer N5,030 to N5,750 p.a. Lecturer N2,760 to N4,830 p.a. (N1 sterling=N1.46). The British Government may supplement salaries in range £750 to £1,500 p.a. (sterling) for married appointees or £250 to £1,000 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation scheme; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than September 6, 1974 to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to the Inter-University Council, 90/91, Tottenham Court Road, London, W1P 0DT. Further particulars may be obtained from either address. (677)

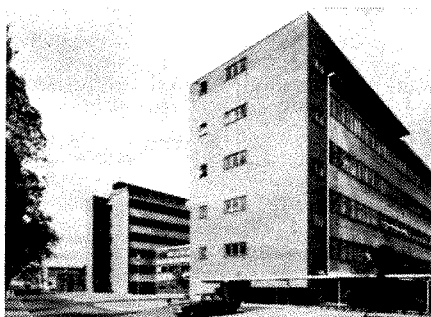
May & Baker Ltd. Drug Metabolism.

A vacancy exists in the Drug Metabolism Division for a graduate, preferably with relevant post-graduate experience and degree. The work of the Division involves studies on the metabolism and pharmacokinetics of new medical products and as such, requires familiarity with metabolic processes, instrumentation applicable in the field and the ability to devise new analytical methods. Preference will be given to a candidate who has a good knowledge of pharmacokinetics and the associated use of computers.

Publication of appropriate work is encouraged. There are active links with a number of university departments, units of clinical pharmacology and physicians involved in

drug trials. Various attractive residential areas are within easy reach of the research site and help will be provided with removal expenses where appropriate.

Applications, giving details of qualifications and experience should be addressed to the Personnel Manager, May & Baker Ltd, Dagenham, Essex RM10 7XS, quoting reference number 194/N/1. (674)



M&B May & Baker



FOOD & AGRICULTURE ORGANISATION
OF THE UNITED NATIONS

Viale delle Terme di Caracalla, Rome, Italy

FISH CULTURIST

Applications are invited for the post of a Fish Culturist for the Khmer Republic under the F.A.O./U.N.D.P. Programme from candidates with a University degree or equivalent in fisheries sciences and several years' experience in the field of fish culture in tropical countries. Very good knowledge of English or French is essential. Duty Station—Phnom Penh.

The duties and responsibilities of the post include (1) to assist in the establishment of a fish hatchery/demonstration fish farm station; (2) to set up and demonstrate modern methods of aquaculture in fish cages; (3) to train counterpart personnel and fish farmers in the techniques of pond and cage fish culture.

Salary: US\$16 542 to US\$21 294 net (tax free), plus Cost of Living Adjustment and other allowances.

Further details can be obtained from the Personnel Officer, Department of Fisheries, F.A.O., Rome. Closing date for receipt of applications: September 15, 1974. (661)

GUY'S HOSPITAL MEDICAL SCHOOL

Applications are invited for the post of

Graduate in Research Assistant in Cytogenetics

The work will be concerned with diagnostic chromosome analysis but, in addition, the successful applicant will be encouraged to participate in research projects.

Applications should be sent to the Administrative Secretary, Paediatric Research Unit, Guy's Hospital Medical School, London Bridge, SE1 9RT, as soon as possible. (658)

THE POLYTECHNIC, WOLVERHAMPTON

DEPARTMENT OF BIOLOGICAL SCIENCE WELCOME TRUST POST DOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited from suitably qualified persons to assist in investigations of the function of intestinal microperoxisomes with special reference to factors influencing lipid and cholesterol synthesis. Applicants should hold or expect to hold a Ph.D. in Biochemistry (or related area). The appointment is tenable for 3 years.

Salary in the range £2,223 by increment to £2,553.

Further details and application forms may be obtained from: The Establishment Officer, The Polytechnic, Wolverhampton WV1 1LY. Telephone 27371 Ext. 94. (673)

UNIVERSITY OF THE WEST INDIES JAMAICA

Applications are invited for the following posts in the Faculty of Medicine:—

1. SENIOR LECTURER / LECTURER / ASSISTANT LECTURER IN THE DEPARTMENT OF MICROBIOLOGY. Duties of appointee, will include routine Clinical Microbiology work for the University Hospital and instructions in Microbiology to students working for the medical degree of the University. Duties to be assumed as soon as possible.
2. LECTURER IN HAEMATOLOGY in the Department of Pathology. Appointee will be attached to the University Hospital and will teach medical students for the M.B., B.S. degree of the University, in co-operation with the Consultant staff of the Hospital.

Salary scale: Medically qualified: Senior Lecturer J\$10,992 to J\$14,472 p.a. Lecturer J\$7,860 to J\$10,752 p.a. Assistant Lecturer J\$6,300 to J\$6,900 p.a. Non-Medically qualified: Senior Lecturer J\$8,460 to J\$12,492 p.a. Lecturer J\$6,168 to J\$9,768 p.a. Assistant Lecturer J\$5,006 to J\$5,468 p.a. (£1 sterling = J\$2.17). F.S.S.U. Unfurnished accommodation at rent of 10% of salary for maximum of three years. Thereafter 20% of salary paid in lieu of housing. Family passages; triennial study leave. Detailed application (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica. Detailed particulars are available and should be obtained from the same source before an application is made. (679)

Pharmacologists/ Toxicologists

Honours Graduates with some experience required for Research and short and long term toxicological studies of compounds of potential therapeutic importance. Excellent opportunities for advancement in modern, well-equipped laboratory. Pension and Assurance Scheme. Application Forms from: The Secretary, Biorex Laboratories Limited, Biorex House, Canonbury Villas, London N1 2HB. (654)

THE UNIVERSITY OF SHEFFIELD
DEPARTMENT OF PHYSICS
POSTDOCTORAL RESEARCH
ASSISTANT

Applications are invited for a postdoctoral Research Assistant to work in collaboration with Dr. J. W. Tucker on a theoretical investigation of phonon scattering by paramagnetic ions. The appointment, which is financed by the Science Research Council, will be for a period of two years. The post is available from August 1, 1974. Initial salary £1,929 a year rising to £2,118 on October 1, 1974 with F.S.S.U. provision. Applications, stating qualifications and experience together with the names and addresses of two referees should be sent as soon as possible to Dr. J. W. Tucker, Department of Physics, The Hicks Building, The University, Sheffield S3 7RH. Quote Ref R. 125/G. (675)

UNIVERSITY OF THE WEST INDIES
TRINIDAD

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP IN AGRICULTURAL EXTENSION in the Faculty of Agriculture. Appointee should be a specialist in Agricultural Extension and should have experience in teaching and research in the field. Salary scales: (a) TT\$17,304 to TT\$25,824 p.a. (b) TT\$12,612 to TT\$20,316 p.a. (£1 sterling=TT\$4.8). F.S.S.U. Unfurnished accommodation at rent of 10% of salary for maximum of three years, thereafter 20% salary in lieu of housing. Family passages; study leave. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible to the Secretary, University of the West Indies, St. Augustine, Trinidad. Further particulars will be sent to all applicants. (678)

ZOOLOGISTS

Zoology graduates are invited to join the editorial team working on Aquatic Sciences and Fisheries Abstracts, an international publication. Starting salary is £1,725 per annum. Write to: Dr. E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London W1V 5FG. (682)

UNIVERSITY OF MELBOURNE
LECTURESHIP
(LIMITED TENURE—3 YEARS)
DEPARTMENT OF PHYSIOLOGY

Applications are invited for this position. Qualifications: Applicants should have teaching and research experience in Physiology, and a Ph.D. in Physiology, a medical degree, or an equivalent qualification.

Duties: The appointee will participate in the departmental teaching activities, including the organising and supervision of laboratory exercises for undergraduate students.

It is anticipated, but not essential, that the appointee's research interests relate to one of the fields being studied at present in the department; these include lipid metabolism, renal, respiratory and cardiovascular function, haematology and physiology of the nervous system with emphasis on sensory processes.

Salary: \$A9,002 to \$12,352.

Commencing date: January 1, 1975 or as soon as possible thereafter.

Further details may be obtained from Professor I. Darian-Smith, Department of Physiology in the University.

Conditions of appointment and application procedure from the Registrar, University of Melbourne, Parkville, Victoria 3052, Australia, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Applications close on September 6, 1974.

(684)

MEMORIAL UNIVERSITY OF
NEWFOUNDLAND
Department of Geology

Applications are invited for the post of RESEARCH ASSOCIATE (ELECTRON PROBE MICROANALYSIS).

Duties: Will include routine care, maintenance and supervision of an automated electron probe micro-analyser. The appointee will also be required to train microprobe users and assist in the preparation of specimens.

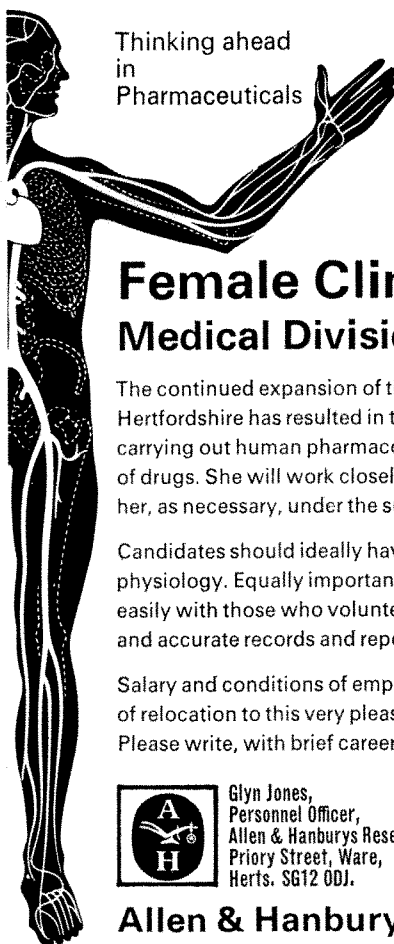
Qualifications: An appropriate degree or diploma, preferably an M.Sc. or Ph.D. Experience in electronics, microprobe analytical techniques and computing procedures would be an advantage.

Tenure: Initially for a two-year period.

Salary: To be negotiated, depending on qualifications and experience.

Application: Written application stating full personal particulars and details of qualifications and experience together with names and addresses of three referees should be sent to Dr. K. D. Collerson, Department of Geology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada. (685)

Thinking ahead
in
Pharmaceuticals



Female Clinical Assistant Medical Division

The continued expansion of the activities of our Medical Division at Ware in Hertfordshire has resulted in the need to recruit a graduate, who will assist in carrying out human pharmacological investigations associated with the testing of drugs. She will work closely with a Senior Scientific Officer and deputise for her, as necessary, under the supervision of a Senior Medical Officer.

Candidates should ideally have a background in pharmacology and/or physiology. Equally important is the ability to understand and communicate easily with those who volunteer to take part in the tests, and to maintain concise and accurate records and reports of her work.

Salary and conditions of employment are attractive. Assistance with the cost of relocation to this very pleasant rural area will be given, where appropriate. Please write, with brief career details, to



Glyn Jones,
Personnel Officer,
Allen & Hanburys Research Ltd.,
Priory Street, Ware,
Herts. SG12 0DJ.



Allen & Hanburys
MAKERS OF FINE PHARMACEUTICALS

(689)

Lakehead University

THUNDER BAY, ONTARIO, CANADA

NUTRITION AND FOOD SCIENCE

Applications invited for position, rank open, in Nutrition and Food Science; Ph.D. or equivalent, with concentration in community nutrition preferred—to teach undergraduate courses, conduct research and assist in curriculum development.

Salary commensurate with experience and rank. 1974 floors: Professor \$21,630; Associate Professor \$17,235; Assistant Professor \$13,865.

Send curriculum vitae and names of three referees to:

Mr Donald E. Ayre
Secretary of the University
Lakehead University
THUNDER BAY, Ontario
P7B 5E1

(687)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the following vacancy:—

LECTURE

in Stratigraphy in the Department of Geology. Salary Range: £2,118 to £4,896. Duties to commence January 1, 1975. Closing date September 14, 1974.

Applications, together with the names and addresses of two referees, should be forwarded to The Registrar, University College, P.O. Box 78, Cardiff, CF1 1XL, from whom further particulars may be obtained. Please quote ref. 0628.

(689)

UNIVERSITY DURHAM DEPARTMENT OF BOTANY

An S.R.C. Research Technician (Grade 3) is required for 3 years from October 1, 1974, to work with Dr. J. A. Pearson and Mr. G. H. aBnury on ferritin and nucleic acid synthesis in *Phycomyces blakesleeanus*. A graduate with experience in microbial biochemistry would be preferred, but other suitably qualified persons may apply. Starting salary £1,605 p.a. plus threshold payments.

Applications in writing, naming two referees, should be sent to the Deputy Personnel Officer, University of Durham, Old Shire Hall, Durham, DH1 3HP, to arrive by Friday, August 23.

(692)

Research Biologist

Cell Biology is one of the newer activities of our Research Function, involving cell and tissue culture and microbiological work. We are now seeking a senior technical assistant to join a small well knit group working in these fields.

The main scientific duties of the appointee would be concerned with fermentation and antimicrobial testing. In addition, he would be responsible for several junior technicians and for general laboratory management of the Cell Biology Unit.

The senior assistant is likely to be aged 25-35 with at least ONC and preferably HNC in applied biology. Experience of either fermentation or antifungal/antibacterial screening over a period of at least 3 years would be a distinct advantage although general experience in a microbiological laboratory would also be useful.

If you would like to be considered for this post please telephone (Welwyn Garden 28128) or write to the Personnel Manager quoting reference RH25 for a Confidential Record Form and further information on the Company.



Roche Products Limited
Welwyn Garden City Hertfordshire AL7 3AY

(706)

THE UNIVERSITY OF THE WEST INDIES—TRINIDAD EXECUTIVE DIRECTOR

PROPOSED CARIBBEAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE

Applications are invited for the post of Executive Director of the Caribbean Agricultural Research and Development Institute (CARDI) which is to be established by the member Governments of the Caribbean Community as the successor organisation to the Regional Research Centre of the Faculty of Agriculture of the University of the West Indies. The Institute has been established to serve the research and development needs of the region and will have its headquarters at the St. Augustine, Trinidad, campus of the University of the West Indies.

Applicants should possess a good degree in agriculture or one of its related fields. A higher degree, though not essential, would be a distinct advantage.

The person appointed will have had considerable experience in tropical agriculture or agricultural research and development and/or be an administrator of exceptionally high calibre. Experience with the region would be an advantage.

The Executive Director will be responsible for the day to day control, management and administration of the Institute.

The appointment will be on contract for five years in the first instance. Salary will be negotiable based on qualifications and experience. Other allowances are payable. A gratuity in lieu of pension will be paid. Unfurnished accommodation will be provided at a cost of 10% of salary. Alternatively a housing allowance of 20% salary will be paid in lieu of accommodation. Four weeks annual leave will be granted. Up to five full passages will be provided on appointment and on normal termination.

The person appointed would be expected to assume duties as soon as possible and preferably by October 1974.

Applications giving full details of date of birth, marital status, qualifications and experience and the names and addresses of three referees should be sent by airmail, as soon as possible to the Secretary, University of the West Indies, St. Augustine, Trinidad from whom further particulars can be obtained.

(676)

UNIVERSITY OF RHODESIA LECTURESHIPS IN THE DEPARTMENT OF BOTANY

Applications are invited for the posts of LECTURER IN MICROBIOLOGY AND LECTURER/SENIOR LECTURER IN PLANT PHYSIOLOGY. Special consideration will be given to applicants for the microbiology post with experience in plant virology and serology. The appointee to the plant physiology post will have prime responsibility for all teaching and research in plant physiology.

Salary Scales (Approximate £ Sterling equivalents): Senior Lecturer: £5,484 by 219 to £7,239. Lecturer Grade I: £4,984 by 184 to £5,720. Lecturer Grade II: £3,071 by 158 to £3,545 by 175 to £3,895 by 185 to £4,635 by 174 to £4,809.

Family passages and allowance for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel allowance. Superannuation and medical aid schemes.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience and publications, and naming three referees, should be submitted by **August 31, 1974**, to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. Applicants from outside Southern Africa should send a copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. (687)

UNIVERSITY OF DURHAM DEPARTMENT OF PHYSICS

Applications are invited for the post of Post Doctoral Research Assistant from October 1, 1974 or January 1, 1975. The successful candidate will be expected to pursue a research and development programme involving the application of neon flash tubes to nuclear particle detection with special reference to high energy γ -radiation. The programme will involve work at the Science Research Council's high energy accelerators.

The appointment, which is funded by the Science Research Council, will be for a period of two years.

The salary will be on the scale from £2,055 to £2,793 with F.S.S.U. benefits.

Applications (3 copies) including the names and addresses of three referees should be sent by September 2, 1974, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained. (691)

DEPARTMENT OF BIOPHYSICS TECHNICIANS Grade 3

required to assist with research project as follows:

(1) **BIOCHEMISTRY TECHNICIAN** with experience in protein separation techniques.

(2) **ELECTRON-MICROSCOPE TECHNICIAN** with experience in autoradiography.

Salary in range £1,650 to £1,920 plus £228 London Weighting plus Threshold.

Application form from Personnel Officer (N) (Technical Staff FD1), University College London, Gower Street, WC1E 6BT. (696)

CHELSEA COLLEGE UNIVERSITY OF LONDON

(In association with St. George's Hospital Medical School and the Royal Dental Hospital of London School of Dental Surgery)

LECTURER IN BIOCHEMISTRY

Applications are invited for the post of LECTURER in the Biochemistry Department of the Basic Medical Science Group of Chelsea College, from October 1, 1974. The person appointed must be willing to become wholeheartedly involved in the undergraduate and postgraduate teaching of an active expanding department, and be prepared to participate in the work of the other sections of the Group and of the collaborating Medical and Dental Schools. The successful candidate will also be expected to maintain an active commitment to research, preferably in collaboration with other workers in the Group or associating Schools. Salary Scale: £2,118 to £4,896 per annum plus £213 London Allowance. Further particulars and application forms from the Personnel Officer N, Chelsea College, Manresa Road, London SW3 6LX. Closing date August 28, 1974. (697)

THE UNIVERSITY OF MANCHESTER

Department of Medical Oncology
(Christie Hospital and Holt Radium Institute)

Applications are invited for the following posts in the new Department of Medical Oncology at Christie Hospital.

1: LABORATORY TECHNICIAN

Duties will include cryopreservation of cells and assistance with research in the field of human tumour immunology. Applicants should have an O.N.C. or equivalent qualification and at least 3 years' background experience preferably in tissue culture or cellular immunology. Salary scale £1,650 to £1,920 per annum.

2: MEDICAL LABORATORY TECHNICIAN IN HAEMATOLOGY

Work will involve a number of research projects in the field of tumour immunology in patients with malignant diseases of the haematopoietic system. Applicants should have the A.I.M.L.T. in haematology and at least 2 years' experience in haematology following qualifications. Commencing salary up to £1,905 per annum on scale for Medical Laboratory Technicians (£1,557 to £2,451 per annum).

Applications with full details of age, qualifications and previous experience should be sent to Professor D. Crowther, Dept. of Medical Oncology, Christie Hospital and Holt Radium Institute, Withington, Manchester M20 9BX. (688)

UNIVERSITY OF OXFORD DEPARTMENT OF BIOCHEMISTRY Research Assistantship in Marine Biochemistry

Applications are invited for an S.R.C. post-doctoral research assistantship to work on the control of fat metabolism in fish and other lower animals for three years. The assistant would spend the first year in the Biochemistry Department, Oxford, learning and developing techniques associated with enzymes of fat metabolism. The following two years would be spent at the Marine Biology Laboratory, Plymouth. It is expected that close collaboration between Oxford and Plymouth will be maintained during the course of this work. Preference will be given to candidates who have had a research training in Biochemistry but who have also had some experience of Zoology or Marine biology.

The salary will be on the scale £1,920 to £3,636 p.a. with F.S.S.U. membership.

Applications, giving brief curriculum vitae and names of two referees, should be addressed to Dr. E. A. Newsholme, Department of Biochemistry, South Parks Road, Oxford. The closing date for applications is September 14, 1974. (693)

NATIONAL INSTITUTE FOR MEDICAL RESEARCH MILL HILL—LONDON

GRADUATE BIOCHEMIST required in the Division of Biophysics to assist in the research of enzyme structure and function. Experience in enzyme preparation and assay preferred. Salary on scale £1,650 to £2,343 p.a. plus £125 p.a. threshold agreements. Please apply, quoting ref: JTO/BP to J. H. Woodcock, Personnel Officer, National Institute for Medical Research, The Ridgeway, Mill Hill, NW7 1AA. Tel: 959-3666. (701)

BIRKBECK COLLEGE (University of London)

RESEARCH ASSISTANT IN PALAEOBOTANY

Applications are invited for a post of Research Assistant in Palaeobotany and Palynology. Honours degree in Botany required, ideally with experience in palaeobotanical methods.

Salary within range £1,686 to £2,412; appointment for up to three years, beginning October 1, 1974.

Further details and application forms from the Deputy Secretary, (N), Birkbeck College, Malet Street, London WC1E 7HX. Closing date August 31, 1974. (695)

UNIVERSITY OF BRITISH COLUMBIA DEPARTMENT OF PHARMACOLOGY

Two assistant or associate professors to be appointed by July 1, 1975:

- (a) one PHARMACOLOGIST, PhD and/or MD;
 - (b) one CLINICAL PHARMACOLOGIST, MD and PhD or equivalent plus specialty qualifications.
- Salary to be negotiated. Duties include teaching and research. Applications including curriculum vitae and the names of three referees to be sent to Dr. M. C. Sutter, Head, Department of Pharmacology, University of British Columbia, Canada, V6T 1W5, by December 1, 1974.

An Equal Opportunity Employer, M/F. (702)

CSIRO AUSTRALIA DIVISION OF ANIMAL HEALTH SYDNEY, N.S.W. RESEARCH SCIENTIST

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: EXPERIMENTAL PATHOLOGY

General: The Division has three major laboratories—the Animal Health Research Laboratory, Parkville, Victoria; the McMaster Laboratory, Sydney, N.S.W.; and the Long Pocket Laboratories, Indooroopilly, Queensland. The appointee in this instance will be located at the McMaster Laboratory, the Divisional centre for studies into gastro-intestinal disease of which helminths of sheep and cattle constitute a major component.

Duties: To work on aspects of gastro-intestinal pathology with particular reference to the scouring diseases of young stock and enterotoxaemia in sheep and cattle. To collaborate and interact with scientists studying the pathophysiology of helminth infections and with helminth immunologists currently working at the McMaster Laboratory.

Qualifications: A degree in veterinary science or equivalent qualifications, and a Ph.D. degree in a relevant discipline or equivalent qualifications, supported by satisfactory evidence of research ability and experience in bacteriology.

Salary: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a.

Tenure: An indefinite or fixed-term appointment carries Australian Government Superannuation benefits.

Applications stating full personal and professional details, the names of at least two professional referees, and quoting Reference Number 201/461, should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
64-78, Kingsway, London WC2B 6BD.

by the 6th September, 1974.

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A. (700)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY BABRAHAM, CAMBRIDGE, CB2 4AT SCIENTIFIC OFFICER

required in the Director's Unit. Applicants should have a pass degree, H.N.C. or equivalent. The successful candidate will assist in an interdisciplinary research program involving the use of electrophysiology and histological techniques, bioassay of hormones and specialised care of experimental animals. Experience in one or more of these fields would be an advantage, but enthusiasm and adaptability are regarded as of equal importance.

Salary in the scale £1,592 to £2,675 according to qualifications and experience. There is a non-contributory Superannuation Scheme. Applications with full details should be sent to the Secretary of the Institute as soon as possible quoting reference D.U.2. (703)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF ZOOLOGY

A position exists immediately for a POST-DOCTORAL RESEARCH WORKER in mammalian cell mutagenesis to join a group working on the cell cycle. Applicants should have a Ph.D. and experience in cell culture techniques and mutagenesis; some biochemical experience would be useful. The post is funded by grants from the Cancer Research Campaign and will initially be for two years with the possibility of further support. Salary within the range £2,800 to £3,800 p.a. according to age and experience, and superannuation with the F.S.S.U.

Written applications giving the names of two referees and including a curriculum vitae should be sent to Dr R. T. Johnson, Department of Zoology, Downing Street, CAMBRIDGE CB2 3EJ. (709)

Senior Technician

£2346-£3147

Applications are invited from persons qualified to HNC or HND level and possessing relevant experience for the post of Head Technician in the Division of Bacterial Products in this Institute. The primary functions of the Division are to control, on behalf of the DHSS, the quality of all bacterial vaccines and antibacterial sera used in the U.K. This work is integrated with a related research programme.

The person qualified will be responsible for the smooth and effective operation of all technical services in the Division and will, in addition, undertake a defined aspect of the work under the supervision of a member of the Senior Scientific Staff.

Please apply, giving brief details (an application form will be sent to you) quoting reference 0035 to R.S. Dunn, Personnel Officer, National Institute for Biological Standards & Control, Holly Hill, Hampstead, London, NW3 6RB Telephone: 435 2232.

NIBSC

National Institute for Biological Standards and Control

(699)

NATIONAL COAL BOARD

Mine Geologists

Applications are invited for the post of Mine Geologist, each to serve 2 or 3 collieries in the East Pennine Coalfields.

The successful candidates will be responsible for recording geological information and participating in investigations into the effects of the geological environment on mining operations. The work will mainly involve underground duties.

Applicants should have an honours degree in Geology. Training in underground geological techniques will be given to the successful candidates.

Starting salary will be paid, in accordance with qualifications and experience, within the scale £2,345-£2,885.

Write for details to:—

**Recruitment Education and Training Branch,
National Coal Board,
Hobart House,
Grosvenor Place,
London SW1X 7AE**

NCB

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY
BABRAHAM, CAMBRIDGE, CB2 4AT
HIGHER SCIENTIFIC OFFICER

required in the Department of Applied Biology to work in a laboratory concerned with Animal Behaviour and Physiology. Minimum qualifications pass degree or H.N.C. and considerable laboratory experience preferably in one or more of the following subjects: physiology, experimental psychology, ethology. Salary in scale £2,461 to £3,371. The post is pensionable. A new modern superannuation scheme is being devised. Application forms and more details may be obtained from the Secretary of the Institute. Please quote reference B.A.4. Closing date August 25, 1974. (704)

UNIVERSITY OF MELBOURNE CHAIR OF ELECTRONICS AND COMMUNICATIONS

The University of Melbourne invites applications for the newly created Chair of Electronics and Communications, which is a second chair in the Department of Electrical Engineering. The appointee will be expected to develop teaching and research and to participate in departmental and faculty activities.

Salary: \$A19,614 per annum.

Further information about the position, application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment is available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on **November 1, 1974.**

(707)

CONNECTIVE TISSUE RESEARCH

A post-doctoral position is available for a young biochemist to collaborate in studies on connective tissue catabolism. A first-class background is essential for this interesting and varied research programme and a knowledge of some aspect of connective tissue research would be an advantage. The position would be most suitable for someone who has recently finished his thesis.

The appointment is for one year in the first instance, and may be renewable. Salary on M.R.C. scale depending on qualifications and age. Write, with full curriculum vitae and the names of two referees to Dr. John J. Reynolds, Strangeways Research Laboratories, Wort's Causeway, Cambridge CB1 4RN. (705)

FELLOWSHIPS AND STUDENTSHIPS

THE POLYTECHNIC OF NORTH LONDON CHEMISTRY DEPARTMENT

POST-DOCTORAL RESEARCH FELLOWS

Applications are invited from suitably qualified research workers for two Research Fellowships, to work on the following projects, which are supported by the Science Research Council.

1. A Biogenetically-patterned Corrin Synthesis. Experience in synthetic organic chemistry would be an advantage for this project.
2. Structural Studies of the Coordination Template Effect of Metal Ions. Experience of X-ray crystallographic work is desirable for this project.

The appointments will be temporary, initially for two years. The salary scale is £2,151 by £90 to £2,421 and paid part-time teaching work may be available in addition.

Applicants should reply giving details of qualifications and experience, and the names of two referees to Dr A. P. Johnson (for post 1) or Dr. P. G. Owston (for post 2), Chemistry Department, The Polytechnic of North London, Holloway Road, London N7 8DB. (634)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for the following:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

POSTS IN THE DEPARTMENT OF GENETICS

Several positions are available in the Department (Head: Professor W. Hayes, F.R.S.), for appointment as Senior Fellow, Fellow, Research Fellow or Postdoctoral Fellow, to initiate a programme of research on regulation and differentiation in lower eukaryotes. Applicants should have experience of biochemical as well as genetical methods. The department, with about twelve academic staff, is well equipped and has only research responsibilities. Current research topics include regulatory mechanisms in phage, bacteria and *Neurospora*; developmental genetics and transgenesis in plants; and evolutionary studies on bacteria and vertebrates.

Closing date: **September 14, 1974.**

FELLOW: DEPARTMENT OF GENETICS

Applicants should have special knowledge of, and expertise in, bacterial and phage genetics with particular reference to membrane biology.

Closing date: **August 30, 1974.**

Salaries: Salary on appointment to the posts will be in accordance with qualifications and experience within the ranges: Senior Fellow \$A14,724 to \$A16,921 p.a.; Fellow \$A10,771 to \$A14,704 p.a.; Research Fellow and Postdoctoral Fellow \$A9,002 to \$12,269 p.o. Current exchange rates are approximately \$A1=67p=US\$1.49.

Other Conditions: Tenure: Senior Fellow and Fellow for five years in the first instance with the possibility of extension to retiring age; Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years; Postdoctoral Fellow for not less than one year and not more than two years. Postdoctoral Fellows would normally have recently completed the Ph.D. degree.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation (where applicable) is on the F.S.S.U. pattern with supplementary benefits.

Travel expenses from overseas (except New Zealand) are not provided for posts at the level of tutor but assisted passage can be considered for a person offered appointment from the United Kingdom who intends to settle permanently in Australia.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should write to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (636)

LA TROBE UNIVERSITY

MELBOURNE, AUSTRALIA

RESEARCH FELLOW IN GENETICS AND HUMAN VARIATION

(one or two positions)

Applicants will be appointed for one or two years, with possible extensions up to a total tenure of three years. They will be expected to work in one of the areas of interest of the Department which include behavioural, cellular, ecological, human, microbial, population and quantitative genetics and cytogenetics. Enquiries may be directed to Professor P. A. Parsons, Chairman of the Department, in the University.

Salary: \$A7,545 to 2 by \$A292 to 3 by \$A291 to 4 by \$A479 to 5 by \$A478 to \$A12,352.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, La Trobe University, Bundoora, Victoria, Australia 3083.

Applications close on **September 13, 1974.**

(529)

THE CITY UNIVERSITY DEPARTMENT OF CHEMISTRY

Applications are invited for a

POSTDOCTORAL RESEARCH FELLOWSHIP

for work on the combustion and flammability of hydrocarbon mists. The Fellowship will be available for one year in the first instance, and the initial salary will be about £2,500. Further details may be obtained from Professor C. F. Cullis, Department of Chemistry, The City University, St John Street, London EC1V 4PB, to whom applications (together with the names of two referees) should be sent before August 31, 1974. (606)

European Molecular Biology Organization (EMBO)

Short- and long-term fellowships in molecular biology

The European Molecular Biology Organisation intends to award to scientists working in laboratories within the European area both short-term (from a few days to several weeks) and longer-term fellowships for collaborative research or advanced training in molecular biology.

The Short-term Fellowships are to support visits to other laboratories for the purpose of carrying out experiments with special techniques or of other forms of scientific collaboration or advanced training, and especially to support developments arising at short notice.

The Long-term Fellowships will usually be for a period of one year, but applications for renewal will be considered. They will be awarded upon individual application, at the "junior" level to promising young research workers who may then spend prolonged periods in other laboratories working under the guidance of leaders in the field of molecular biology. At the "senior" level, they may be awarded to enable established research workers to gain experience in new approaches and new problems. Upon application by European Institutions fellowships at the "senior" level may also be awarded to specialists who can assist in the initiation and development of research programmes in the sponsoring Institution. Such "sponsored" awards may be made to established workers at all stages of their career beyond the postdoctoral stage.

Application forms and further details may be obtained from Dr J. Toozé, Executive Secretary, European Molecular Biology Organisation, 6900 Heidelberg 1, Postfach 1022.40, Germany.

(521)

UNIVERSITY OF LEEDS

DEPARTMENT OF INORGANIC AND STRUCTURAL CHEMISTRY

Applications are invited from suitably experienced chemists or physicists for a post of **POSTDOCTORAL FELLOW** to investigate the mechanisms of **FAST IONIC TRANSPORT IN INORGANIC SOLIDS** primarily by utilising the radioactive tracer technique supported by possible evidence of defect structure from high temperature diffraction measurements, and interpreted in conjunction with the resonance studies already being undertaken.

S.R.C. funds are available for 2 years from October 1, 1974; salary on the scale £2,118 to £2,412 plus F.S.S.U., depending on age and experience. Further information from Dr A. T. Howe, Dept. of Inorganic and Structural Chemistry, The University, Leeds LS2 9JT, to whom applications, including the names of 2 referees, should be sent as soon as possible. (608)

UNIVERSITY OF LIVERPOOL

UNIT OF REPRODUCTIVE BIOLOGY

Applications are invited from honours graduates who are interested in physiological research and who have a degree in either Physiology or Zoology, for a graduate studentship in this Unit.

Applications should be received as soon as possible by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/276164/N. (638)

UNIVERSITY OF MANCHESTER

Department of Geology
in collaboration with

Selection Trust Ltd.

N.E.R.C. C.A.S.E. Studentship

Applications invited for a studentship tenable from September to work for a higher Degree on the relationship between mineralisation and geological setting in Carboniferous rocks. Applicants should hold an Honours Degree or equivalent qualification in an appropriate discipline; some mineral exploration experience an advantage. Applications with curriculum vitae and names of two referees as soon as possible to the Secretary, Department of Geology, The University, Manchester M13 9PL, from whom further details are available. (665)

UNIVERSITY OF WESTERN AUSTRALIA PERTH

UNIVERSITY RESEARCH FELLOWSHIPS (POSTDOCTORAL)

Five Research Fellowships will be offered, to be taken up as soon as possible. Appointment will be for one year in the first instance with the possibility of renewal for a second year. The Fellowships could possibly be renewed also for a third year, but in competition with any new applications. They will be tenable in the following academic departments for work in the areas stated below:

Italian: Italian High Renaissance, c. 1480-1520 (1600).

Philosophy: Locke.

Economics: Economic Theory.

Politics: Evaluation of federal-state politics in government in Australia.

Mechanical Engineering: Environmental Fluid Mechanics OR Control Systems.

Electrical and Electronic Engineering: Integrated Optics—Optical Communications.

Botany: Plant Fine Structure.

Psychology: Child Development OR Vision.

Biochemistry: Mechanisms of Lactation and

Parturition OR Developmental Biochemistry.

Microbiology: Antigenic Variation in Viruses.

The Fellowships are intended primarily for Ph.D. graduates (normally from other universities), or those with equivalent qualifications, who by publication and in other ways have demonstrated significant research capability. Initial salary will be within the range \$A7.54 to \$A9,002 p.a., and appointees may in certain circumstances be considered for admission to an F.S.S.U. superannuation scheme. Fellows may be given the opportunity to participate in teaching. An overseas appointee would be entitled to appointment expenses of up to \$A1,000 and an appointee from within Australia to fares for self and spouse.

Applications in duplicate setting out full personal particulars, qualifications and experience, and describing the details of the research interest should reach the Staffing Officer, University of Western Australia, Nedlands, Western Australia 6009, by **September 30, 1974**. Candidates should request three referees to write immediately to the Staffing Officer. (645)

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF BIOCHEMISTRY
RESEARCH STUDENTSHIP IN PHYSICAL
BIOCHEMISTRY

Applications are invited from honours graduates in Chemistry and Biochemistry for studies on the physical biochemistry of enzymes. The nature of the award will be equivalent to an S.R.C. Studentship and the candidate will be able to register for a Ph.D. degree.

Applications giving curriculum vitae and the names of two referees should be sent by August 31st to Superintendent, Department of Biochemistry, Tennis Court Road, Cambridge CB2 1QW.

(X664)

BRUNEL
UNIVERSITY

(Department of Polymer Science
and Technology)

Applications are invited for the CEMENTATION CHEMICALS RESEARCH STUDENTSHIP from graduating and recently graduated chemists or material scientists.

Work will be in the field of solid polymer characterisation but aimed at products of civil engineering end use.

This studentship is unique in that very close liaison will be encouraged between the student/University and the industrial sponsor.

The work, initially for one year, will lead to the degree of M.Phil. The salary of £900 p.a. will be paid. In addition fees and reasonable expenses will be met.

Candidates—Male or Female should apply to:—

Mr G. R. Southern,
BRUNEL UNIVERSITY
Department of Polymer Science,
Kingston Lane,
Hillingdon, Uxbridge.

(708)

THE MEDICAL COLLEGE OF
ST BARTHOLOMEW'S HOSPITAL
WEST SMITHFIELD, LONDON EC1A 7BE
DEPARTMENT OF BIOCHEMISTRY
POSTDOCTORAL RESEARCH
FELLOWSHIP

for work of the Mechanisms of Action and Control of Thiol Enzymes.

Applications are invited for the above post tenable for 2 years from October 1, 1974, from graduates with a Ph.D. and experience in kinetic and/or fluorescence work with enzymes.

The successful applicant will work with Dr K. Brocklehurst on the reactivity characteristics of thiol groups in enzyme catalytic, regulatory and reporter sites. Use will be made of novel reagents tailor-made by Professor H. Suschitzky's groups in the University of Salford. Salary on the Scale £2,118 to £2,412 p.a. plus £213 London Allowance. Curriculum vitae and names of 2 referees to The Secretary of the Medical College, quoting reference 669.

(639)

UNIVERSITY OF EDINBURGH
RESEARCH FELLOW
DEPARTMENT OF THERAPEUTICS

A Research Fellow with or without medical qualifications is required for a research project for 2 years on cancer immunology relating to the immunological status of patients with lung or breast cancer and the assessment of immunological tests for cancer under the direction of Dr. W. J. Irvine, Immunology Laboratories, Department of Therapeutics, Royal Infirmary and 2 Forrest Road, Edinburgh.

The salary scale is for non-clinical or clinical lecturers (£2,118 to £3,474) according to age, qualifications and experience.

Applications, together with the names of two referees, should be sent to the Secretary to the University, University of Edinburgh, Old College, South Bridge, Edinburgh, EH8 9YL. Please quote reference number 5042.

(694)

AUSTRALIAN NATIONAL
UNIVERSITY

Applications are invited for appointment to the following:

RESEARCH SCHOOL OF BIOLOGICAL
SCIENCES

PROFESSORIAL FELLOWSHIP
Department of Neurobiology

The Department (Head: Professor G. A. Horridge) has an active programme of research in the neural basis of behaviour and perception in lower animals, mainly insects and crustacea. Applicants should be capable of a strong research programme in an aspect of neurobiology which is either complementary or supplementary to the present interests, which include: (a) mechanisms of arthropod vision; (b) growth and regeneration of insect nerves; (c) control of movement in crustaceans; (d) biochemistry of insect neurons; (e) insect sound production and hearing; (f) establishment of connections between nerve cells.

The University is looking for a man with an ambitious project who finds himself restricted by lack of assistance, research time or equipment. Vertebrate neurobiology is not excluded. The position is tenured, with opportunity to train graduate students.

Closing date: **September 21, 1974.**

JOHN CURTIN SCHOOL OF MEDICAL
RESEARCH

SENIOR RESEARCH FELLOW,
FELLOW OR SENIOR FELLOW IN
PHYSICAL BIOCHEMISTRY

Appointment will be in the Department of Physical Biochemistry (Head: Professor L. W. Nichol). Applicants should be interested in setting up a small research group to carry out theoretical and experimental studies involving biological macromolecules or systems. The Department is well equipped for the study of macromolecular properties in solution; the fields of nuclear magnetic resonance spectroscopy and X-ray crystallography will not be pursued as major interests within the Department in the near future.

Closing Date: **October 21, 1974.**

SALARIES: Salary for a Professorial Fellow is \$A18,131 p.a. Salary on appointment to the other post will be in accordance with qualifications and experience within the ranges: Senior Fellow \$A14,724 to \$16,921 p.a.; Fellow \$A10,771 to \$14,704 p.a.; Senior Research Fellow \$A13,163 to \$15,548 p.a.. Current exchange rates are approximately \$A1: 67NP: \$US1.49.

OTHER CONDITIONS; tenure: Professional Fellow to retiring age (65 years); Senior Fellow and Fellow for five years in the first instance with the possibility of extension to retiring age; Senior Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should apply to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (686)

NATIONAL RESEARCH
DEVELOPMENT COUNCIL
N.R.D.C. FELLOWSHIP

Applications are invited for an N.R.D.C. Fellowship. The successful candidate will work under the direction of Dr K. Jewers at the Tropical Products Institute on the isolation and transformation of biologically active compounds from tropical plants. The Fellowship is tenable for one year in the first instance with the possibility of renewal subject to satisfactory progress being made.

Applicants should have a degree or equivalent in chemistry plus at least two years postgraduate experience.

Salary in the region of £2,689 per annum plus an annual leave allowance of 22 days.

N.R.D.C. RESEARCH ASSISTANTSHIP

Applications are also invited for an N.R.D.C. Research Assistantship. The successful candidate will work under Dr K. Jewers at the Tropical Products Institute on the pharmacology of biologically active constituents isolated from tropical plants. This post is also tenable for one year in the first instance with the possibility of renewal for a further period subject to satisfactory progress being made.

Applicants should have a degree or equivalent in pharmacology. Salary £1,820 to £2,040.

Application forms for both these posts from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 8DB. (635)

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Studies in History and Philosophy of Science is a quarterly journal which analyses the genesis, evolution and logic of scientific thought. It attempts to unite historical and philosophical approaches to science.

Recent papers include:

Maxwell's Methodology and his application of it to Electromagnetism, A. P. Chalmers. **The Astronomy of Eudoxus: Geometry or Physics?**, Larry Wright. **Conceptual Structures and Scientific Change**, Garry Gutting. **Whewell's Theory of Scientific Language**, Morton L. Schagrin. **Speculation in Physics: The theory and practice of Naturphilosophie**, Barry Gower. **Feyerabend and Galileo: the interaction of theories, and the reinterpretation of experience**, Peter K. Machamer. Edited by Gerd Buchdahl, Reader in History and Philosophy of Science, University of Cambridge and Professor L. L. Laudan, Chairman, History and Philosophy of Science, University of Pittsburgh. Published by Macmillan Journals Limited.

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Differentiation

Differentiation, published bi-monthly, provides the opportunity for interdisciplinary communication. This journal offers up to date information and synthetic views of a problem underlining the whole biological phenomenon — differentiation. It aims to cover the following areas of study: embryonic differentiation, normal cell growth and division, carcinogenesis and the cancer problem as an aspect of cell differentiation, inter-tissue reactions in vivo and in vitro, genetic mosaicism, nucleo-cytoplasmic interactions, nuclear transplants, cell hybridization, evolutionary biology, membrane controls of the cell, plant evolution and differentiation, immunological events relevant to differentiation.

Recent papers include:

Degree of differentiation in non-proliferating cells of mammary carcinoma, C. V. Wylie, P. K. Nakane and G. B. Pierce. **DNA synthesis and the production of antibodies by lymphoid tissues**, G. Harris. **The principle of sequential dependence in cellular differentiation**, P. A. Riley. Edited by: Dimitri Viza. Faculté de Médecine Pitié Salpêtrière, Paris.

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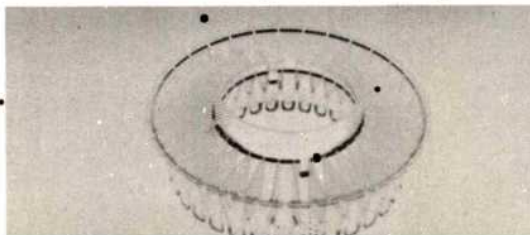
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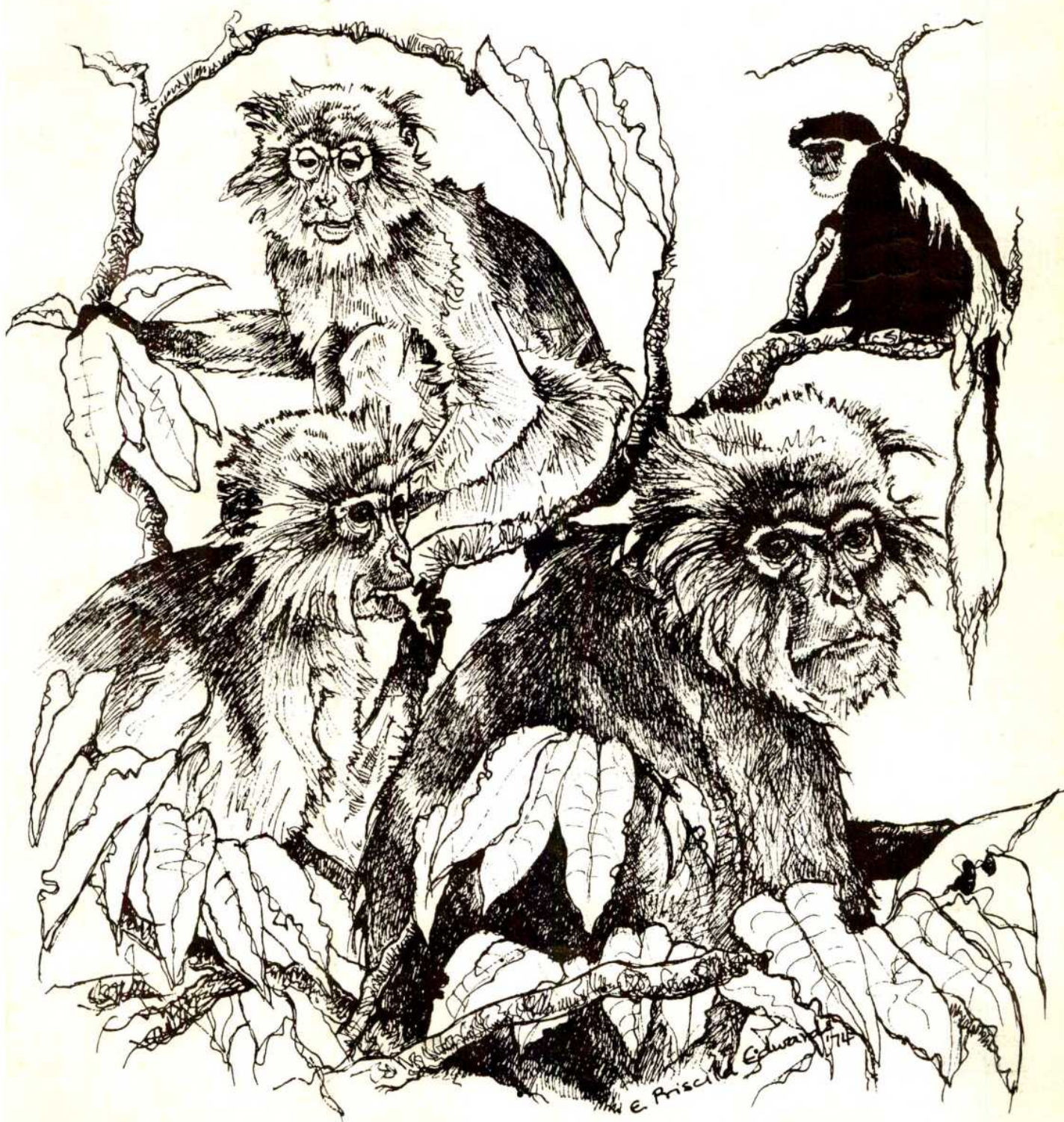
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A N.E.R.C.-funded C.A.S.E. studentship is available for a project concerning the effects of weathering on the physical and chemical properties of clays. The work, which will be submitted for a higher degree, is to be carried out partly in the Reading Geology Department and partly with the I.G.S. Engineering Geology Unit. Apply immediately to Dr R. Till, Geology Department, University of Reading, Whiteknights, Reading RG6 2AB. (Ref. M.N.40). (644)

REGIONAL NEUROLOGICAL
CENTRE,
NEWCASTLE UPON TYNE

Post-doctoral biochemists required for research work on biochemical aspects of neuromuscular disease. The posts are Research Associateships of the University of Newcastle upon Tyne and will be for one year in the first instance. Salaries will be at or near the bottom of the University Lecturers' scale. Experience in protein chemistry, enzymology, subcellular fractionation, and radio-active tracer techniques will be an advantage. Applications (with two referees) should be sent to Dr. R. J. T. Pennington at the Regional Neurological Centre, General Hospital, Newcastle upon Tyne, NE4 6BE, from whom further information may be obtained (Telephone: Newcastle 3-8811, Ext. 483). (681)

THE JACK CHARRINGTON
MEMORIAL FELLOWSHIP 1974/75

The closing date for applications for the above fellowship, as advertised in July 11, 1974 issue of this Journal, has been extended to August 31, 1974. (683)

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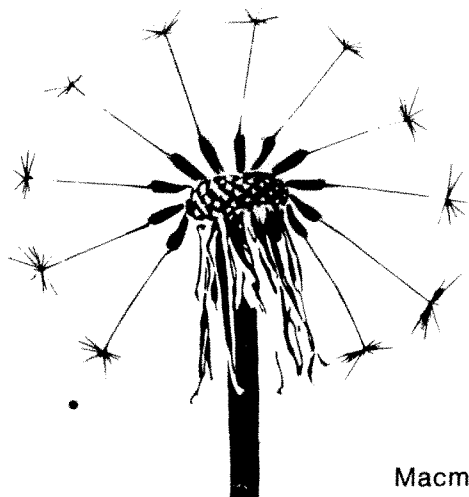
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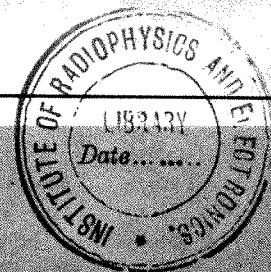
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Cover picture

Three red colobus monkeys feeding
with a black and white colobus
monkey in the background. Differ-
ences in the dispersal of their food
supply may account for the different
social organisations of these two
species, as discussed on page 539.

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- Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.

- Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).

- 'Matters Arising' permits occasional short discussion of papers that have previously appeared in *Nature*. A limit of 300 words is placed on contributions in this category.

Manuscripts may be submitted either to London or Washington. Three typed copies should be submitted, each including lettered copies of figures. Typing (including references) should be double spaced. The title should be brief and informative. Pages should be numbered. References, tables and figure legends should start on separate pages. Experimental detail vital to the paper yet which would interrupt the narrative is best placed in the figure legends. Units should conform to the *Système International*. Greek characters should be identified in the margin on their first appearance. Equations should occupy single lines if possible. $\exp(a)$ is preferred to e^a if 'a' is more than one character. Articles should be accompanied by an abstract of not more than fifty words, and the abstract should list the main conclusions that are drawn.

References are indicated by superscripts in the text. The style may be gleaned from any contemporary *Nature* with the following two changes:

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Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

A fuller guide appeared in *Nature* (246, 238; 1973).

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Cambridge : its manpower and money

THE University of Cambridge, in an annual gesture calculated to bring some relief to an editor beleaguered in the August silly season when government and universities vanish, has just released a small mountain of statistics. Two issues of the *Reporter*, issued on August 7, deal with student numbers and with sponsorship of research within the university. At ten pence, the pair provide a remarkably detailed profile of a university which has often claimed, with some justification, to be particularly distinguished in the sciences.

For several years Cambridge's undergraduate numbers have remained relatively constant. There is at the moment still a swing towards admitting more women but total numbers of students rose only 0.5% last year. On the other hand the numbers of those applying to enter is falling rather rapidly as Table 1 (compiled with the help of a *Reporter* of last year) shows. At present roughly half of those who apply get accepted, not long ago it was barely a third.

Table 1 Trends (percentages) in student numbers from 1973 entry to 1974 entry.

	Applications	Acceptances
English	-18	+3
History	-7	+3
Modern languages	-18	+2
Law	+5	+4
Mathematics	-9	+2
Natural sciences	-16	-10
Engineering	-8	+2
Medical sciences	-3	-1

The swing away from science is manifested in an interesting way. If we can assume certain standards of rationality in the selection procedure we must conclude that the swing is more in quality of student than in quantity of applications. All of this is well known on a national and international scale, and those in provincial universities can be excused a wry smile that Cambridge is beginning to feel the breeze that is a gale in their own laboratories. Once the trend has started downwards, though, the question must be whether the resources of Cambridge are capable of reversing it. The news can only be good for teachers of science in schools, getting increasingly used to being courted by other universities and now, at long last, to be wined and dined at High Table for the favours of their shrinking brood.

The other report puts together expenditures, by sources outside the university, of research projects for the financial year 1972-73. At the detailed level it is a delight. The Department of the Environment spent £150 with Applied Mathematics of "Effect of wind on people". The

Veterinary School received £522 worth of equipment and materials from "Broilers-various". The Science Research Council cheerfully gave £88,651 for "Observational and theoretical astronomy" but needed the justification of "Spectroscopic studies of some metal deficient and some strong line *g* and *k* type stars" before forking out another £40. And what does one make of the Ministry of Defence's total expenditure—£114,000 by our addition, £14,000 by the *Reporter's*? Misprint or deceit?

Table 2 Sources of research funding 1972-73.

	£
Government bodies	2,125,000
Charities, trusts, foundations	480,000
Overseas bodies	98,000
Four industries*	81,000
The rest of British industry	60,000
<i>Studentships and capital expenditure excluded</i>	

* Mullard, Beecham, Rolls-Royce, Tobacco Research Council.

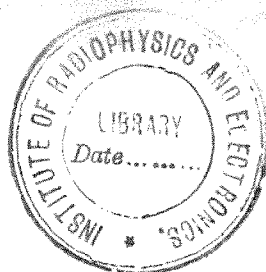
Table 2 brings together the sources—our interpretation and our addition. It is worrying, to say the least, that the university has such a restricted financial relationship with industry. Here, surely is a pointer to the mutual suspicion between industry and academics which is such a disagreeable feature of British scientific life. And here, also, is a good place from which an improvement in relations could grow. It would be quite wrong to point a blaming finger specifically at either side, but perhaps when the schoolteachers have had their meal at High Table there might be something left over for industrialists. If not, Cambridge's days as an outstanding university could be numbered.

100 years ago



M. MAREY has recently published the results of experiments undertaken to determine by the graphic method what is the true movement of the legs in walking. His results prove convincingly that the brothers Weber were wrong in assuming that the oscillation of the leg which is not in contact with the ground is the same as that of a pendulum; for when it is represented on a uniformly moving plane, the line drawn is a straight and not a curved one. The movement of the suspended foot is therefore uniform, depending on muscular action, in combination with that of gravity.

From *Nature*, 10, 306, August 20, 1874.



The legacy of the Nixon years

A few hours before he announced his resignation as President of the United States, Richard Nixon vetoed the appropriations bill for the Environmental Protection Agency because he considered it to be inflationary. It was a symbolic last act for an Administration which spent a good deal of time doing battle with Congress over spending priorities. Colin Norman discusses how, for that small section of the population known as the scientific community, which has often been caught in the middle of the budgetary battle, Nixon's last veto was a reminder of what has passed and probably a taste of things to come.

IN his five and a half years in the White House, Nixon did not exactly win the undying support of scientists for his handling of scientific affairs. In fact, throughout his Presidency, cries of alarm from parts of the scientific community have been clearly audible above the background grumblings from most of academe. Among other things, the departed president and his lieutenants have been accused of plunging science into a financial crisis, of relegating some scientific disciplines to second place in the world pecking order, of failing to foresee situations in which science and technology could have been marshalled to help out (such as the energy crisis) and even of ignoring science completely.

The record, however, is a good deal better than many of the criticisms allow, and a good case can be made for the argument that some of the disquiet in the scientific community has its origins in events which took place well before Nixon set foot in the oval office. And it can equally well be argued that many of the science policies and institutional arrangements laid down by President Nixon will survive long after his departure.

Underlying most of the criticisms of Nixon's stewardship of the scientific enterprise is the fact that federal expenditures on science and technology during the past five years have increased at a rate barely sufficient to keep pace with inflation, and in some cases funding has even declined. On top of that, the Administration has gone for some of the scientific community's most cherished programmes, of which biomedical training was perhaps the most prominent. And the final straw

came early last year when Nixon announced that he no longer needed a full-time science adviser in the White House, so he abolished that post along with the Office of Science and Technology and assigned some of its duties to the Director of the National Science Foundation.

Thus, the Nixon Administration's policies were clearly not designed to bring joy to the country's science and engineering laboratories. Nevertheless, scientists and technologists have fared rather better than their colleagues in many other disciplines, and the federal budget for science and technology, which stands at nearly \$19,000 million (\$4,600 million more than it was five years ago) is immense by any measure.

When the Nixon Administration came to power, the unquestioned growth of the science budget which took place during the Eisenhower and Kennedy Administrations had already come to a virtual halt. Thus, the first couple of years of so of Nixon's tenure were marked by painful adjustment from a period of burgeoning growth in science budgets to a period of almost static funding. One consequence was that the job market for scientists rapidly became very tight just when record numbers of scientists were emerging from the academic pipeline, a situation which did little to enhance the new Administration's standing in the eyes of the academic community.

The Administration's response was to cut back on funding for programmes designed to increase the supply of scientists and technologists. In 1971, for example, the proposal was made to eliminate the National Science Foundation's institutional support programme

and to phase out its graduate training programmes. Since those two items were highly cherished sources of funds for universities, which were then facing huge financial deficits, the Administration's academic support slipped a few more notches.

But the budget announced in January 1971 represented, for the first time since 1968, a real increase in federal support for research and development. This was carried through with another boost for science and technology in the budget announced a year later. But, in January 1973, just after his re-election, Nixon slammed the brakes on public expenditure and withheld considerable sums of money promised in the pre-election spending spree, with the result that funding for science again took a hammering. Nixon's support in the scientific community hit another low.

Finally, the budget announced just six months ago promised another round of increases in research and development expenditure, chiefly for the development of energy resources and for the Department of Defense.

The overall pattern of expenditure has therefore been one of stagnation, growth, cutbacks and growth—a situation which has not been conducive to university planning, and which has been unsettling for those who are forced to depend on the government for research grants. Whether or not the fabric of science in the United States has been damaged in the process, is, however, open to question.

The upshot of this slow and sporadic growth in the science budget has been to force a number of painful priority decisions in the basic sciences, as, for example, in space research where a number of promising satellite missions have been dropped, the High Energy Astronomy Observatory has been considerably scaled down in cost and performance and other missions have been deferred to accommodate development of the shuttle in a tight overall budget.

Similarly, particle accelerators have been shut down in order to accommodate the rapid growth in the budget of the National Accelerator Laboratory, and many research grant applications which have been designated as scientifically worthwhile by peer review groups at the National Institutes of Health have gone unfunded for lack of money. In the days of burgeoning growth, such painful choices probably not have been needed.

It has been in the biomedical science community, however, that the cries of anguish over the Administration's science policies have been loudest, and once again, the problems have been

Ford: inherits unhappy science community



White House science adviser
Guyford Stever (left)
and Edward E. David, jun. (right).



exacerbated by a painful transition from periods of rapid growth to stagnation.

Superimposed on the situation, however, is the fact that in the past three years two highly publicised crusades—against cancer and against heart disease—have been launched and enthusiastically supported by both Congress and the Administration. The result has been that the budgets of the National Cancer Institute and the National Heart and Lung Institute have been allowed to grow relatively rapidly, while other NIH institutes have been held back so that many are now receiving less money than they got in 1968. Moreover, biomedical scientists both at NIH and in the Universities have complained bitterly that the Administration has been trying to run the cancer and heart programmes like NASA-style operations to land men on the moon by highly targeted research programmes which have drawn money away from basic research.

On top of all those complaints, the biomedical community has been upset by repeated vetoes by President Nixon of the appropriations bills for the Department of Health, Education and Welfare, which have held up funds for months, and which have kept funding at the levels proposed by the Administration rather than at the more generous levels approved by Congress.

These funding decisions and policy changes have not, however, been taken entirely in a vacuum, for Mr Nixon has also made substantial changes in the science policy machinery in the top echelons of the federal government. In fact, it is those changes which have done most to upset the elder statesmen of the scientific community.

When he arrived in the White House, Nixon inherited an extensive science policy apparatus whose origins dated back to the second World War, but when he departed last week, virtually none of it was left intact.

On the President's immediate staff was a science adviser, and a small policy office called the Office of Science and Technology which provided him with staff support. Lines to the scientific community were kept open by means of the President's Science Advisory Committee, chaired by the science adviser, and filled with a raft of luminaries from the universities. Nixon appointed Lee A. DuBridge, President of MIT as his first science adviser.

DuBridge and the Office of Science and Technology never became a powerful force in the Nixon White House, however, partly because President's

Science Advisory Committee took a number of stands on military matters which were in direct opposition to the Administration's policies, and partly because the rest of the White House just wasn't interested. And, as the influence of the science apparatus waned, the power of the New Office of Management and Budget (OMB) increased, so that it is now the focal point through which White House policy is conveyed to the departments and agencies. It wields tremendous power through the budgetary process.

DuBridge was eventually succeeded by Dr Edward E. David, jun., an engineer from Bell Labs, who departed in January last year after trying to interest the rest of the White House in science, with little success. Within two weeks of David's departure, Nixon scrapped the Office of Science and Technology, the post of Science Advisor to the President and the President's Science Advisory Committee, and designated the Director of the National Science Foundation, Dr H. Guyford Stever, as science adviser to the White House.

Stever has since established in NSF a science policy office and an energy policy office, and he has been given a budget three times larger than that of the defunct Office of Science and Technology. He has also established lines to OMB, and is generally credited with doing a commendable job, given the limits to his power.

The changes irked many scientists, however, who felt that science had been downgraded in national affairs, and many people have also criticised the fact that Stever has specifically been given no mandate to advise on military technology. In response to such complaints, Dr Philip Handler, President of the National Academy of Sciences, established recently a special

blue ribbon panel under the chairmanship of Dr James Killian, Eisenhower's first science adviser, to look into the workings of the present science policy apparatus.

Not surprisingly, the Killian panel recommended that a science advisory council should be re-established in the White House, and that the present arrangement is unsatisfactory since Dr Stever, being head of a small science agency, is not in a strong enough position to orchestrate the vast federal science bureaucracy. Since there was absolutely no chance that Nixon would reinstate the post in the White House, the recommendations were clearly aimed at his successor.

Thus, President Gerald Ford has inherited an unhappy scientific community, a strong feeling among the scientific establishment that science should be reinstated in the White House, and a host of problems such as the energy crisis and food shortages which will require large injections of science and technology.

Clearly, the science policy apparatus is not going to be one of Ford's immediate concerns, and little change can be expected in the short term. Ford himself has said that his immediate concern is to curb inflation, however, and that, indirectly could have a bearing on science.

Ford is a self-confessed fiscal conservative, believing in balanced budgets and decreased federal expenditures. Since only a relatively small proportion of the federal budget can be decreased—the vast majority of the budget is for such items as salaries, pensions and welfare payments which cannot be tinkered with—and since science expenditures mostly fall into the controllable category, the pressure on the science budget is unlikely to decrease under President Ford.

Two approaches to scientific aid for disaster areas

Scientists and nonscientists alike are becoming increasingly aware of the need for science to be 'relevant'. In human terms, perhaps the most important applications of science are to the problems facing the less developed parts of the world, and the need for relevant science can be seen most clearly at times of crisis—such as the recent floods in Bangladesh. But how effectively is science being used in such situations? John Gribbin and John Wilson have been looking at two contrasting approaches to these problems.

ESTABLISHED charitable organisations such as Oxfam are making increasing use of science and technology in their work; it is becoming accepted that provision of food to survivors of a disaster is no more than a temporary solution to any problem, and that famine and disease can only be prevented by more fundamental help. But still, only a tiny fraction of Oxfam's income, for example, is spent on research — although that small budget seems to be used remarkably effectively.

Among projects Oxfam is working on are:

- A completely new sewage disposal system which can be flown straight to disaster areas and is, according to Oxfam, cheap, simple and easy to erect.
- A new building technique to provide rapid emergency housing after an earthquake, flood or other catastrophe.
- An investigation of bicycle 'pedal power' in the poorer countries.

These are all projects with a sound scientific pedigree. The sewage scheme, for example, required fundamental research on the biology of the cholera vibrio. But money allocated by Oxfam to such research was only £3,000 in 1973, out of a total 'income' from public donations of £4.2 million, more than 80% of which was spent overseas.

The Deputy Director of Oxfam, Mr Guy Stringer, explains that the best use is made of this £3,000 by using Oxfam money only to prime the financial pump of a project. Once a plan has shown potential, Oxfam seeks help from other sources. This falls into line with the organisation's avowed policy

of spending as much of the public's money as possible 'over there' rather than on research at home. And the people and firms that Oxfam approaches are eager to help. "They have never refused us", says Mr Stringer.

Oxfam undoubtedly benefits enormously from this goodwill; but part of its success must surely lie in the simple direct approach which it adopts. The charity first became interested in sanitation schemes as a result of its experience of the refugee camps in Bengal during the Indo-Pakistan conflict of 1971. Oxfam workers there realised that most of the relief effort was being spent on the treatment of diseases arising from the insanitary conditions within the camps. Even so, this preventive medicine was often ineffective—particularly against cholera.

At first Oxfam simply tried to contain the excrement and other wastes of the refugees. It found that one possible container (a 30,000 gallon collapsible fuel tank belonging to the RAF) provided almost instant anaerobic conditions. From that discovery sprang the idea of destroying the cholera and dysentery bacteria anaerobically.

As no one knew the viability of *Vibrio cholerae* in anaerobic sewage, Oxfam asked Mr Barry Lloyd of the University of Surrey to find out. To keep costs as low as possible, Mr Lloyd presented the project to two final year students as the topic for their degree theses. Although each thesis 'cost' about £800, Oxfam paid only a tenth of this for the results.

The survival of the vibrios was found to depend on the temperature of the sludge and the proportions of solid matter in it. Vibrios were usually eliminated after 7 days at 37°C but at 25°C took 12 days to disappear. Once Oxfam had an estimate of how long the sewage should be retained in anaerobic conditions, it was able to seek the advice of the Water Pollution Research Laboratory at Stevenage, and the University of Loughborough on the general layout and hydraulics of the unit. The Plastics Research Group at the Atomic Energy Research Establishment, Harwell, designed a mould for a cheap stackable plastic squatting unit—the Asian equivalent of a toilet seat.

Mr Jim Howard, Oxfam's Industries Officer, emphasises the importance of

this squatting unit. Produced now for a matter of shillings, they replace heavy vitreous china items that cost about £50.

The sanitation package on which Oxfam has now decided consists of two or three large butyl rubber tanks holding some 4,500 gallons each, connected in series to a group of 20 squatting units. During October, a team from Oxfam will be taking an example of each unit to Bangladesh. With the cooperation of the Cholera Research Laboratories in Dacca, the two-bag unit will be tested on hospital effluent known to be rich in cholera bacteria and the three-bag system will be set up in a Bihari refugee camp to assess its impact on an existing community.

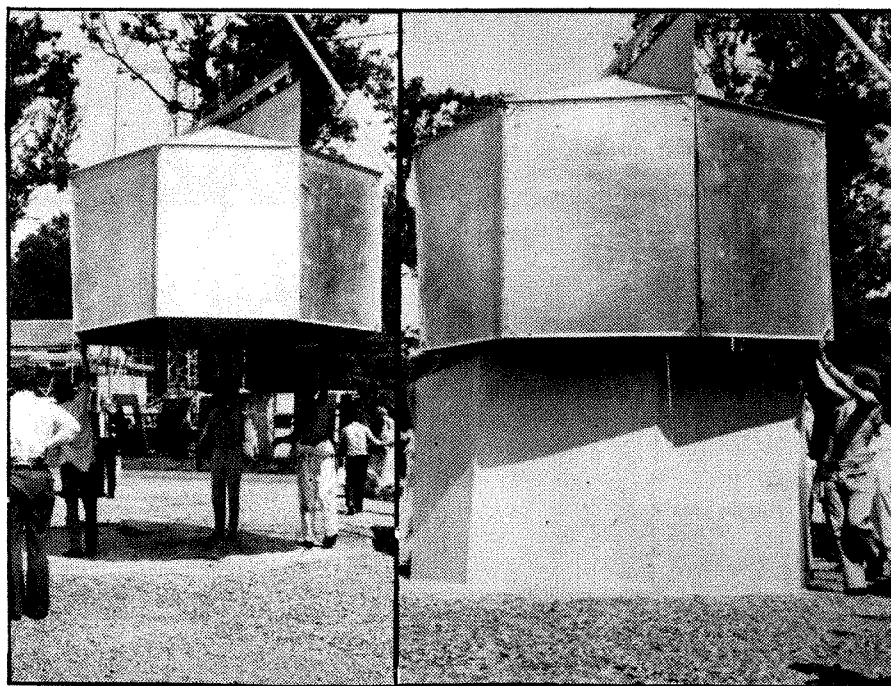
Even including the cost of this visit, Oxfam says it has spent less than £1,500 on the entire sewage project. Once the scheme was underway the Leverhulme Trust provided a grant of £19,000 and the British Government gave £6,500. The charity holds British, United States and Canadian patents on the system so it may still recoup what little it spent. With the addition of antifreeze the technique might be adapted for use in the Arctic or Antarctic.

The sanitation scheme is probably the most ambitious technical project that Oxfam have tackled and it typifies the organisation's direct approach to disaster relief. In the same vein, Oxfam is pioneering a building technique which it hopes will provide warm weather-proof shelters quickly and cheaply. Polyurethane foam is sprayed on to the inside of a lightweight aluminium mould. When the foam hardens, the mould is removed leaving a house with approximately 70 square feet of living space.

The mobile factory to spray the foam costs about £4,500 and Oxfam claims that the chemicals needed for one house may be bought for £30. Polystyrene granules are cheaper—and are rather more fire resistant—but the apparatus needed to steam them into place is six or seven times as expensive as the polyurethane apparatus. In September, Oxfam will be training two volunteer teams in the spray building technique.

In many underdeveloped countries a great proportion of the labour force—as much as 15% in some cases according to Mr Howard—spends its time moving water for irrigation. Oxfam is now trying to develop a portable

*Native huts, Oxfam style.
An aluminium shell is lowered into
place (left) and removed after serving
as a mould (right).*



water pump that could be powered by pedals in the same way as a bicycle. It is also investigating this use of pedal power to drive simple winnowing or grinding machines. With Mr Stuart Wilson, of the University of Oxford, it is developing a superior rickshaw, and a four wheel drive pedal platform or 'Pedalrover' which could carry half a ton across country; Zambia has already expressed an interest in this idea.

Oxfam does not always design its schemes for its own use. In 1971 it began a project to turn Britain's surplus potato crop and skimmed milk into a balanced emergency diet. Although Oxfam persuaded Cadbury Schweppes to let it use their powdered potato production lines at cost price it still spent £6,250 of its own money. Mr Howard feels that since Oxfam's new food is now accepted by the World Food Programme, it is up to the government to use the recipe when and if a surplus occurs again.

But Oxfam is not without its critics. Some people have expressed concern, for example, that any temporary housing provided after a disaster might remain occupied when conditions returned to normal, degenerating into instant slums. Oxfam admits that further work is needed to determine an optimum spacing of such units to minimise the fire hazard while housing as many people as possible—but it is fundamental to their philosophy that such housing is needed, although a case can be made that in many places, such as Bangladesh, the local population already possesses both the skills and the material needed for the rapid erection of cheap lightweight shelters.

At a more fundamental level, questions might be asked about whether such organisations as Oxfam are in fact the best bodies to organise scientific aid for disaster situations. The point is debatable; but it is true that Oxfam is not run by scientists, and that its scientific activities are still very much an appendage to the main work. So it is interesting that a group of London-based scientists is trying just the opposite approach. The London Technical Group (LTG) is a group of scientists from various disciplines which is looking at problems of disaster relief solely from the scientific and technological point of view.

The most widely available tangible product from the group so far is an

annotated bibliography of papers relating to *Disaster Technology* (LTG, 55 Evelyn Gardens, London; 1974). This typifies one aspect of the group's activities to act as a clearing house for relevant information and, hopefully, to ensure that such information does not disappear from the general awareness. Members of the group say that they have been astonished at how often 'new' ideas turn out to have been presaged years or even decades ago. They cite the example of the compression effects which cause internal injuries in victims pulled from collapsed buildings and have been "discovered" after almost every major earthquake affecting built up areas. In fact, they say, these problems were thoroughly investigated more than three decades ago, when many people were trapped in collapsing buildings during the mass bombing of the Second World War.

But the LTG is not just concerned with collecting and distributing information. Members of the group (now some 30 strong) see the LTG as something of a centre of expertise in terms of field experience of disaster and famine situations, eager to hire out their skills to anyone who can use them.

The opportunity for scientists with such expertise to meet regularly and discuss problems provides the third string to LTG's bow, as a 'disaster think tank'. Such ideas as mixing dried skim milk with oil to form a drinkable and nutritious emulsion (with the addition of appropriate substances to make it palatable) and an emphasis on rugged simplicity for all field equipment emerge from these meetings. Individual field trips made by members of the LTG have provided the essential training in

basics which encourages the group to think that it now has the experience to, say, carry out surveys of nutritional problems on behalf of governments or other bodies, who would put up the money and collect the results on a contractual basis. These trips also, of course, provide valuable information in their own right. Mr John Rivers, a member of the LTG, has just returned from Ethiopia, where he carried out a nutritional survey with Dr John Seamen as part of a UNICEF project, partially funded (to the tune of £1,000) by Oxfam. It seems from this work that the general feeling that protein deficiency may not be the greatest nutritional problem in famine areas may well be correct; the normal food of the nomads in Ethiopia includes milk, cereal and a little meat. In the present famine situation they are forced to eat other foods, such as beans. But there is no evidence that the quality of these foods is inadequate, whatever the problems of finding enough food; and this means that any emphasis on protein supplements as a high priority in famine relief is wrong, at least in this case.

It remains to be seen whether any charity or other organisation will jump at the opportunity of hiring the LTG team of scientific specialists as a group to do this kind of work, or whether the LTG will continue simply as a collection of concerned scientists devoting their spare time to efforts aimed at improving the way science is used to combat disasters. The approach they advocate is, however, worthy of serious consideration if only as a reminder that there are alternatives to the existing systems which have, by the nature of things, become 'the establishment'.

international news

CITING a sheaf of evidence which indicates that the pesticide dieldrin is highly carcinogenic to mice and rats, and that it now resides in the tissues of virtually every man, woman and child in the United States, the Environmental Protection Agency (EPA) last week abruptly halted further production and recommended that it be phased out of use as rapidly as possible.

The decision is the latest, and most significant, milestone in a four-year legal battle fought by a Washington-based environmentalist organisation to get dieldrin off the market, but it is far from the final word on the matter. Officials of the Shell Chemical Company, the sole manufacturer of the pesticide, erupted with indignation at the EPA's action, charged that "there is no evidence whatsoever to associate this chemical with cancer in man", and demanded a public hearing to state its case. The hearing is now going on, and a final decision is expected by the end of August.

If all that sounds familiar, it is. The history of the battle against dieldrin bears a strong resemblance to the long and bitter fight against DDT, although according to opponents of the pesticide the case against dieldrin is even more cut-and-dried than that against DDT. In both cases, the central issue is the ubiquity of the pesticide in the environment and the possibility that it may cause cancer in man.

Although it is a pesticide in its own right, dieldrin is also the breakdown product of the chlorinated hydrocarbon pesticide aldrin; the two agents are considered together in the EPA decision.

Firm favourites in the eyes of growers of corn and citrus fruits, the pesticides are used chiefly to control soil insects. They are applied directly to the soil in the spring, essentially as an insurance against the possibility of an outbreak of soil pests later in the year. Their longevity is thus a prime requisite, since they may have to kill insects weeks after they are put into the soil and, according to Shell and the United States Department of Agriculture which staunchly defends the use of aldrin and dieldrin, no other pesticide can remain active for long enough to do the job.

But the persistence of these pesticides is a mixed blessing, for dieldrin now contaminates many foods and is stored in high concentrations in human fatty tissues. According to a survey carried

EPA halts dieldrin production

by Colin Norman, Washington

out by the Food and Drug Administration last year, for example, measurable amounts of the pesticide were found in 83% of all dairy products, 88% of all garden fruits, 96% of all meat, fish and poultry, and in 12 to 14% of samples of grain, potatoes, fruit and some other foods. Moreover, in 1971, the EPA found that 99.5% of human tissue samples taken during autopsy or therapeutic surgery contained amounts of dieldrin averaging 0.29 parts per million.

There is thus no denying that man is constantly exposed to the pesticide. But there is considerable debate about whether this exposure carries a risk of cancer, and it is on that point that the case really hinges.

The EPA issued its order last week chiefly on the basis of studies which have shown that dieldrin "definitely causes significant increases of tumours in two and possibly three strains of mice tested", and that the pesticide has also raised tumours in two different strains of rats. The tumours, which "have been diagnosed unequivocally as malignant", appear in the liver, lungs, lymphoid tissue, thyroid, uterus and mammary glands, and have resulted from feeding tests involving doses as low as 0.1 parts per million. The EPA also cited evidence that exposure to dieldrin for periods as brief as a few weeks has caused significant carcinogenic effects in test animals.

The case against dieldrin was brought out during weeks of public hearings conducted by the EPA to determine whether or not the pesticide should be removed from the market. Those hearings are not expected to be completed until late this year or even early next year, but while they have been going on, Shell has been free to continue manufacturing both aldrin and dieldrin.

In April, however, EPA officials considered that the evidence against the pesticides—which came from studies conducted at the Food and Drug Administration, the National Cancer Institute and in Shell's own laboratories—was so compelling that they contacted

Shell officials and asked them to halt production of aldrin and dieldrin voluntarily until the public hearings have been completed. But Shell officials maintained that there is no evidence that the pesticides are harmful to man, declined to accede to the EPA's request and announced that they would begin producing next year's batches of aldrin and dieldrin on September 1. Thus the EPA moved last week to force Shell to stop manufacturing the chemicals, at least until doubts about their safety are cleared up.

For its part, Shell maintains that the animal feeding studies have little applicability to man, and that the safety of aldrin and dieldrin is assured by the fact that workers in factories manufacturing the pesticides have shown no signs of developing cancers. Lawyers for Shell have yet to present their case in full at the public hearings, and are annoyed that the EPA is trying to halt production of the chemicals before all the facts have been argued.

If Shell is allowed to go ahead with its production in September, millions of pounds of the chemicals will be distributed around the United States, and if the public hearings eventually conclude that the pesticides do pose a serious health risk, it would be virtually impossible to call all supplies in. Thus, the EPA is trying to ensure that if it bans the pesticides at the end of the public hearings, they will not continue in use for another year. □

More radiation protection

MICROWAVES and lasers are to be the first two types of non-ionising electromagnetic radiation to be taken under the wing of the British National Radiation Protection Board (NRPB) under an order extending its functions.

Since its inception as a result of the National Radiological Protection Act of 1970, the board has confined its advisory and research services to the fields of ionising radiation. Although it has no statutory powers, it advises the government and other responsible authorities on standards and safeguards against radiation hazards.

The recent order, by the Secretary of State for Health and Social Services, came into effect on August 1 and extends the functions of the board to cover potentially all electromagnetic

radiation. Its interest is being concentrated on lasers and microwaves in the first instance, say the board, not because these pose any widespread health hazard but because their use has increased enormously in the past few years and some national authoritative reference is now desirable.

Already, there is a generally agreed safety limit for continuous microwave exposure of not more than 10 mW cm⁻² average power density. This is rigorously observed by present users of radar, one of the main commercial applications of microwaves in Britain.

But there is another growing commercial use of microwaves, in quick microwave ovens which are not yet big business in Britain but whose use will probably increase. The board is setting up an advisory and research service at its Leeds centre, where ovens can be tested for radiation leakage. The second part of the NRPB's extended brief is the use of lasers. □

Taxing your sabbatical

UNTIL the end of the tax year on April 5, 1974, visitors to Britain on sabbatical leave were taxed by the British authorities on a so-called 'remittance basis'. The sum of money actually brought into the country was the base for taxation and in the six years before a sabbatical became due, many academics had learnt how to prevent most, if not all of their income of the seventh year (assuming it came from their permanent institution) from entering Britain. Legislation that has just been passed radically alters the tax position.

It is assumed in what follows that the work done in Britain is not entirely unconnected with normal duties of employment in the home country—an assumption which the tax officer is almost certain to make if the permanent institution is paying.

In the case of visitors from the United States, half of their pay will be liable to tax in Britain for any tax year in which they are in the country for 183 days or more; these days need not necessarily be consecutive. Any tax year in which visits do not add up to 183 days is not considered. A double taxation convention applies with the United States and so credit for British tax paid can be claimed against the United States tax on the same income. If a visitor becomes liable for tax through being in Britain for six months or more, he is entitled to claim full personal allowances.

Similar arrangements are in force with many other countries but tax authorities point out that it should not be assumed that arrangements are

identical and detailed information should be sought from embassies.

This change in the tax situation has been widely asserted to drive potential visitors away from Britain. In fact the most likely to suffer from it are those who have been able to find a way to avoid remitting income to this country, say by borrowing for their visit. Anyone who has had, of economic necessity, to bring his income with him, has previously had to pay tax on the full amount remitted in tax years in which he has been here for six months or more. Now he pays tax on only half that amount. □

Business: CEGB awash with capacity

by Roger Woodham

WHATEVER the Central Electricity Generating Board's financial position may be—it made a loss of £87.4 million in 1972–73—the board evidently stands no risk of running short of generating capacity as it did in the winter of 1969–70. At that time the CEGB's maximum output capacity was some 46,000 MW and the maximum demand to be met was about 38,000 MW, but for a string of reasons that were investigated by the Select Committee on Science and Technology insufficient capacity was actually available when it came to the crunch and the result was a series of voltage reductions—'brown outs'.

The situation as of March 1974 was much different in that the maximum output capacity had soared to 58,000 MW whereas the maximum demand that winter had been about 40,000 MW. The latter figure had not changed appreciably for three years (*CEGB Statistical Yearbook 1973–74*).

The inescapable conclusion is that the CEGB has an unrealistic amount of surplus capacity which it busily added to in 1973–74 at a cost of £189 million, representing 1,600 MW. And the plant now under construction which should be available by the end of 1978 will provide a further 12,000 MW, bringing the total to perhaps 67,000 MW if allowance is made for the demise of old plant. If the growth of electricity demand were about 3% a year in the next few years, the maximum call on the CEGB's services would only rise to some 45,000 MW by 1978. At that stage the board would be capable of meeting a peak demand of half as much again. Naturally some of that 'overkill' must be regarded as a provision for plant temporarily out of service, but the margin is different altogether from the 21% which proved to be insufficient—just—in exceptional circumstances in 1969–70. In 1966–67 the CEGB was getting by without bother on a margin of about 12%. □

Qualified approval for Aspartame

by Colin Norman, Washington

THE United States Food and Drug Administration (FDA) last week gave its approval for a new artificial sweetener to be used in a variety of products. Called Aspartame, it is 180 times sweeter than sugar and its official debut is significant since it comes right in the middle of an investigation of the safety of saccharin—the only other artificial sweetener on the market in the United States—which is suspected of causing bladder tumours in mice.

Aspartame loses its sweetness on prolonged cooking, however, and thus it has only been approved for such uses as sweetening tea and coffee, adding to breakfast cereals and for sweetening puddings, gelatins and artificial cream. So far, it has not been approved for use in so-called diet drinks, and it will therefore not challenge saccharin in its largest market. But FDA officials suggest privately that soft drink manufacturers will soon petition the government to allow them to use Aspartame.

So far, few people have expressed doubts about the safety of Aspartame, chiefly because it is broken down in the body into two essential amino acids, L-aspartic acid and L-phenylalanine. Furthermore, FDA's approval was based on the results of feeding studies which involved dogs and rats for two years and a number of rats which were exposed to Aspartame *in utero* and throughout their lifetimes. The studies gave no indication of tumorigenicity, and suggested that at least 2 grams per kilogram of body weight are required before any toxic effects are evident. That level is more than 100 times greater than the likely average daily intake of the sweetener in foods for for which it has so far been approved.

The FDA's decision will be closely studied in several other countries—including Britain—where applications have been made by G. D. Searle and Co., the manufacturer of Aspartame, for permission to market the sweetener. Its importance in the United States is that it gives the FDA more flexibility in dealing with two particularly sticky problems. First, the FDA has been forced to re-evaluate its decision to ban cyclamates from the market on the basis of suspected carcinogenicity (a decision is expected early next year) and, second, saccharin is now under investigation and the FDA will probably have to decide its fate within the next couple of months. Before Aspartame came along, the FDA was facing the problem of perhaps removing from the market all artificial sweeteners, but now at least it has a third product to insert into the cost-benefit equation. □

correspondence

Victimisation

SIR,—What Professor Burhop in effect is saying (*Nature*, August 9) is that the "Declaration on the Rights of Scientists", made by the World Federation of Scientific Workers in 1969, is really no more than an expression of pious hopes, since there is nothing that can be done about victimisation by governments, left, right, or centre, if the governments responsible are themselves disinclined to cooperate.

As President of the W.F.S.W., Professor Burhop is no doubt well placed to judge of this. But when he goes on to say that "... the close contacts maintained between our affiliated organisations in different countries are beneficial to scientists and for science itself", one wonders whether there might not be long-term benefits for science and scientists in particular countries, if the W.F.S.W. were to make it clear that although "Our aim must be to strengthen these contacts", that policy would have to be applied selectively, where there was reason to believe that the "Declaration" was not being adhered to by the governments or affiliated organisations concerned.

Yours faithfully,

C. B. GOODHART

Cambridge, UK

Plasmid engineering

SIR,—The appeal by a committee of the National Academy of Sciences for restraint with respect to certain types of genetic experiment (*Nature*, July 19) deserves the generally favourable response accorded it (*Nature*, July 26). The potential hazards associated with the creation of artificial recombinant DNA molecules are clearly outlined in the committee's statement. However, there is an unfortunate lack of clarity in defining the types of experiment which it is recommended should be deferred. The statement concerning experiments of type I is confusing. This is particularly so if one does not persevere to the last three lines of the inordinately long sentence which excludes "plasmids containing such combinations of antibiotic resistance determinants (which) already exist in nature." The hazards associated with these latter plasmids are not potential but have already been amply demonstrated and are well understood, as explained by Dr Anderson. The NAS committee's recommendation is concerned with unnatural recombinant plasmids and it would be unfortunate if, as seems likely from parts of Dr Anderson's comment,

the lack of clarity in the definition of experiments of type I led to the inclusion in the recommended ban of experiments concerned, not with future potential hazards, but with those already with us and in need of attention.

Much work is in progress, including my own, with the specific objective of seeking means for the effective elimination of plasmids from bacteria. It would be against the spirit of the NAS committee's aims if misinterpretation of the nature of experiments of type I brought to a halt, even temporarily, experiments carrying no potential hazard from new recombinant DNA molecules, but concerned with already existing hazards in clinical practice and animal husbandry.

Yours faithfully,

G. R. BARKER

Manchester, UK

Scientists don't move

SIR,—Your editorial of July 26 on Sir Hermann Bondi's report on the interchange of scientists was very critical of the lack of mobility of scientists, and could scarcely believe that they are inhibited from changing jobs because of the difficulty of moving house. My recent experiences may help you to understand the problems.

Last December I moved from a civil service post in Farnborough to a research fellowship at Leicester University. It took the Civil Service Department over four months to determine the transfer value of my pension, but this was certainly no obstacle to me. Indeed I think that the preservation or transferability of pension rights is of little concern to the young scientist contemplating a move. My new employers seem to be unusually generous by contemporary standards in that they reimbursed a large part of my "removal expenses" and also provided a small second mortgage loan at low interest rate. Even so we have been faced with unreimbursable expenses in the present dormant housing market of more than £2,000—probably nearer £3,000 by the time the flat is sold.

My present contract lasts for only three years, and, with the present tendency of university science departments to contract, it is entirely possible that I shall have to move again in 2½ years' time, with, no doubt, a repetition of these expenses.

In North America or any other country of Western Europe, people

in our position find it quite natural to rent a house for a few years; here it is impossible to find unfurnished accommodation. Even worse, the 1974 Rent Act, by giving security of tenure to furnished tenants as well, has made us abandon any idea of letting our empty flat in this way. It seems to be the policy of both major parties that every family should either own its own home or be a council tenant, but there can be little doubt of the severe effects of this policy on the mobility of all labour, and not just scientists.

While I have no regrets at the change in my working environment which I have bought at the price, perhaps, of two years' salary, I feel unable to criticize the many scientists who do not choose to follow my example. They too may count the cost, and they may feel that boldness in pushing back frontiers is not incompatible with financial solvency.

Yours faithfully,

C. G. PAGE

Leicester, UK

Collecting egg-whites

W. R. P. BOURNE (*Nature*, 249, 793; 1974), seems determined to continue to hound Professor Charles Sibley for his comparatively minor indiscretion (with the connivance of others) in obtaining the eggs of certain birds unlawfully.

Professor Sibley is not, and never has been, an egg collector in the accepted sense as the title of Bourne's article suggests. Indeed his interest in eggs has been confined to the analysis of egg-white protein and the taxonomic value of such analysis as far as avian relationships are concerned.

Whatever the final results of Professor Sibley's researches may be, it is certain that they will be studied by all who are interested in the many unsatisfactory aspects of the classification of birds, typified, for instance, by the Babblers (Timaliidae), for too long the despair of orthodox systematists.

R. WAGSTAFFE

Bluntisham, Huntingdon, UK

How many deer?

SIR,—How many deer have been 'sacrificed' (which is, I believe, the correct scientific term) in order to provide linings for "attractive space-saving files for your *Nature* ... beautifully labelled"?

Yours faithfully,

Hove, UK

B. JOVE

news and views

Current work with the voltage clamp

THE present view of the ionic basis of the action potential in nerve cells depends largely on the classic voltage-clamp experiments of Hodgkin and Huxley, published in 1952 (*J. Physiol.*, **116**, 449). Although it is remarkable how well this analysis has withstood the test of time, recent work has shown that the changes in potassium permeability are more complex than previously realised and that Ca^{2+} ions may play an important part in the inward current in many neurones.

The action potential of nerve cells is essentially a transient reversal of the voltage across the cell membrane. Hodgkin and Huxley showed that it is generated by brief, sequential increases in the membrane permeability first to Na^+ ions and then to K^+ ions. They used a voltage-clamp system to control the membrane potential of a squid giant axon, and were able to measure the currents flowing across the membrane. Following a large depolarisation the current first flowed inwards, and then rapidly reversed and was maintained in an outward direction, as shown in the figure (a). This membrane current could be separated into an inward one carried by Na^+ , and an outward one carried by K^+ . The Na permeability was rapidly switched on, or activated, and then inactivated after a short delay. The K permeability was activated only after a short delay, but was apparently not inactivated. It remained constant until the depolarisation ended.

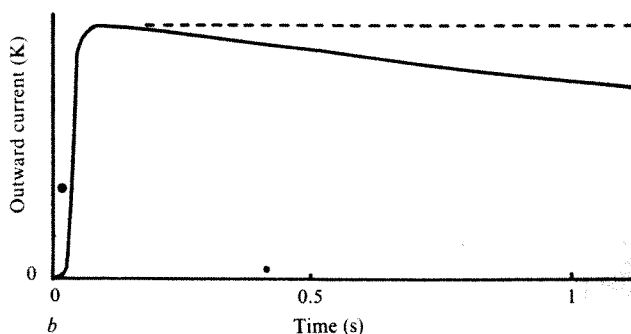
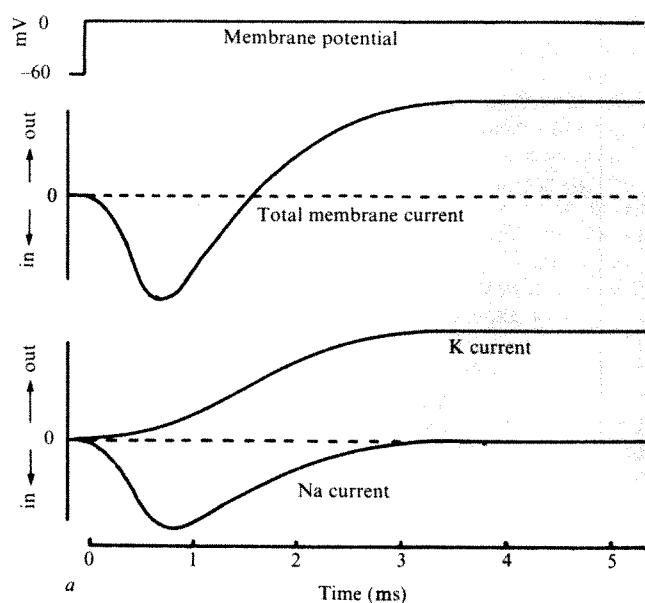
Within a few years of this elegant work it was shown in several preparations, particularly in mollusc nerve cell bodies, that the removal of external Na does not block action potentials. Voltage-clamp studies have now demonstrated that the inward current can be carried by Ca^{2+} as well as, or instead of, Na^+ . Some Ca^{2+} may pass through the standard Na permeability system (Baker, Hodgkin and Ridgway, *J. Physiol.*, **218**, 709; 1971), but it is not yet clear whether there is a separate permeability system which handles most of the Ca current. Geduldig and Greuner (*J. Physiol.*, **211**, 217; 1970) reported that the activation properties of Na and Ca permeabilities in *Aplysia* neurones are different, suggesting separate systems. Kostyuk and colleagues (*Pflügers Arch. ges. Physiol.*, **348**, 83; 1974) and Standen (*Nature*, **250**, 340; 1974), both using snail neurones, have recently presented evidence, however, that the two ions use either the same system or, possibly, parallel ones with identical kinetics.

Even more involved than these complications of the early inward current system are those concerning the K permeability. As well as the well-established (or slow) outward K current, Neher (*J. gen. Physiol.*, **58**, 36; 1971) found a fast outward K current in snail neurones with kinetics similar to those of the inward current. Connor and Stevens (*J. Physiol.*, **213**, 31; 1971) also separated the potassium current that they measured in dorid neurones into a fast component that had both an activation and inactivation mechanism and the more usual slow component. This fast K current seems to be particularly important in controlling pacemaker activity, and may play an important part in repetitive firing.

Another aspect of the K current which has recently

come to light is the observation of a K inactivation mechanism. Whereas Hodgkin and Huxley's K current was maintained indefinitely during a depolarising step, several recent experiments have shown that it can decline (see figure b). Connor and Stevens found that the slow component of the K current inactivated with a time constant of several seconds. They also showed that it was a distinct phenomenon (that is, not a simple inactivation of the fast K current) by demonstrating differences in the voltage-dependent characteristics and the responses to TEA. Many examples of K inactivation have now been reported: to cite only two of them; Leicht, Meves and Wellhoner (*Pflügers Arch. ges. Physiol.*, **323**, 63; 1971) measured an inactivation of the delayed K current in snail neurones with time constants of 0.5–1.75 s and Ehrenstein and Gilbert (*Biophys. J.*, **6**, 553; 1966) found a similar inactivation in squid axons with a time constant of 11 s. Such slow processes, although obviously of no importance in the generation of single action potentials, could play an important part in the long-term firing characteristics of the cell.

One difficulty with the voltage-clamp technique is that it only measures the net membrane current. It is not always easy to determine whether changes in the recorded current



Hypothetical voltage-clamp records of voltage or current against time.

result from changes in an inward or an outward component. Although it is relatively easy to establish that the inward current is carried by Na^+ or Ca^{2+} ions by removing them from the external solution, it is difficult to show directly that K^+ ions carry the outward current. An elegant way of doing this was recently reported by Neher and Lux (*J. gen. Physiol.*, **61**, 385; 1973). They used a K^+ -sensitive microelectrode to measure changes in K^+ outside snail neurones, and showed that the total amount of K^+ released by the cell during a clamp pulse was in good agreement with that calculated assuming that the outward current was carried only by K^+ ions. Lux and Eckert (see page 574 of this issue of *Nature*) have now applied this ingenious technique to a study of apparent K inactivation. When the outward current was reduced by procedures expected to produce K inactivation, the actual release of K^+ , as determined by the K^+ -sensitive microelectrodes, was not equally reduced. They conclude that there must be a slow inward current as well as the normal slow outward current. Part of the apparent K inactivation was the result of this unsuspected inward current, which would make the outward current as recorded by the clamp smaller than the true outward current. The ion or ions carrying this slow inward current are so far unidentified, but are presumably Na or Ca.

ROGER C. THOMAS
L. DONALD PARTRIDGE

Germline antibody genes

THE genetic basis for antibody diversity is a subject of continuing interest though the argument between germline and somatic models of antibody diversity has abated recently. The large number of different amino acid sequences so far determined for V regions of mouse Kappa chains, point to there being at least a hundred V_{Kappa} genes and there is probably a similar minimum number of V_{H} genes. The question as to whether somatic processes are used to increase the diversity encoded in the multiple germline V genes remains open. Now the major effort is aimed at defining the way in which the V and C genes are arranged in a chromosome and the way in which they function.

At present it is thought that the genes which code for H chains (V_{H}) are present on the same chromosome with the set of about ten C_{H} genes, one C_{H} gene for each class of antibody. In this model any V_{H} gene can be paired with any C_{H} gene to give a gene pair coding for a single heavy chain. Although there is considerable evidence to support this scheme, at present it is only an outline of what must be a fascinating genetic process. The C genes for both light and heavy chains map as single genes in a Mendelian fashion. Similar mapping of individual V genes has been limited by the lack of suitable phenotypic markers. Recently individual antibodies have been characterised by either their idiotype (characteristic antigenic determinants defined by antisera raised against the antibody under study) fine specificity (definition of an antibody combining site by the quantitation of cross reactions) or spectrotpe (characteristic isoelectric focusing spectrum of an antibody). Using these genetic markers six V_{H} genes have been identified in the mouse and a search is now being made for recombinants so that a map of the V_{H} region of the chromosome can be drawn. Each of these V_{H} genes, in agreement with the model I have mentioned, has been found to be closely linked to the C_{H} loci.

Eichmann, Tung and Nisonoff reported in last week's edition of *Nature* (250, 509-511; 1974) strong evidence for a recombinational event separating two of the known V_{H} genes *ARS* and *A5A*. These genes had been identified by

the idiotype present on A/J strain mouse antibodies directed against *p*-azophenylarsonate (*ars*) and group A streptococcal carbohydrate respectively. A/J mice can make a variety of different antibodies directed against each of these two antigens. The initial response of A/J mice to *ars* always includes an antibody (or a family of antibodies) carrying the *ARS* idiotypic determinant. The *A5A* idiotype has been found to be associated with a particular antibody spectrotpe and cells producing that antibody have been cloned *in vivo* (*Eur. J. Immunol.*, **2**, 301; 1972). The inheritance of *A5A* in the C_{H} linkage group was shown by Eichmann and Berek (*Euro. J. Immunol.*, **3**, 599; 1973) who mated A/J mice against BALB/c mice, a strain which efficiently makes antibody against group A carbohydrate but has never shown that *A5A* idiotype; the F_1 progeny were backcrossed against BALB/c. Of the backcross offspring 16/29 were homozygous for the BALB/c C_{H} genes; all but one of those 16 were, as expected, negative for the *A5A* idiotype on their anti-group A carbohydrate antibody. The odd mouse BB♂7 showed a recombinant phenotype with the *A5A* gene apparently present in the BALB/c C_{H} linkage group.

In Eichmann *et al.*'s latest study, BB♂7 has been again backcrossed against BALB/c and the progeny support the idea that a new linkage of *A5A* to the BALB/c C_{H} loci has occurred as the result of a recombinational event. Many of the progeny of BB♂7 have been tested for the *ARS* idiotype. In this respect the backcrosses behave just like the parental BALB/c mice and make anti-*ars* lacking the *ARS* idiotype. The one recombinational event observed among the three genes under study is consistent with a simple linear map *A5A*—X—*ARS*— C_{H} —where X shows the position of the crossover. Further crossovers are needed in order to estimate map distances between these three genes.

The occurrence of a recombinant in only sixteen progeny in which it could be detected is either a very fortuitous event or else it is indicative of a large map distance between *A5A* and the other two genes. One map distance in the H chain linkage group of the mouse was reported by Riblet at the workshop on immunoglobulin variable region genetics (Bethesda, Maryland, March 25 and 26, 1974). Riblet studied the *DEX* gene which is characterised by an idiotypic specificity found on two BALB/c myeloma antibodies binding α -1,3 dextran. Only two recombinational events were observed in a total of 530 crosses in which a recombination could have been detected. At the time of writing their article, Eichmann and his colleagues had screened 73 meaningful crosses and BB♂7 remains the only recombinant. The next recombinant is keenly anticipated since the measurement of the distance between *A5A* and *ARS* will be indicative of the number of V genes which might lie between these markers.

ALAN R. WILLIAMSON

Reappearance of hexosaminidase A

SINCE it became possible to fuse somatic cells using Sendai virus people have been investigating the complementation (supply of the other's needs by each parental cell) of closely similar genetic defects. Complementation, in hybrids and heterokaryons (the multinucleated cells produced by fusion) has provided useful information about recessive mutants, in cases, for example, where the precise metabolic lesion is not known and genetic heterogeneity has been neatly demonstrated (see, for example, Kao *et al.*, *Science*, **164**, 312; 1969; de Weerd-Kastelein *et al.*, *Nature*

new Biol., 238, 80; 1972). If different genes are defective in each parent then the normal gene product from the opposite parent in each case will be present in the hybrid with comitant 'normal' or 'heterozygote' phenotype—namely intergenic complementation. If on the other hand the two parental cell lines have a mutation at the same gene locus, complementation does not usually occur; the hybrid line also shows the defect.

Different mutations at the same locus, however, can it seems sometimes complement each other (interallelic complementation) as the result of the formation of functional proteins (enzymes) made up of two different types of defective subunits, the variant gene products from each parent. Although interallelic and intergenic complementation are indistinguishable when the primary product of the relevant gene is not identified, in cases where an enzyme deficiency has been observed in the parental cells, the newly synthesised 'hybrid' enzyme produced as a result of interallelic complementation would probably show differences in property from the normal enzyme.

Genetic complementation has now been pleasingly demonstrated in heterokaryons made by fusing skin fibroblasts from patients with Tay-Sachs and Sandhoff's disease, two clinically very similar diseases characterised by the excessive accumulation of the ganglioside GM₂ in the brain. In the tissues of patients with Tay-Sachs disease the enzyme N-acetyl β -D hexosaminidase A is absent and in Sandhoff's disease both major forms of N-acetyl β -D hexosaminidase (A and B) are deficient. What Thomas *et al.* have done (see page 580 of this edition of *Nature*) is to demonstrate that hexosaminidase A (as well as B) is synthesised in the heterokaryons. Unfortunately, this finding does little to quell the dispute about the relationship of the hexosaminidase isozymes, because it can be explained by all the current models.

Until the past week or two only two main models had been considered in the literature. The first is that B, the precursor enzyme, determined by locus 1, is enzymatically converted secondarily (by the product of locus 2) into A; and the second and perhaps most favoured theory is that A and B possess similar and dissimilar polypeptide subunits ($A=(\alpha\beta)_n$; $B=(\beta\beta)_n$ or $(\beta\gamma)_n$) α being deficient in Tay-Sachs and β in Sandhoff's disease (see, for example, Carroll and Robinson, *Biochem. J.*, 137, 217; 1974; Srivastava and Beutler, *J. biol. Chem.*, 249, 2054; 1974). Both of these models invoke at least two separate gene loci, a different one being affected in each

disease, and if either is right the synthesis *de novo* of hexosaminidase A in heterokaryons and hybrids would be predicted. If, however, only one gene were involved, the finding could still be explained by the formation of a hybrid enzyme by interallelic complementation. Yet another lengthy article on the interrelationship of the hexosaminidases has just appeared in the *Journal of Biological Chemistry* (Tallman *et al.*, 249, 3489; 1974) and these authors conclude that the A and B enzymes are conformational isomers—a one gene model. They propose that the mutation leading to Sandhoff's disease affects the active site of the enzyme, thus both conformers are absent, and that that leading to Tay-Sachs disease causes instability of the 'A' conformation. In the light of this new claim it might then be worth studying the properties of the heterokaryon hexosaminidase A in rather more detail. It would not, however, seem surprising if the task were unrewarding because despite the wealth of arguments concerning the biochemical and immunological properties of the enzymes one simple yet important set of findings is not readily compatible with this one gene model. This also comes from somatic cell hybrid studies. In human/mouse hybrid cells (in which many of the human chromosomes are lost) the human hexosaminidase A could be lost without B, and its loss has been associated with the loss of a particular human chromosome (Lalley *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, 71, 1569; 1974).

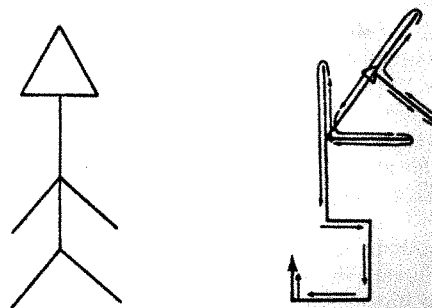
Complementation then of these two mutant lines does not seem to have solved the hexosaminidase puzzle, but it does provide another example of virus fusion in which interesting observations can be made from heterokaryons without the use of a system to select for mononuclear hybrid cells. The success of this experiment should provoke somebody into attempting the same thing with other mutant hexosaminidase lines—which might just turn out to be more informative.

From a Correspondent

Bugs are beautiful

from our Experimental Psychology Correspondent

It might seem that using the computer as a theoretical vehicle in the exploration of the mind constitutes about as reductionist a strategy as one could devise: likening thought to processes involving punched cards and electronic circuits. But to judge from the discussions at the Artificial Intelligence Society of Britain (AISB) Summer Conference held at the University of Sussex from July 8-10, many of the



Left: intended drawing of stick man; right: attempt by program with bugs (arrows represent tracks of pen).

practitioners of artificial intelligence take up on the contrary a very mentalist stance indeed. A couple of speakers who described their work as simulating neural processes, or making computational models of the cortex, attracted energetic comment from other participants.

Although the size of the conference indicated that artificial intelligence is now well established in Britain, the stars of the show were undoubtedly the speakers from the large American centres of AI, and in particular from the Massachusetts Institute of Technology. In that region at least it is neither the potential for stimulating neurones, nor the automation efficiency of the computer that is a source of light on the nature of intelligence at present. The elucidation emanates from an examination of 'bugs'—the programming errors which prevent the actuality of programs from reflecting the aspirations of the programmers.

Typical of this kind of work at MIT is I. P. Goldstein's program which takes a specification of a simple picture, in descriptive terms, including the names of its parts (for example, head, body, legs) and relational statements such as that the legs and body are connected, the arms are below the head and so on. It also has as part of its input a program intended to draw a stick man, which represents an approximate solution, but which has bugs in it (see figure). What the debugging part of Goldstein's program knows is a good deal about the structure of simple programs in which parts (for example, procedures) can be corrected and executed independently of other parts. It also has some problem specific knowledge about how to relate descriptions in the non-procedural descriptive statement of the specification to the primitives of the graphical procedures which can draw pictures. What the whole program does in effect is to edit and rewrite parts of itself in the light of its failures to achieve its specific performance.

This provides a view of the problem solving process in which a coherent strategy, albeit containing misconcep-

tions and shortcomings, is executed. The mistakes so generated are regarded as valuable rather than unfortunate, because they can give the information necessary to improve the program's or the programmer's grasp of the process. This view is, for instance, quite different from the approach to problem solving taken in the General Problem Solver, or in most game playing programs, where absurdly large numbers of microscopic actions are searched through to see if the program can stumble across a sequence of moves that provide a solution. That approach is the domain of the combinatorial explosion: the number of moves to be searched grows astronomically as the program peers rather blindly ahead.

Programs which debug themselves, like Goldstein's, or G. J. Sussman's program called 'Hacker' whose skill improves with practice, or the construction of a programming apprentice which is being designed by C. Hewitt and his colleagues (MIT) to cooperate in the design and testing of a program, represent what looks like a productive approach to problem solving. Mistakes are valuable in that they represent interaction of a program with its environment, the experimental disconfirmations generated by a programmed theory about how to do something. An initial strategy for designing a process will almost certainly fall short in some way, but if the programmer gets about it properly, the nature of the failure can indicate a new and better theory of how that process works or can be designed.

The emphasis on bugs and debugging constitutes another new departure in AI. Bugs provide a further way in which interaction with computational forms extends the understanding of intelligence. The task is not really to simulate the brain, nor to discover that intelligence is so hopelessly complex that we continually find ourselves trying to defuse one combinatorial explosion, after another. It is more a case of providing ourselves with better ways of thinking about thinking: more penetrating and productive metaphors for the mind than Freud's bubbling cauldron of the unconscious, or the self-operating supermarket door of stimulus-response theory. New insights into thought processes may emerge from looking at the nature of bugs and the ways they are made, and from the attempt to formalise the process sufficiently to write programs that debug themselves. It can then be seen if the experience and understanding gained encourages thought not just about program bugs, but how people do learn, or could learn, from their mistakes.

Sahara spreads south

from Peter D. Moore

DURING the past century the Sahara Desert has expanded very considerably, particularly in a southerly direction. As a result the lands along the southern edge of the Sahara, which have long been used for grazing by nomadic tribes, no longer support savanna grasslands but have been engulfed by the desert.

Recent attempts at explaining this expansion have centred around the concept of climatic change; for example, Lamb (*Phil. Trans. R. Soc.*, **A276**, 195; 1974) has reviewed evidence for climatic changes during the past 5,000 years. Although he considers it impossible as yet to construct vegetational maps for early civilised times, there is evidence which suggests that the Sahara has enjoyed higher levels of rainfall. Rock drawings of animals have been found in western and central areas of the Sahara which suggest that game was once available where it no longer exists. Skeletons of elephants have even been found, so some surface water must then have been available. The level of Lake Chad (southern Sahara, 13°N) was more than 30 m above present day levels 5,000 yr ago; also, at that time, the level of the Nile floods was far greater than has regularly been experienced since. In Lamb's opinion, the equatorial rains ranged farther north during their seasonal migration which could have resulted in a regular rainy season to 20°N and erratic rains further north still. As a result one would expect a greater abundance of subsoil water than is now the case.

A consideration of rainfall patterns in the area during the past 70 yr (Winstanley, *Nature* **245**, 190; 1973) has shown that there has been a considerable decrease in rainfall in the southern Sahara since 1960 (from 165 mm to 100 mm in 1970, using 5 yr running means). In Winstanley's view this represents a southerly shift in isohyets of 9 km per year. Since there is a steep spatial gradient of rainfall in this area this could account for the spread of desert conditions.

Back in 1952 a rather different view prevailed. Richards (in, *The Tropical Rain Forest*, Cambridge, 1952) considered that the expansion of savanna grasslands at the expense of rain forest south of the Sahara was the result of an increased use of fire by man in the area. Similarly the spread of the desert has often been attributed to human mismanagement. This view has recently been reiterated by Cloudsley-Thompson (*Envir. Conserv.*, **1**, 5; 1974). He considers that the use of fire by man, which can now be traced back

more than 50,000 yr in East Africa, is a critical factor in the development of savanna and the subsequent spread of desert, but that the additional pressures imposed by overgrazing also deserve attention.

Undoubtedly overgrazing has been an important factor, especially where goats have been involved, and it can result in the production of desert-like conditions even in situations where rainfall is moderate, for example 635 mm in Karamoja, Northern Uganda. In addition to the removal of vegetation cover, the influence of trampling on soil erosion may also be considerable.

It is, however, impossible to ignore Winstanley's data or to regard them as unimportant. The response of vegetation to climatic change in the absence of human-induced factors is essentially slow, as the result of the long generation time of many climax-dominant species and to the influence which plants have on local microclimate because of their canopy structure. Thus vegetation, particularly if it is structurally complex as in the case of forest, possesses an inertia which buffers it against minor climatic fluctuations. One of the most important influences of man on world vegetation has been the simplification of the structure of habitats by felling, burning and grazing, with a resulting modification of microclimatic conditions close to the ground. Soil hydrology and nutrient content are also affected by such processes. It is likely that the high intensity of grazing imposed on the savanna south of the Sahara has rendered the vegetation of the area particularly sensitive to the changes in rainfall pattern. The result has been the spread of the Sahara.

Galactic chemistry

from Virginia Trimble

THE problems of determining the abundances of the chemical elements and accounting for them in terms of a theory of nucleosynthesis and the evolution of our Galaxy involves almost every branch of astronomy, and many related sciences, from objective prism spectroscopy and neutron activation techniques to nuclear shell models and computerised histories of the Galaxy. It is not, perhaps, surprising then that no single, clear picture of nucleosynthesis and evolution emerged from the NATO Advanced Studies Institute on the origin and abundance of the chemical elements, held at the Institute of Astronomy (Cambridge) from July 22 to August 9. If there is any unifying principle at all, it seems to be that, wherever one looks, there is evidence that more than one

process has been at work, and that, therefore, no one mechanism or theory can hope to explain all the observed chemical and isotopic abundances.

Very broadly, one must think in terms of stars more massive than the Sun undergoing nuclear reactions throughout the history of the Galaxy and returning the products of those reactions to the interstellar medium. Successive generations of stars, including the Sun and its planetary system (whose composition is, by definition, normal and the standard with respect to which enhancements or deficiencies elsewhere are measured) will then be formed from the material thus enriched in elements heavier than hydrogen and helium.

Within our Solar System, abundances can be determined for meteorites of various types, the solar photosphere, the solar corona and wind, and particles ejected by solar flares, as well as for the Earth (which is, however, so chemically fractionated as to be almost useless as an indicator of general abundance). The carbonaceous chondrites (which are the type richest in volatile materials and therefore the closest to the primitive solar nebula in composition) have, to first order, compositions and structure which can be understood in terms of temperatures at which various compounds condense from a gaseous to a solid phase, with some later remelting and fractionation of their parent body, according to E. Anders (University of Chicago). But there are additional components, amounting to 1 or 2% of the masses of the meteorites concerned which, from their isotope ratios (in one case a deficiency of ^{20}Ne and ^{21}Ne relative to ^{22}Ne , and in the other an excess of ^{16}O relative to the other isotopes), seem to represent interstellar grain material incorporated into the meteorites without ever having been vaporised and recondensed in the solar nebula, according to R.N. Clayton (University of Chicago). The Earth on the basis of its oxygen isotope ratio may also contain about 1% of this unvaporised component.

High energy particles ejected from solar flares exhibit strong variations in composition with energy below a few MeV per nucleon, but above that converge to abundances which are not significantly different from those in the solar photosphere or corona. This suggests, according to P. B. Price (University of California, Berkeley) that, at sufficiently high energies, the composition of cosmic rays coming from outside the Solar System might also reflect that of the objects which are their sources (supernovae or supernova remnants in most theories). In fact, at those energies which have been studied, the cosmic ray composi-

B and H

from Peter J. Smith

As far as units are concerned, geomagneticians have traditionally had an easy life. Geomagnetic measurements are usually made in media having relative permeability $\mu_r \sim 1$; and in the c.g.s. e.m.u. system the permeability of free space $\mu_0 = 1$. Thus the induction B is related to the field intensity H by $B = \mu_r \mu_0 H = H$ for all practical purposes. As a result of this simple equation geophysicists have seldom had to think very carefully about the physical difference between B and H ; few, if any, have really cared whether their magnetometers have measured B or H ; the gauss, the c.g.s. unit of B , has by usage become totally interchangeable with the oersted, the c.g.s. unit of H ; and as Lowes points out (*Geophys. J.*, **37**, 51; 1974), it is no longer even clear whether the gamma was introduced as 10^{-3} gauss or 10^{-3} oersted.

The recent recommendation by the International Association of Geomagnetism and Aeronomy

shows unmistakable signs of spallation (which greatly enhances the amount of Li, Be, and B among other things) as a result of passage through the interstellar medium. After corrections for spallation, the apparent cosmic ray source composition, as determined by M. Shapiro (Naval Research Laboratory) and others, still has a great enhancement of heavy elements in general over hydrogen and helium and of the iron peak elements with respect to C, N and O. There may, in addition, be an excess of U and Th and other nuclei produced in the r process (in which neutrons are captured rapidly by iron peak nuclei and the resulting unstable products then beta decay back to stability). The cause of these anomalies must be apportioned between the processes which produce the nuclei that become cosmic rays and the processes that accelerate the nuclei to relativistic energies. It is not clear how this apportionment should be made.

A recently discovered, low energy component in the cosmic rays has a quite different set of anomalies (O and N enhanced with respect to C, and enhanced He which is pure ^4He). These have been attributed on the one hand to processes in the sources, assumed to be ordinary novae, by D. Clayton (Rice University) and F. Hoyle (University of Manchester) and, on the other hand, to preferential leakage into the heliosphere of atoms with high ionisation potential, by R. Ramaty and

(IAGA) that the geophysical community adopt the SI system has given cause for a little thought, although insofar as the conversions from c.g.s. may be made by simple numerical factors, life will probably go on much as before. Indeed the basic decision about whether to express results in terms of B or H (necessitated by the fact that in SI, $\mu_0 = 1$) was made by IAGA in favour of B in the "interest of achieving... the least possible disruption of existing numerical usage" and in spite of protestations that "matters of physical fact are involved" (Whitworth and Stopes-Roe, *Nature*, **234**, 31; 1971).

In any event, Lowes has now shown that, whatever it may say on the label, real magnetometers have calibrations which depend on the permeability of the medium in which the field is measured. Magnetometers thus actually measure neither B nor H exactly; and so from the instrumental point of view there is no reason for preferentially choosing either for expressing magnetic field data. Not that this will prevent controversy in the future, of course!

others at Goddard Space Flight Center, where the new component was discovered.

The most striking recent progress in measuring abundances has been made by a group at Princeton University as a result of Copernicus observations of ultraviolet interstellar absorption lines. They have obtained a relatively coherent picture, in which the elements that are most depleted from the general interstellar gas onto grains are those that condense at the lowest temperatures. This is reminiscent of the situation in meteorites.

Among the stars, the extraordinary enrichment of the surfaces of certain peculiar A stars in Eu and other rare earths seems, from the work of G. Michaud (University of Montreal), to be attributable to diffusion in the outer layers of stars with initially normal composition. A new class of CH subgiants, discovered by H. Bond (Louisiana State University) has, on the other hand, overabundances of C, Nd, Ce and La, combined with a deficiency of other metals, which seem to reflect mixing to the surface of material which has undergone nuclear reactions in the interior of the stars.

The theoretical models of element production tend to reflect the multiplicity of components in the observations. Several theorists, including I. Iben (University of Illinois) and S. Woosley (California Institute of Technology) have followed the assorted processes (hydrogen and helium burning; hydro-

static and explosive carbon and oxygen burning; explosive silicon burning; and the rapid, slow, and equilibrium capture of neutrons) which should occur in the various layers of normal stars. By a judicious mixing of the products of the various processes (corresponding roughly to the masses in each of the layers), they have succeeded in reproducing the observed abundances in the Solar System over a wide range of atomic numbers while using a rather small number of adjustable parameters.

On a still larger scale, W. D. Arnett (University of Illinois) and R. Talbot (Rice University) and B. Tinsley (University of Texas, Dallas) have formulated models of the evolution of our Galaxy as a whole which can reproduce the variations of total metal abundance with time and position in the galaxy as well as the percentages of matter now present in the form of gas and of stars of various masses and compositions. The models are by no means unique.

Lizards and dune buggies

from our Animal Ecology Correspondent

NOBODY can doubt that increased leisure time available to people living in affluent societies is on the verge of creating immense environmental hazards. As it seems likely that time and money are going to be increasingly plentiful in the future, it is high time that ecologists and politicians got together to formulate useful legislation. This must be tailored to suit the specific ecosystems under pressure and no area, however barren, should be thought to be unworthy of inclusion.

The vast Mojave desert of the southwestern United States is one such barren area that has been used for more than a century for mining and domestic stock grazing. Today it is an important playground for the people of California and, as might be expected, the huge pressure so induced is taking its toll. At a conservative estimate there are 1.2 million motor cycles and 500,000 dune buggies in California most of which are used for scrambling and other off-road pursuits. A most useful pilot study of the impact of both vehicles and sheep on the Mojave has been made by Busack and Bury, and it is to be hoped it does not escape the attention of environmental decision makers (*Biol. Conserv.*, **6**, 179-183; 1974).

Dove Springs canyon, 23 km N and 9.5 km W of California City, was chosen as a study site since it is a popular place for buggy racers to display their talents. Three 1 ha plots representing heavy use, moderate use and no use were established and all the

resident lizards in each were collected during a 3-day period. The conditions under which the census was made satisfy the restrictions as to effectiveness laid down by Zippin (*Biometrics*, **12**, 163-189; 1956). Some lizards were caught by hand (the rare ones which were subsequently released), some were shot with 0.22 dust shot and others were shot with elastic bands. In the heavily used area, where the vegetation was severely reduced, only two lizards (*Uta stansburiana*) were caught. In the moderately used area 15 lizards were taken adding *Callisaurus draconoides* and *Cnemidophorus tigris* to the list. The biomass of lizards in this area was more than 48 times greater than that in the first area. The unused plot yielded 24 lizards (the three species mentioned plus *Crotaphytus wislizenii*) and had a biomass almost double that of the moderately used area. If lizards can be taken as indicator species of general ecological complexity—and being insectivorous this seems not unreasonable—there seems to be a case for speedy legislation to restrict dune buggy racing only to certain areas.

Grazing has different effects on lizards. Previous studies of desert vegetation have indicated that grazing does not greatly affect the diversity of plant species (Blydenstein, Hungerford, Day and Humphrey, *Ecology*, **38**, 522-526; 1957; Gardner, *Ecology*, **31**, 44-50; 1950). Protection from grazing serves to increase biomass without seriously affecting ecological complexity. In Gardner's study 30 years' protection from grazing brought about a 110% increase in biomass. The ungrazed study plot used by Busack and Bury yielded 36 lizards with a total biomass of 690 g ha⁻¹; of this 6 *C. draconoides* comprised less than 10% of the total biomass. The plot which had been heavily grazed by sheep in the previous year revealed 17 lizards with a biomass of 185 g ha⁻¹. Interestingly, of this 17, 11 were *C. draconoides* which comprised almost 70% of the total biomass. *Phrynosoma platyrhinos* and *C. wislizenii* were absent from the grazed plot. The likely interpretation of these observations is that the vegetational changes associated with grazing reduces to below an acceptable exploitable level the population of insects required by these species. *C. draconoides* is an agile lizard that favours open areas with scant plant growth and small rocks. The effect of grazing is to produce these conditions.

Both motor cycling and buggy racing have an important influence on community structure which could result in irreparable ecological damage. Strict control over who does what where in the Mojave will be required if the whole place is not to become unfit for lizards and thus, in the long term if not in the short, unfit for any kind of human use.

Lattice defects in Freiberg

from John Walker

THE latest in the series of international conferences on lattice defects in semiconductors, held at Freiberg from July 22-25, was opened by G. D. Watkins (General Electric, Schenectady) with his traditional review of spin resonance studies in silicon. The self-interstitial has still not been isolated, but seems to be produced in a positive-charge state during irradiation. It is then trapped by and changes place with the negatively-charged acceptors in p-type material. This trapping does not occur in n-type material, so the damage rate is lower, though boron counterdoping increases it. The importance of oxygen in radiation damage was emphasised by the identification of the Si-G3 and Si-G4 centres as vacancies perturbed by oxygen atoms. Both defects anneal to give the well-known A-centre (an oxygen-vacancy pair).

R. P. Messmer (General Electric, Schenectady), in his review of the theory of point defects in semiconductors, described the X α scattered wave theory which has been used to illuminate the controversy between many-electron and one-electron treatments of the diamond vacancy. Apparently the electrons are not sharply localised at the defect, but spill out into the surrounding lattice. Hence electron-electron interaction is reduced and the one-electron calculations are adequate. This is consistent with experimental results on silicon, but the data are not available for diamond.

In the diamond session, L. A. Vermeulin and colleagues (Universities of Reading and the Witwatersrand) reported the observation of sharp photoconductivity peaks in irradiated diamond crystals, which suggests the existence of bound states within the conduction or valence bands. J. W. Van der Sande (University of the Witwatersrand) has used the low temperature thermal conductivity of irradiated diamond to demonstrate the presence of interstitial clusters 200 Å in diameter; these indicate that defects are mobile below room temperature.

Phosphorus ions, when ion-implanted into silicon in a random direction, penetrate further than expected. Explanations which have been suggested are diffusion and the scattering of ions into channelling directions. P. Blood and colleagues (Mullard Research Laboratories, Redhill and AERE Harwell), in a very elegant experiment, prepared a thin silicon crystal and implanted radioactive phosphorus ions into it. Diffusing ions would necessarily remain in the crystal,

whereas channelled ones could emerge from the back face and be trapped in a second, adjacent crystal. Radioactive ions were found in the second crystal, so channelling was indeed occurring.

D. V. Lang and L. C. Kimerling (Bell Laboratories) introduced a powerful new tool for the study of defect states within the forbidden gap. Deep level transient spectroscopy can detect the activation energy for thermal emission from a defect level to the band edge, the defect concentrations, their spatial profile and the electron and hole capture cross sections. All these parameters are of obvious interest to device manufacturers as well as academic physicists. A voltage pulse fills with charge carriers the defects within the depletion region of a p-n junction. The trapped carriers are then released and cause a capacitance transient whose magnitude and time constant, combined with the device temperature and the width of the depletion region, indicate the above parameters. The technique is also relatively cheap and simple to operate.

Genetical hazards of pollutants

from A. D. Bradshaw

HUMAN beings are rather ambivalent to problems of pollution. On the one hand people can get very worried by them, and yet on the other hand they all too easily dismiss them. It was for this reason that the Genetical Society held a symposium on the subject on July 10-12 during their summer meeting at Lancaster. Eight invited speakers provided an assessment of the current state of knowledge about the genetical effects of pollution.

These effects fall into two distinctive parts—selection and mutation. It was clear that a great deal is now known about the selective effects of pollutants. Indeed, they provide some of the most elegant examples of evolution in action as was shown in the accounts of industrial melanism in moths by L. M. Cook (University of Manchester) and J. A. Bishop (University of Liverpool) and of heavy metal tolerance in plants by A. D. Bradshaw (University of Liverpool). These two examples are remarkably similar in the way they demonstrate the powerful effects of selection in the formation of highly localised adapted populations, particularly in organisms with limited powers of dispersal. In both cases the observed clinal patterns can be correlated with observed values for gene flow and selection.

Although the rapidity of the evolution of insecticide resistance is now well known it is doubtful if many

people realise the remarkable rate of appearance over just a few years of resistance to first one new insecticide and then another. This problem was outlined by R. J. Wood (University of Manchester) who showed how much the evolution of insecticide resistance is ruining insect control. The evolution, however, is determined by the availability of appropriate variation as well as by selection, for only some, and not all, species have evolved resistance.

It is easy to appreciate that evolution of resistance to insecticides or heavy metals, being so much a matter of survival or extinction, will be very rapid: one or two years seem to be sufficient for substantial evolutionary change. It was therefore interesting to hear from R. W. Snaydon (University of Reading) that even the quiet fields of Rothamsted Experimental Station can provide examples of equally far reaching and rapid evolution in the grass *Anthoxanthum odoratum*, in response to the different liming and fertiliser treatments of the Park Grass Experiment. Here indeed is an example which shows the very pervasive effects of evolution on all characters of an organism, and the fact that an ordinary meadow can readily generate coefficients of selection as high as 0.5 or more.

By contrast, contributions on the mutational aspects of pollution did not paint quite such a clear picture, not because of lack of importance of the subject or of lack of work, but because of the inherent difficulties of mutation research. C. E. Purdom (Fisheries Laboratory, Lowestoft) described the history of research on the effects of ionising radiation, from the early days when only the linear relationship between mutation and dose was known to the present day when it is realised that the relation is not so simple and that different species including man can show very different patterns of sensitivity.

This theme was taken up by M. F. Lyon (MRC Radiobiology Unit, Harwell) for the particular case of the effects of radiation on mammals. It now seems that repeated small doses give a lower mutation rate than the same total dose given in a single exposure. Since environmental radiation is received almost entirely in small doses or at low rates it may therefore be less hazardous than was previously thought. At the same time the yield of mutation in mice, and guinea-pigs and hamsters, suggests that mutation yield decreases with time after irradiation. All this is comforting to human beings, but there is still, for obvious reasons, no direct evidence.

The last but not least effect of environmental mutagens, particularly chemicals, is to cause chromosome change. There is enormous pressure to

produce effective means for testing the effects of new chemicals on human tissue. M. L. O'Riordan and H. J. Evans (Western General Hospital, Edinburgh) showed the value of peripheral blood lymphocytes for this purpose. But the problem is to know how far it is possible to extrapolate from such data to effects on germ cells. Certainly germ cells and embryos in human beings have their own particular properties. E. Alberman (Guys Hospital, London) very effectively brought the symposium to a close with an analysis of the causes of spontaneous abortion in human beings. It is very clear from the relation between foetal death and the presence of chromosomal abnormalities in the foetus, and between mean ovarian X-ray dose and foetal death, that abortion is a process by which human beings get rid of much newly acquired genetic abnormality.

Pacific motions

from Peter J. Smith

DEEP sea sediment cores—unlike rocks from continents, oceanic islands and seamounts—have not generally been used to reconstruct lithospheric plate motions. There are several reasons for this: azimuthal orientations of sediment cores have not usually been available, thereby making it impossible to obtain the complete palaeomagnetic information; cores have often been short, thereby limiting data in time; and there are the usual problems of dating and determining sedimentation rates, especially beyond the well defined continental polarity-time scale. Perhaps even more importantly, the sediments easier to recover are obviously the younger ones within whose span plate motions have been small. Ocean cores have therefore been most useful in polarity studies, in elucidating time-stratigraphic relationships and in determining geomagnetic field properties, although some years ago Sclater and Cox (*Nature*, **226**, 934; 1970) did show that cores can be used to trace palaeolatitude variations given adequate time coverage.

Another attempt to determine plate motions from sediments has now been made by Hammond *et al.* (*Earth planet. Sci. Lett.*, **22**, 22; 1974) using 7 cores from the central equatorial Pacific. Taken together the cores spanned completely the period 0.1–21 million years (Myr) ago, a range determined partly from magnetic and partly from biological data. For the younger sediments, dating was based primarily on comparison with the continental polarity-time scale. Sediments in the range 5–10 Myr were dated by compar-

ing the relative lengths of polarity epochs in the cores with those of dated ocean floor anomalies. For the period beyond 10 Myr ago, dates were based largely on the correlation of observed radiolarian zones with the tropical radiolarian zoning previously established.

Such dating is easier to describe than to carry out; but within the limitations of the various methods, Hammond and his colleagues go on to plot palaeolatitude (from the measured palaeomagnetic inclinations) as a function of age throughout each core on the assumption that during the relevant period the geomagnetic field has been axially dipolar. In each case, the variation of palaeolatitude with time indicates northward motion. The overall average rate of movement, based on linear least-squares regression analyses of the latitude-age data, is 8.4 cm yr^{-1} during the past 21 Myr. The average, however, conceals a significant mid-period change in motion, for about 12 Myr ago the rate of northward movement seems to have slowed from about 11 to 6 cm yr^{-1} . This could represent a genuine decrease in the absolute rate of motion, a change in direction (which could reduce the northward component of motion without necessarily reducing the actual rate), or both.

Numbers apart, the important general point demonstrated by Hammond *et al.* is that the method of reconstructing plate motions using deep sea cores can be made to work as long as the cores cover a sufficiently long interval of time. They also note that in view of the limited number of cores studied and the difficulties in dating them accurately, their own particular results must be regarded as tentative. Nevertheless, they draw attention to the fact that their overall drift rate of 8.4 cm yr^{-1} is in excellent agreement with the rate of about 8 cm yr^{-1} obtained by Grommé and Vine (*Earth planet. Sci. Lett.*, **17**, 159; 1972) from a palaeomagnetic study of Miocene basalts from Midway Atoll.

On the other hand, Hammond and his coworkers do not mention that Winterer (*Bull. Am. Ass. Petrol. Geol.*, **56**, 63; 1972) concluded (on the basis of eastern Pacific data) that the Pacific plate has been moving northwards at a rate of only 3 cm yr^{-1} for the past 30 Myr, nor that Heezen *et al.* used western Pacific data to obtain an even lower rate of 2 cm yr^{-1} . But Forristall (*Geophys. Res. Lett.*, **1**, 131; 1974) does mention this previous work, and uses it to expand some ideas of his own concerning Pacific motions.

Forristall's ultimate conclusion is that the asthenosphere beneath the central Pacific is about twice as thick as the lithosphere—a result which will cause little surprise insofar as it is in

general agreement with majority views favouring shallow convection. But the assumptions and data upon which the conclusion is based are much more contentious. For example, many will surely quarrel not only with Forristall's view that the concept of hot spots fixed with respect to each other has been conclusively rejected but also with his more fundamental assertion that "it is hard to escape the idea that linear chains of volcanic features . . . are the expression of isolated subsurface hot spots".

Be that as it may, the more important point in the present context is that in his calculations Forristall uses the northward drift rates proposed by Winterer and Heezen *et al.*, apparently rejecting the higher rate obtained by Grommé and Vine. It thus becomes clear that not only are different workers obtaining quite different figures for recent Pacific plate motion, the variety of values available provides a basis for wider conclusions. The reasons for the discrepancies in drift rate are not difficult to imagine; but until they are sorted out the confusion is likely to increase.

Observing the millenium

by John Gribbin

ON the face of things, the achievement of 1,000 issues of a journal may seem unremarkable by *Nature's* standards—this journal is now well into our sixth 'millenium' of continuous publication. But when the journal in question only appears bimonthly at best, and has recently suffered more than most from the rigours of the British three-day week, there is perhaps some excuse for dropping the mask of scientific sobriety for one celebratory issue.

The journal to which I refer is *The Observatory*, which is the house journal of the Royal Astronomical Society. The sense of humour needed to run such a journal for a body of scientists as free-ranging in their ideas as astronomers is often apparent in ordinary issues of the journal, where sober scientific papers rub cheek by jowl with letters which are sometimes decidedly peculiar (but must presumably be published if they come from Fellows of the RAS?) and with verbatim reports of meetings which are so deadpan that they have on occasion been known to reduce an astronomical coffee break to something approaching hysteria.

To anybody who has been present at the meeting being reported in any particular issue of *The Observatory*, the favoured game is to spot who has taken the opportunity (offered in the

best traditions of scientific accuracy) to change what they actually said at the meeting to what they would have said if they had either remembered, or had time, or read the papers they should have read in advance. Together with the frequent interjections from "A Fellow" (anybody present who was not recognised by the RAS scribes), this makes the meeting reports just about beyond improvement—so it is a pity that the editors of the journal thought it necessary to start their celebratory 1,000th issue with a spoof meeting report.

The spoof scientific papers are more successful, although most of them hinge upon 'in' jokes which will mean little to non-astronomers (but then, who else will be reading them?). But a couple of contributions deserve at least passing notice from the wider world of science. These are concerned with two newly discovered manifestations of the 11-year cycle which seems to be linked to many terrestrial phenomena, and may be triggered by solar activity. Research into this subject is still seen as a contentious issue in some quarters, and one of the contributors hides behind the pseudonym "Disgusted, Tunbridge Wells" (presumably A. Fellow?). What Disgusted has discovered, among other things, is that an analysis of the number of pages in each issue of *The Observatory* shows "a suggestion of the eleven-year cycle which occurs in most astronomical data". But the second communication is even more remarkable.

According to Mathews (*Observatory*, **94**, no. 1,000, 13P; 1974) there is a correlation between sunspot activity and the political "colour" of British governments. Labour governments, it seems, tend to be returned at times of sunspot minimum, and Conservative governments at times of sunspot maximum. Best of all, in a prediction made before the most recent British election but only now appearing in print, Mathews said "regions of political instability may well be triggered in the next few months, resulting in the election of a Labour Government", and also predicted "the continuation of the Liberal revival". Those predictions, of course, may well be considered still relevant today, and if Mr Harold Wilson is a reader of *The Observatory*, there might well be another election in Britain in the very near future.

This area of sunspot research is clearly an important field of astronomy today (see *Nature* **246**, 453; 1973). If there are still doubters who are unconvinced of the reality of the solar-terrestrial links let them ponder on a quote from *The Observatory* of 1877 (**1**, 370): "M. Tempel supposes that the spiral shapes (of Nebulae) are only creatures of fantasy".

Primate social organisation and ecology

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Attempts to relate interspecific differences in social organisation among primates to gross differences in habitat or diet type have been largely unsuccessful. This is probably partly because distantly related species have adapted to similar ecological situations in different ways and partly because much finer ecological differences are important.

THE accumulation of primate field studies in the course of the last two decades has emphasised the extent to which social organisation varies between species. Marked inter-population differences have been observed in a number of cases¹, but most species possess characteristic modal patterns of social organisation. Members of some species are solitary² for much of their time, while others live in family groups^{3,4} and others in semi-stable groups of fifty or more animals^{5,6}. Groups may include approximately equal numbers of males and females⁷ or, as is more usual, there may be a preponderance of females^{8,9}. In a number of species, groups regularly split up into parties which forage separately^{10,11} while in other all members of the group stay together^{6,8}. The average size of the area used by groups differs from <0.01 to >50 km² (ref. 12).

At present, little is known about the adaptive significance of these differences. Several reviews have attempted to relate them to gross differences in diet or habitat type¹²⁻¹⁸. The first and perhaps the most influential of these was produced by Crook and Gartlan¹³ in 1966. Primate species were divided into five "grades" according to the nature of their diets, the kind of habitat they occupied and the pattern of social organisation which they displayed. The paper emphasises the similarity of social organisation in species sharing the same diet and habitat type and is widely quoted as evidence that social organisation and ecology are closely related. But when it is viewed in the light of current knowledge, differences between groupings are less impressive than differences within them. For example, *Saimiri sciureus*, *Colobus* spp., and *Gorilla* are all placed in the same grade, despite the gross differences in diet, foraging behaviour and social organisation which exist between them¹⁹⁻²⁶. In addition, the paper illustrates a fundamental problem in classifying primate social systems. Since different aspects of social organisation are not well correlated across species, categories defined by a single criterion will include social systems which differ widely in other ways.

Jolly¹² avoids this problem by considering different aspects of social organisation separately. She groups species into six ecological divisions ("nocturnal", "arboreal leaf-eaters", "arboreal omnivores", "semi-terrestrial leaf-eaters", "semi-terrestrial omnivores" and "arid country species"), and compares troop size, range and territory size, population density, inter-group behaviour and day-range size separately between ecological groups. Again, differences within most groupings are more striking than differences between them. For example, between species allocated the "arboreal leaf-eaters" category, average troop size differs from <10 to >50 and average range size from <0.02 to >1 km², while other categories show even more variability.

To criticise these reviews in the light of current knowledge is not to discount their value. The paper by Crook and

Gartlan in particular has stimulated much interest and a considerable body of research. There are, however, theoretical reasons why social organisation should not be expected to be closely correlated with gross ecological variation.

Phylogeny, ecology and adaptation

One probable reason for the absence of close correlation is that different species tend to react to similar environmental pressures in different ways. When a novel adaptation evolves, its form will be partly determined by the various environmental factors through which selection is operating and partly by the species' phylogenetic inheritance^{27,28}. Consequently, distantly related species are likely to evolve different traits with similar functions. For example, anatomical specialisations permitting the digestion of foliage have evolved in several primate families. In some this has been achieved by the development of a large caecum (for example, *Alouatta*, *Indri*)²⁹, in others by chambered stomachs with bacterial symbionts (for example, *Colobus*, *Presbytis*)³⁰. Similarly, many interspecific differences in social organisation may well prove to represent different methods of overcoming the same ecological problems.

Another reason is that the wrong kind of ecological variation has been assumed to be important. Within habitat categories as broad as "forest", "woodland" and "savanna", and dietetic categories such as "insectivore", "frugivore", "folivore" and "omnivore" there is room for vast ecological differences. Food supplies may be static or mobile, sparse or dense, heavily clumped or evenly dispersed, reasonably

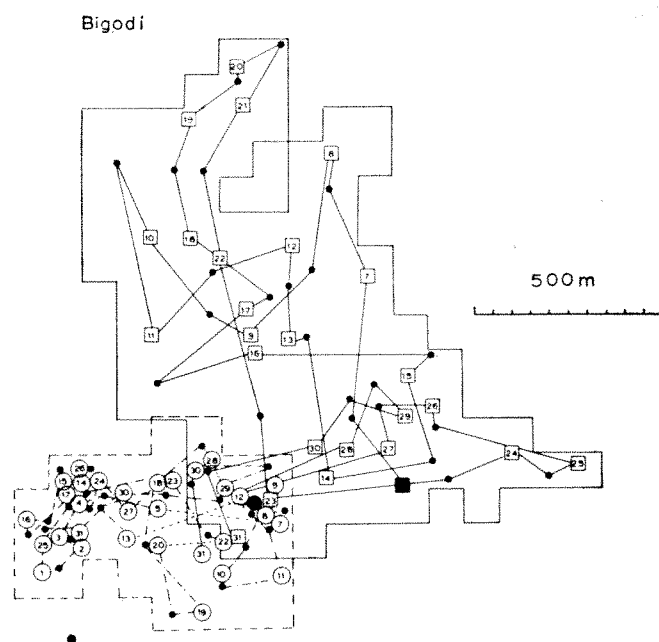


Fig. 1 Movements of a troop of red colobus (squares: September 6 to 31, 1970) and one of black and white colobus (circles: August 29 to September 31, 1970) about their ranges in Kibale Forest Reserve, Uganda. Squares and circles show the troops' night-resting positions, the figures inside them the dates. Points show the positions of the troops at 12 noon on each day.



Fig. 2 A male black and white colobus watches an intruder.

stable throughout the year or extremely variable¹⁷. Intensity of predation may vary in the same ways. If ecological differences at this level affect social organisation (and, by analogy, the plentiful literature on birds suggests that this will be found to be the case³¹) one should expect social organisation to vary widely within habitat and diet types.

A recent study of two species of colobus monkeys in East Africa³² provides an example of the level at which differences in social organisation may be related to ecology. Both the red colobus (*Colobus badius*) and the black and white colobus (*C. guereza*) are widely distributed across tropical Africa³³. The two species are sympatric throughout much of their range, though the black and white colobus is found both in wet and dry forest while the red colobus is less commonly found in dry forest^{25,34}. Although both species are largely folivorous, arboreal and forest-dwelling,³⁵ their characteristic patterns of social organisation differ widely. Red colobus live in large, multi-male troops of 40 or more animals which occupy extensive ranges of around 1 km² in size (see Fig. 1). Their different calls intergrade³⁶. Oestrous females show pronounced swelling of the perineal region. Infants have a black natal coat and are apparently handled only by the mother during the first months of life. In contrast, black and white colobus live in small troops of five to 10 animals which often contain only one adult male²⁴⁻²⁶. The troops occupy defended territories usually <0.2 km² in size and inter-troop relationships are normally hostile²⁴. Males give a booming roar which may serve to space groups³⁷ while intragroup vocalisations intergrade as in the red colobus. Females show no obvious oestrous swellings. Infants have a white natal coat and are handled by females other than their mothers from the day of birth³⁸.

Between August, 1969, and June, 1970, I observed one troop of red colobus in the Gombe National Park for 9

months³². Afterwards, for approximately a month each, I watched one troop of red colobus and one of black and white colobus in each of two areas in Kibale Forest, Uganda. I measured the amount of time which the animals spent feeding on different foods by recording, at quarter-hourly intervals, the foods that all visible animals were eating³⁹. In each area, I also measured the relative abundance of the different tree species.

The feeding behaviour of all three troops of red colobus was very similar. The animals ate the flowers, fruit, shoots and leaves of a variety of tree species. They were extremely selective in their choice of food, regularly choosing certain parts of particular tree species. Most tree species were not evenly distributed through the forest, with the result that the animals fed on different foods in different parts of their ranges. In addition, the availability of food on most species varied seasonally and this was reflected in seasonal changes in the animals' diet. When shoots, flowers and fruit were less abundant, the animals fed to a greater extent on mature leaves, though in no month did they spend more than 60% of their feeding time eating mature leaves. In all months of the year at Gombe, and in both study areas at Kibale, the animals fed on a wide variety of food species. To measure the variability of their diet, in each month I ranked the animals' foods on the amount of time spent feeding on them. At Gombe the proportion of time spent feeding on the top-ranking food species varied from 13-42% between months, that on the second ranking species from 11-21%, while the amount of time spent feeding on the top five varied from 51-80%. Figures for the two Uganda troops were similar. In one study area the red colobus spent 11% of their time feeding on the top-ranking food species, 10% on the second and 46% on the top five. In the other they spent 16% of their time on the top species, 14% on the second and 50% on the top five.

The diet of the black and white colobus troops differed in two important ways from that of the red colobus. Both troops fed almost exclusively on two tree species (*Celtis durandii* Engl. and *Markhamia platycalyx* (Bak.) Sprague). In one area *Celtis durandii* accounted for 71% of all feeding records and *Markhamia platycalyx* for 19%. In the other, *Celtis durandii* accounted for 88% and *Markhamia*



Fig. 3 A female red colobus leaps from one tree to another, her infant clinging to her chest.

platycalyx for 5%. These species were utilised to a lesser extent by the red colobus troops in both areas and the difference in feeding behaviour was not a product of reduction in the availability of alternative foods to the black and white colobus³⁹. Second, during the first weeks of the Uganda study, when few *Celtis* trees had yet come into flower or shoot, the black and white colobus fed largely on the mature leaves of *Celtis*. At the same time, the red colobus were feeding on the shoots, flowers and fruit of a variety of other food species, but were not observed to feed on mature *Celtis* leaves. Subsequently, many of the *Celtis* trees came into flower and shoot, and the black and white colobus switched to feeding on these parts.

These differences in feeding behaviour may be closely related to the differences in distribution and social organisation between the two colobus species. Black and white colobus may be able to exist on a diet of mature leaves when only these are available. This would allow the species to colonise areas of dry forest where strongly seasonal rainfall produces a high degree of production synchrony across different tree species (so that shoots, flowers and fruit are only available at certain times of year). Second, it would allow them to exist on the products of a small number of tree species throughout the year, since these would provide acceptable food in all seasons. Consequently, a small area of forest would be able to support animals throughout the year. In this situation, small troop size might permit the animals to minimise range size and thus to increase their ability to defend their food supply efficiently^{17,40}. It might also allow them to minimise the distance which they would have to travel each day to collect food (both because the total demands of the troop would be less and because they would be able to utilise food sources too small for larger troops).

In contrast, red colobus may need to maintain a high proportion of shoots, flowers and fruit in their diet throughout the year. Consequently, the species may be limited to wetter forests where some tree species carry these parts in all seasons. Since different tree species will carry acceptable foods at different times, the animals would need to maintain access to a wide variety of food species. As the distributions of most tree species are heavily clumped, a large sized range would be necessary in order to provide sufficient supplies of acceptable food in all months of the year. A square kilometre of forest can support a considerable population of red colobus. Theoretically, the animals could either aggregate into a single group or split up into several small groups with extensively overlapping ranges. In the red colobus, minimisation of group size may be less advantageous because the range size necessary to support even a small group throughout the year would be too large to be efficiently defended. Aggregation may have a number of advantages: the presence of other feeding animals in the immediate area may show individuals where to find food⁴¹, while the troop may provide a reservoir of knowledge about the distribution of food and of predators in the past⁴². It also may allow the animals to develop a pattern of regular use of the different parts of the range which would maintain leaf growth at an acceptable stage and maximise the reaction of the tree species to being cropped⁴³. Finally, large troop size may enhance the animals' ability to detect and defend themselves against predators. Certainly chimpanzees at Gombe were regular predators of red colobus (R. Wrangham, personal communication) and several instances of cooperative defence by the colobus were observed.

The origins of several other differences between the two species are probably linked with these differences in social organisation. The contrasting pelage of the black and white colobus and the males' roars may help to demarcate troops' territories (J. F. Oates, quoted in ref. 44). Differences between the two species' reactions to intruders are well



Fig. 4 Female red colobus feeding on flowers of *Commersonia bartramia*.

adapted to the difference in group size. When disturbed, black and white colobus tend to move to thick cover and remain silent while red colobus escape noisily and males may attack potential predators, including chimpanzees. Other differences, such as the absence of obvious sexual swellings in the black and white colobus and of infant-swapping in the red are less easily explained.

This reconstruction is clearly speculative. Few colobus troops were observed and, except at Gombe, they were watched only at one time of year. Current studies of the two species by T. T. Struhsaker and J. F. Oates may throw further light on the problem. But large group and range size is associated with clumped, unstable food supplies in several other animal groups. Studies of *Presbytis entellus* and *P. senex* in Ceylon show that *P. entellus*, which lives in larger troops in larger ranges than *P. senex* also feeds more on fruit and less on mature foliage and utilises a wider range of foods. Among East African ungulates, most of the plains-dwelling, grazing species, whose food supplies tend to be clumped and relatively unpredictable, aggregate in large herds which range widely (for example, most of the *Alcelaphinae*). In contrast, most browsing species, whose food supplies tend to be more uniformly scattered and more stable, occupy small ranges and live either in small groups, in pairs or alone (for example, dik-diks, duikers and most of the *Neotraginae*)⁴⁶. With some exceptions, the same association between diet and social organisation is found among European and Asiatic deer. Finally, a high proportion of graminivorous bird species feed in flocks, whereas relatively few insectivorous species do so³¹. The diet of the former is commonly assumed to be more heavily clumped than that of the latter⁴⁷. Within the insectivores, the aerial feeders (for example, swallows, martins and swifts) whose food supplies tend to be more clumped and less predictable, almost all feed in flocks³¹.

The colobus study provides one example of the kind of ecological differences which may be associated with interspecific differences in social organisation. In other primate species, different factors are likely to be important. But if many of them are as subtle as those probably involved in

this case, considerable variation is to be expected within gross diet and habitat categories. This is not to say that high level ecological variation has no effect on social organisation. Indeed, the examples quoted above suggest that in some animal groups there is a relatively close correlation between some aspects of social organisation and certain types of diet. Whether associations at this level can be identified will depend both on the number of species which in ecological categories and on the number of species which can be compared. Lack³¹, working with a vast array of bird species, was able to demonstrate correlations between social organisation and gross dietetic variation despite the presence of considerable variation within ecological categories. In primates, however, this approach is less likely to be useful both because there are relatively few species and because there is evidently wide variation within gross ecological groupings.

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- ¹ Jay, P. C., in *Primates: Studies in Adaptation and Variability* (edit. by Jay, P. C.), (Holt, Rinehart and Winston, New York, 1968).
- ² Charles-Dominique, P., and Martin, R. D., *Advances in Ethology*, **9** (Paul Parey, Berlin, 1972).
- ³ Ellefson, J. O., in *Primates: Studies in Adaptation and Variability* (edit. by Jay, P. C.), (Holt, Rinehart and Winston, New York, 1968).
- ⁴ Mason, W. A., in *Primates: Studies in Adaptation and Variability* (edit. by Jay, P. C.), (Holt, Rinehart and Winston, New York, 1968).
- ⁵ Gautier-Hion, A., *Folia primat.*, **12**, 116-141 (1970).
- ⁶ Altmann, S. A., and Altmann, J., *Baboon Ecology* (University of Chicago Press, Chicago, 1970).
- ⁷ Jolly, A., *Lemur Behaviour* (University of Chicago Press, Chicago, 1966).
- ⁸ Poirier, F. E., *Folia primat.*, **10**, 20-47 (1969).
- ⁹ Hall, K. R. L., *J. Zool.*, **148**, 15-87 (1965).

- ¹⁰ Eisenberg, J. F., and Kuehn, R. E., *Smithsonian Misc. Coll.*, **151**, 1-63 (1966).
- ¹¹ Aldrich-Blake, F. P. G., Thesis, University of Bristol (1970).
- ¹² Jolly, A., *The Evolution of Primate Behaviour* (Macmillan, New York, 1972).
- ¹³ Crook, J. H., and Gartlan, J. S., *Nature*, **210**, 1200-1203 (1966).
- ¹⁴ Crook, J. H., in *Social Behaviour in Birds and Mammals* (edit. by Crook, J. H.), (Academic Press, London, 1970).
- ¹⁵ Crook, J. H., *Anim. Behav.*, **18**, 197-209 (1970).
- ¹⁶ Denham, W. W., *Am. Anthropol.*, **73**, 77-95 (1971).
- ¹⁷ Crook, J. H., in *Sexual Selection and the Descent of Man* (edit. by Campbell, B. G.), (Aldine, Chicago, 1972).
- ¹⁸ Eisenberg, J. F., Muckenkirn, N. A., and Rudran, R., *Science*, **176**, 863-874 (1972).
- ¹⁹ Schaller, G. B., *The Mountain Gorilla* (Chicago University Press, Chicago, 1963).
- ²⁰ Thorington, R. W., jun., in *Progress in Primatology* (edit. by Starck, D., Schneider, R., and Kuhn, H.-J.), (Fischer-Verlag, Stuttgart, 1967).
- ²¹ Baldwin, J. D., *Folia primat.*, **9**, 281-314 (1968).
- ²² Baldwin, J. D., *Folia primat.*, **11**, 35-79 (1969).
- ²³ Baldwin, J. D., and Baldwin, J., *Folia primat.*, **18**, 161-184 (1972).
- ²⁴ Marler, P., *Science*, **163**, 93-95 (1969).
- ²⁵ Kingston, T. J., *E. Afr. Wildl. J.*, **9**, 172-175 (1971).
- ²⁶ Schenkel, R., and Schenkel-Hulliger, L., in *Progress in Primatology* (edit. by Starck, D., Schneider, R., and Kuhn, H.-J.), (Fischer-Verlag, Stuttgart, 1967).
- ²⁷ Chalmers, N. R., in *Comparative Ecology and Behaviour of Primates* (edit. by Michael, R. P., and Crook, J. H.), (Academic Press, London, 1973).
- ²⁸ Struhsaker, T. T., *Folia primat.*, **11**, 80-118 (1969).
- ²⁹ Hladik, C. M., *Mammalia*, **31**, 120-147 (1967).
- ³⁰ Hollih, von K. W., *Z. Säugetierk.*, **36**, 65-95 (1969).
- ³¹ Lack, D., *Ecological Adaptations for Breeding in Birds* (Methuen, London, 1968).
- ³² Clutton-Brock, T. H., Thesis, University of Cambridge (1972).
- ³³ Rahm, U. H., in *Old World Monkeys* (edit. by Napier, J. R., and Napier, P. H.), (Academic Press, New York, 1970).
- ³⁴ Kingdon, J., *East African Mammals*, **1** (Academic Press, London, 1971).
- ³⁵ Clutton-Brock, T. H., *Folia primat.*, **19**, 368 (1971).
- ³⁶ Marler, P., *Folia primat.*, **13**, 81-91 (1970).
- ³⁷ Marler, P., *Behaviour*, **42**, 175-197 (1972).
- ³⁸ Wooldridge, F. L., *Anim. Behav.*, **19**, 481-485 (1971).
- ³⁹ Clutton-Brock, T. H., *Folia primat.* (in the press).
- ⁴⁰ Goss-Custard, J. D., Dunbar, R. I. M., and Aldrich-Blake, F. P. G., *Folia primat.*, **17**, 1-19 (1972).
- ⁴¹ Krebs, J. R., MacRoberts, M. H., and Cullen, J. M., *Ibis*, **114**, 507-530 (1972).
- ⁴² Kummer, H., *Primate Societies: Group Techniques of Ecological Adaptation* (Aldine, Chicago, 1971).
- ⁴³ Vesey-Fitzgerald, D. R., *E. Afr. Wildl. J.*, **7**, 131-145 (1969).
- ⁴⁴ Marler, P., *Behaviour*, **42**, 175-197 (1972).
- ⁴⁵ Hladik, C. M., Hladik, A., *La Terre et la Vie*, **2**, 149-215 (1972).
- ⁴⁶ Jarman, P. J., *Behaviour* (in the press).
- ⁴⁷ Emlen, J. M., *Ecology: An Evolutionary Approach* (Addison-Wesley, Mass., 1973).

Tectonic segmentation of the Andes: implications for magmatism and metallogeny

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The Andean orogen consists of a series of tectonic segments separated by transverse boundaries that reflect discontinuities on the underlying subduction zone. The characteristics of belts of magmatic rocks and the type, age and size of ore deposits in a series of longitudinal belts may change at tectonic boundaries.

THE central Andes provide a classic example of a volcano-plutonic orogen developed along a convergent plate margin¹. The longitudinal continuity and transverse variability of characteristic volcano-plutonic orogens are commonly emphasised². Although I accept that longitudinal continuity is dominant in such orogens, I stress here the potential significance of a fundamental, longitudinal segmentation of the central Andes and, by analogy, of other comparable orogenic belts above subduction zones.

In Chile a series of longitudinal, physiographic provinces (the Norte Grande, Norte Chico, Central Chile, and Southern Chile) has been recognised for many years. Some of the boundaries between the physiographic provinces must have a seismic significance because they coincide with positions at which the level of seismicity changes^{5,6}. The Norte Grande and Central Chile, with longitudinal, fault-bounded valleys, bordered to the east by lines of recent volcanoes^{3,4,7}, contrast with the Norte Chico from which these features are absent, thereby demonstrating that the physiographic provinces also exert a control over tectonism and calc-alkaline magmatism.

Similar, possibly more important, transverse boundaries which coincide with major features in the east-central Pacific, effectively define the northern and southern limits of the central Andes and mark a single, transverse division⁸.

A study of intermediate and deep focus earthquakes in the Japanese arcs⁹ revealed a series of offsets and changes in strike in the deep seismic zone, which is thus effectively divided into a number of 100–300 km long segments. These discontinuities can be correlated with surface geological features, such as: offsets or changes in the strike of the trench axis or of belts of volcanoes; and lines of volcanoes, prominent structures or topographic changes transverse to the arc⁹. It is proposed here that the well known longitudinal subdivisions of Chile, and the boundaries emphasised by Gansser⁸, are comparable to the transverse geological features in Japan and are probably also coincident with discontinuities on the underlying deep seismic zone.

Using the criteria of Carr *et al.*⁹, together with additional geological evidence, I have subdivided the central Andes into tectonic provinces (Fig. 1).

Definition of tectonic segments

Boundary 1 (Fig. 1) is known as the Amotape zone^{7,10,11}, and marks the northern limit of the central Andes⁸. The overall geology changes markedly at this point; to the north, a coastal belt of ophiolitic rocks appears^{8,12}, together with a line of recent stratovolcanoes associated with a longitudinal graben⁷. The boundary coincides with local transverse strikes⁸ and faults¹³, aligned with the Gulf of Guayaquil and the Carnegie Ridge to the west, and the Amazon depression, perhaps an old shield lineament¹¹, to the east.

Boundary 2 approximately coincides with the Huancabamba deflection¹⁴ at a point where the Andes change their strike from north-west to north-north-east.

Transverse strikes have been noted on boundary 3 (ref. 10), which marks the southern limit of the Coastal Cordillera.

Boundary 4 coincides with the Pisco¹⁴ or Abancay^{15,16} deflection and has been proposed as a line of division in the central Andes⁸. It is marked by a major step in the coastline, which indicates the abrupt northward disappearance of the Precambrian formations of the Coastal Cordillera^{17,18}, and by the offshore Nazca Ridge, and a shallowing of the Peru–Chile trench¹⁹. On the boundary there is a marked northward narrowing and change in direction of the Eastern Cordillera, and north-east–south-west striking formations^{16,20}. Important changes in the Mesozoic palaeogeography have also been observed¹⁶.

Boundary 5 is marked by the northern limits of the belt of recent volcanoes and the Altiplano–Puna block.

Boundary 6 is characterised by a change in Andean strike, from north to north-west, and by a northward narrowing of the Eastern Cordillera, where it has been termed the Ichilo fault zone²¹ or line²², or the Arica Elbow line²³. East of the Andean orogen the Precambrian basement is veneered by Cainozoic sediments to the north of the boundary, whereas to the south, Palaeozoic and Cretaceous formations are also present²⁴. The

westward extension of the Arica Elbow line is less apparent but has been proposed^{22,23,25}.

Boundary 8 is so positioned because of the coincidence of a sinuous portion of the Peru–Chile trench¹⁹ with the increase in width of the longitudinal valley (Fig. 1), the northern limit of the north-north-east trending Cordillera Domeyko²⁶, a westward step in the longitudinal belt of recent volcanoes, and an extension of Quaternary volcanoes in an irregular transverse belt extending as far as the Eastern Cordillera, especially in Sud Lipez in southernmost Bolivia.

Boundary 10, between the Norte Grande and the Norte Chico, coincides with the approximate southern limits of the belt of recent volcanoes, the longitudinal valley and the elevated

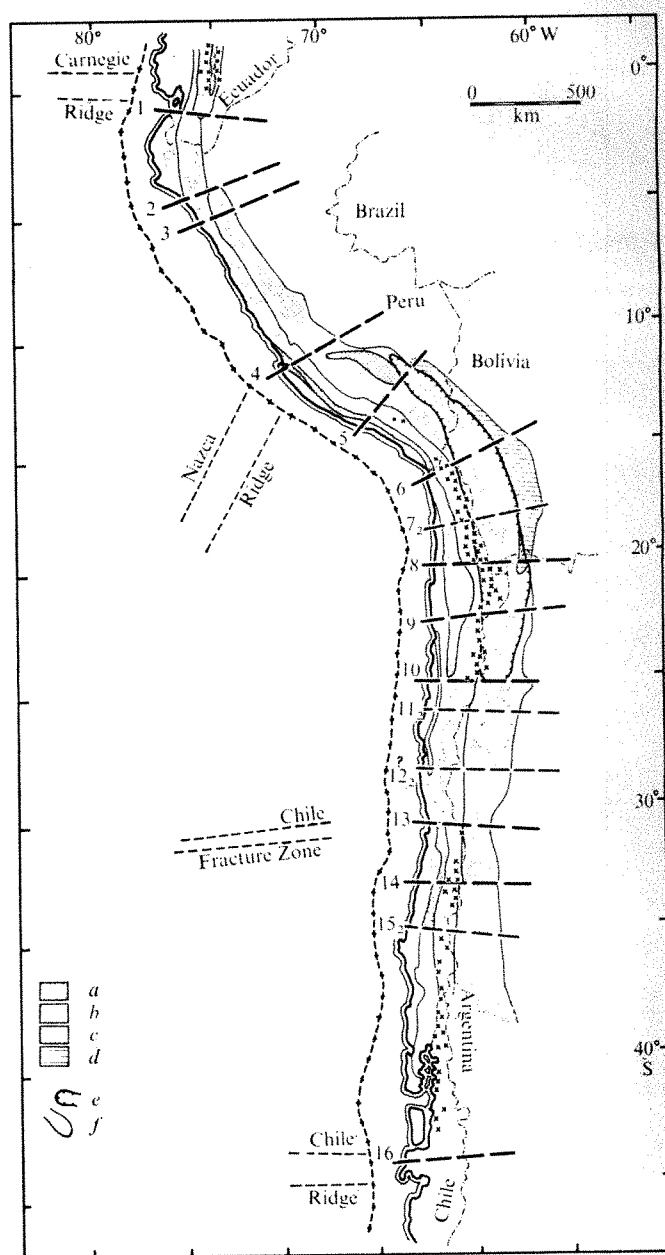


Fig. 1 The tectonic boundaries proposed for the central Andean volcano-plutonic orogen, in relation to certain physiographic features. a–d, metallogenic belts: a, iron; b, copper–gold–molybdenum; c, copper–lead–zinc–silver; d, tin–tungsten–silver; e, Altiplano–Puna; f, Longitudinal and Central Valleys; x, Location of recent volcanoes; +---+, axis of Peru–Chile trench. Heavy dashed lines are the boundaries between the tectonic segments; numbered as referred to in the text; a sub-script 2 denotes a boundary of only secondary importance.

Altiplano-Puna block²⁷ (Fig. 1). To the south there is a distinctive Cainozoic geomorphological development^{28,29}. The boundary also seems to have controlled the southern limit of widespread volcanism in the Jurassic, and the northern limit of widespread submarine volcanism in the Lower Cretaceous³⁰.

Boundary 13 marks the junction between Central Chile and the Norte Chico. It limits the northernmost extent of the belt of recent volcanoes, of the Central Valley—a Palaeogene feature^{31,32}, of the belt of Upper Tertiary andesitic–basaltic volcanics, and of widespread Precambrian rocks and Palaeozoic intrusives in the Coastal Cordillera²⁶. In Argentina, the boundary controls the southernmost extent of the Precordillera³³ and the Sierra de Córdoba. The boundary also lies at approximately the same latitude as the Chile fracture zone in the east-central Pacific (Fig. 1).

Boundary 16 is the southern limit of the central Andes⁸ and of the Central Valley of Chile (Fig. 1), and lies at the same latitude as the spreading Chile Ridge. It also corresponds to the southern limit of volcanism in Upper Cretaceous, Eocene, late Tertiary and recent time³⁰.

Correlations with seismicity

In order to confirm whether or not the present seismic boundaries in the Andes coincide with the geologically defined boundaries suggested here, and in an attempt to recognise additional boundaries, a study of the latitudinal distribution of intermediate and deep focus earthquakes is necessary. Some information on seismic provinces is, however, available and correlates reasonably well with the tectonic segmentation proposed here. Gajardo and Lomnitz⁵ defined provinces in Chile according to the levels of seismicity measured during a 16 yr period. Some of their boundaries correspond to boundaries 6, 8, 10, 14 and 15 (Fig. 1). Stauder³⁴ defined a similar series of boundaries between distinct seismic provinces, which correspond well to boundaries 6, 9, 10, 14 and 16 (Fig. 1). He also postulated that “the lithospheric slab” is apparently “segmented into a series of tongues that are absorbed independently”. South of boundary 15 there is a shift of major seismicity to an offshore position^{34,35}. Kelleher³⁶ studied the rupture pattern of the shallow part of the seismic zone beneath the Andes. He determined that the zone between boundaries 1 and 3 (Fig. 1) has been aseismic this century, whereas that between boundaries 3 and 5 has experienced large earthquakes, and that between boundaries 13 and 16 has ruptured about once each century along its entire length. He also recognised the intervals between boundaries 5 and 9 and 9 and 13 as distinct seismic provinces.

Nature of tectonic boundaries

As favoured by Carr *et al.*⁹ for the Japanese arcs, I believe that the surface geological discontinuities in the central Andes probably correlate with subjacent seismic discontinuities, and reflect transverse boundary zones between separate segments of oceanic lithosphere which are subducted as individual units, perhaps even at different rates. The segmentation of the subduction zone is presumed to result from stresses created by the underthrusting of a cap-like slab of lithosphere, and the pattern produced may well be self-perpetuating. Transform faults, which divide up the oceanic lithosphere into a series of strips, may also play a part in the production of the segments. Shield lineaments could also be involved at the boundaries at which changes in Andean strike are apparent⁸, but they probably exert only a subsidiary influence.

The surface manifestations of the tectonic boundaries suggest that the magnitudes, and perhaps the histories, of their activity

are different. Furthermore, some boundaries (such as No. 13) seem to be fairly abrupt, and others (such as No. 10) more diffuse. It is not clear whether tectonic boundaries are relatively permanent features or whether they tend to migrate slowly or jump around with time. The fact that some of the present boundaries apparently occupied the same positions in early Cainozoic and even Mesozoic times, however, implies some degree of stability, or at least of repeated reactivation.

Although the discontinuities at the surface and on the subduction zone probably coincide, it does not necessarily mean that they are connected by major fault zones. Rather, I believe that the crustal segments overlying the various segments of the sinking slab have been subjected to similar, but clearly distinct, tectonic, geomorphological, magmatic and metallogenic regimes, with zones of transition between them. Consequently, the wholesale extrapolation of geological histories along the lengths of volcano–plutonic orogens should be avoided.

Implications for magmatism

Across the central Andes, post-Triassic calc-alkaline magmas, of probable subduction zone origin^{1,2,37,38}, have been emplaced episodically upon and within the continental crust as a series of discrete pulses^{30,39,40}. Each magmatic pulse has given rise to long, narrow belts of intrusive and extrusive rocks roughly parallel to the continental margin. Successive belts young eastwards in the western and central parts of the orogen^{30,39,40}, as is common in volcano–plutonic orogens⁴¹, whereas little lateral migration of magmatism took place in a second locus of activity along the eastern margin of the Andes at latitudes 14°–24°S.

Radiometric age data^{30,40,42}, together with the mapped distribution of the magmatic belts both in the west and centre of the orogen and further east, support the concept that the belts are not longitudinally continuous. Therefore, magmatism in any particular tectonic segment can be expected to possess its own unique episodicity and spatial distribution, replaced by similar but clearly distinguishable regimes to the north and south. Clear discontinuities in belts of intrusive rocks have been recognised at boundaries 2, 3, 4, 5, 6, 7, 8, 10, 13 and 14, and volcanism, particularly recent activity, only occurs in certain segments at any one time.

Although magmatism in the North American Cordillera is locally episodic, it is essentially continuous if viewed over the full extent of the orogen⁴³. Meso–Cainozoic intrusive epochs cannot be correlated throughout the orogen⁴⁴. This may be simply explained if it is accepted that age patterns are only valid within individual tectonic segments.

Implications for metallogeny

The majority of the principal ore deposits in the central Andes have an inherent spatial and temporal relationship with the magmatic activity, and they are believed to derive their metal contents from the underlying subduction zone^{45–47}. Thus, it might be expected that the regional metallogeny varies between segments. An examination of the distribution of post-Palaeozoic metallogenic belts in the central Andes (Fig. 1, and ref. 46) reveals a coastal belt rich in iron, bordered to the east by a copper–(gold–molybdenum) belt, a copper–lead–zinc–silver belt, and, in the central part of the region, a tin–(tungsten–silver) belt. When tectonic boundaries are crossed these metallogenic belts tend either to end or to undergo changes in their characteristics, including: second-order variations in their metal contents; changes in their widths; and changes in the ages, types and sizes of deposits. The tin belt is restricted to three segments enclosed by boundaries 5 and 8 (Fig. 1), and is subdivided into a narrow, northern segment dominated by vein-type tin–tungsten deposits related to Mesozoic and

early Miocene batholiths, and a broader, southern part in which tin and tin-silver deposits of vein and porphyry⁴⁹ types are largely related to late Tertiary subvolcanic stocks⁵⁰⁻⁵². Contact-metamorphic and vein type iron deposits are located in the coastal zone between boundaries 1 and 2, 4 and 6, and 9 and 12 (refs 30, 53, 54). The copper belt extends northwards from boundary 15 (ref. 53), but loses a good deal of its importance north of boundary 4. The polymetallic province is particularly enriched in silver north of boundary 1 (ref. 13), but is most important, despite its narrowness, from boundary 2 to boundary 4. From boundary 4 to boundary 5 the metal content is anomalous and lead, zinc and silver are overshadowed by copper and iron (Fig. 1, and ref. 54), and south of boundary 8 mineralisation is rather sparse and dominated by copper⁵⁵. Within the copper belt the termination of long lines of distinctive ore types is also controlled by tectonic boundaries. Examples are the chalcopyrite-magnetite-actinolite veins that are known between boundaries 8 and 12 (ref. 53) and the disseminated, stratiform (manto-type) copper deposits in Jurassic volcanics from boundary 8 to boundary 10 (ref. 56). The porphyry copper deposits in the central Andes decrease in age eastwards³⁸ but, in addition, the major deposits change in age along the strike, so that Palaeocene deposits are dominant between boundaries 5 and 6 (refs 40 and 57), late Eocene-Oligocene deposits are dominant between boundaries 7 and 10, and late Miocene-Pliocene deposits are dominant between boundaries 12 and 14 (ref. 58). It is concluded that the definition of metallogenic segments is an important facet of the metallogenic characterisation of volcano-plutonic orogens and effectively explains the longitudinal variability of their metallogeny.

Mineral exploration

The mapping of metallogenic segmentation in both young and ancient volcano-plutonic orogens is clearly of paramount importance in the planning of mineral exploration programmes since metal contents, ore types and even economic importance change at tectonic boundaries.

Although a conclusive statement cannot be made at present, there is evidence from the central Andes that some major ore deposits tend to be located on or close to tectonic boundaries; the Chuquicamata and Río Blanco-Disputada porphyry copper deposits lie on boundaries 8 and 13, and the Oruro and Potosí porphyry tin deposits on boundaries 6 and 7. If magmas and their related metals are derived from the vicinity of the subduction zone, then this possibility seems to be more reasonable than the proposal that the locations of major ore deposits are controlled by megalineaments and their inter-sections (ref. 59 and 60), especially as lineaments in the central Andes are commonly at high angles to the tectonic boundaries⁶¹⁻⁶³ and are relatively superficial flaws in the upper continental crust.

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¹ James, D. E., *Bull. geol. Soc. Am.*, **82**, 3325-3346 (1971).

² Dickinson, W. R., *Rev. Geophys. Space Phys.*, **8**, 813-860 (1970).

³ Brüggen, J., *Fundamentos de la Geología de Chile* (Inst. Geogr. Milit., Santiago, 1950).

⁴ Muñoz Cristi, J., in *Handbook of South American Geology* (edit. by Jenks, W. F.), 187-214 (*Mem. geol. Soc. Am.*, **65**; 1956).

⁵ Gajardo, E., and Lomnitz, C., *Proc. 2nd World Conf. Earthquake Engng.*, **3**, 1529-1540 (1960).

⁶ Lomnitz, C., *J. geophys. Res.*, **67**, 351-363 (1962).

⁷ Gerth, H., *Der Geologische Bau der südamerikanischen Kordillere* (Borntraeger, Berlin, 1955).

⁸ Gansser, A., *J. geol. Soc. Lond.*, **129**, 93-131 (1973).

⁹ Carr, M. J., Stoiber, R. E., and Drake, C. L., *Bull. geol. Soc. Am.*, **84**, 2917-2930 (1973).

¹⁰ Steinmann, G., *Geología de Perú* (Carl Winters Universität, Heidelberg, 1930).

¹¹ de Loczy, L., *An. Acad. brasil. Ciénc.*, **42**, 185-205 (1970).

¹² Goossens, P. J., and Rose, W. I. jun., *Bull. geol. Soc. Am.*, **84**, 1043-1052 (1973).

¹³ Goossens, P. J., *Econ. Geol.*, **67**, 458-468 (1972).

¹⁴ Ham, C. K., and Herrera, L. J. jun., in *Backbone of the Americas* (edit. by Childs, O. E., and Beebe, B. W.), 47-61 (*Mem. Am. Ass. Petrol. Geol.*, **2**; 1963).

¹⁵ Jenks, W. F., in *Handbook of South American Geology* (edit. by Jenks, W. F.), 215-247 (*Mem. geol. Soc. Am.*, **65**; 1956).

¹⁶ Marocco, R., *Cah. ORSTOM, sér. Géol.*, **3**, 1, 45-58 (1971).

¹⁷ Bellido, E., *Bol. Serv. Geol. Min., Lima*, **22** (1969).

¹⁸ Cobbing, E. J., and Pitcher, W. S., *Nature phys. Sci.*, **240**, 51-53 (1972).

¹⁹ Fisher, R. L., and Raitt, R. W., *Deep-Sea Res.*, **9**, 423-443 (1962).

²⁰ Ruegg, W., *Bol. Soc. Geol. Perú*, **38**, 97-142 (1962).

²¹ Rod, E., *Bull. Am. Ass. Petrol. Geol.*, **44**, 107-108 (1960).

²² Radelli, L., *Trav. Lab. Geol. Grenoble*, **42**, 237-261 (1966).

²³ Schlatter, L. E., and Nederlof, M. H., *Bol. Serv. geol. Bolivia*, **8** (1966).

²⁴ Ahlfeld, F., *Geol. Rdsch.*, **59**, 1124-1140 (1970).

²⁵ Sonnenberg, F. P., in *Backbone of the Americas* (edit. by Childs, O. E., and Beebe, B. W.), 36-46 (*Mem. Am. Ass. Petrol. Geol.*, **2**; 1963).

²⁶ *Mapa Geológico de Chile*, 1:1,000,000 (Inst. Invest. Geol., Santiago, 1968).

²⁷ Turner, J. C. M., *Geol. Rdsch.*, **59**, 1028-1063 (1970).

²⁸ Sillitoe, R. H., Mortimer, C., and Clark, A. H., *Trans. Inst. Min. Metall.*, **B77**, 166-169 (1968).

²⁹ Mortimer, C., *J. geol. Soc. Lond.*, **129**, 505-526 (1973).

³⁰ Ruiz, F. C., Aguirre, L., Corvalán, J., Klohn, C., Klohn, E., and Levi, B., *Geología y Yacimientos Metalíferos de Chile* (Inst. Invest. Geol., Santiago, 1965).

³¹ Levi, B. D., Aguilar, A. M., and Fuenzalida, R. P., *Bol. Inst. Invest. Geol., Santiago*, **19** (1966).

³² Carter, W. D., and Aguirre, L., *Bull. geol. Soc. Am.*, **76**, 651-664 (1965).

³³ Herrero-Ducloux, A., in *Backbone of the Americas* (edit. by Childs, O. E., and Beebe, B. W.), 16-28 (*Mem. Am. Ass. Petrol. Geol.*, **2**, 1963).

³⁴ Stauder, W., *J. geophys. Res.*, **78**, 5033-5061 (1973).

³⁵ Lomnitz, C., *Geol. Rdsch.*, **59**, 938-960 (1970).

³⁶ Kelleher, J., *J. geophys. Res.*, **77**, 2087-2103 (1972); Kelleher, J., et al., *J. geophys. Res.*, **78**, 2547-2585 (1973).

³⁷ Dickinson, W. R., *Am. J. Sci.*, **272**, 551-576 (1972).

³⁸ Sillitoe, R. H., *Econ. Geol.*, **67**, 184-197 (1972).

³⁹ Farrar, E., Clark, A. H., Haynes, S. J., Quirt, G. S., Conn, H., and Zentilli, M., *Earth planet. Sci. Lett.*, **10**, 60-66 (1970).

⁴⁰ Stewart, J. W., Evernden, J. F., and Snelling, N. J., *Bull. geol. Soc. Am.* (in the press).

⁴¹ Dickinson, W. R., *J. geophys. Res.*, **78**, 3376-3389 (1973).

⁴² Clark, A. H., and Farrar, E., *Econ. Geol.*, **68**, 102-106 (1973).

⁴³ Gilluly, J., *Bull. geol. Soc. Am.*, **84**, 499-514 (1973).

⁴⁴ Lanphere, M. A., and Reed, B. L., *Bull. geol. Soc. Am.*, **84**, 3773-3782 (1973).

⁴⁵ Sillitoe, R. H., *Bull. geol. Soc. Am.*, **83**, 813-818 (1972).

⁴⁶ Sillitoe, R. H., *Geol. Assoc. Canada Spec. Pap.* (in the press).

⁴⁷ Sawkins, F. J., *J. geol.*, **80**, 377-397 (1972).

⁴⁸ Petersen, U., *Geol. Rdsch.*, **59**, 834-897 (1970).

⁴⁹ Sillitoe, R. H., and Halls, C., submitted for publication.

⁵⁰ Ahlfeld, F., and Schneider-Scherbina, A., *Los Yacimientos Minerales y de Hidrocarburos de Bolivia* (Depto. Nac. Geol. Bol. 5, La Paz, 1964).

⁵¹ Ahlfeld, F., *Miner. Deposita*, **2**, 291-311 (1967).

⁵² Turneaure, F. S., *Econ. Geol.*, **66**, 215-225 (1971).

⁵³ Ruiz, F. C., and Ericksen, G. E., *Econ. Geol.*, **57**, 91-106 (1962).

⁵⁴ Bellido, E., and de Montreuil, L., *Aspectos Generales de la Metalogenia del Perú* (Serv. Geol. Min., Geol. Econ. 1, Lima, 1972).

⁵⁵ Angelelli, V., Fernandez Lima, J. C., Herrera, A., and Aristarain, L., *Descripción del mapa Metalogenético de la República Argentina. Minerales Metalíferos* (Direc. Nac. Geol. Min., Buenos Aires, 1970).

⁵⁶ Ruiz, C., Aguilar, A., Egert, E., Espinosa, W., Peebles, F., Quezada, R., and Serrano, M., in *Proc. IMA-IGOD Meetings '70, IAGOD Vol.* (edit. by Takeuchi, T.), 252-260 (Soc. Mining Geol. Japan Spec. Issue 3, Tokyo, 1971).

⁵⁷ Laughlin, A. W., Damon, P. E., and Watson, B. N., *Econ. Geol.*, **63**, 166-168 (1968).

⁵⁸ Quirt, S., Clark, A. H., Farrar, E., and Sillitoe, R. H., *Geol. Soc. Am. Absts. with Prog.*, **3**, 7, 676-677 (1971).

⁵⁹ Mayo, E. B., *Min. Engng.*, **10**, 1169-1175 (1958).

⁶⁰ Thomas, N. A., *Geol. Rdsch.*, **59**, 1013-1027 (1970).

⁶¹ Segerstrom, K., *Prof. Pap. US geol. Surv.*, **700-D**, 10-17 (1971).

⁶² United Nations, *Fotolineamientos y Mineralización en el Noroeste Argentina* (Exploración Minera de la Region Noroeste, Argentina, inf. téc., New York, 1973).

Structure of yeast phenylalanine tRNA at 3 Å resolution

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The structure of a tRNA has been determined by isomorphous replacement. Some of the interactions which maintain the tertiary structure are of a novel type. Our model differs significantly from one which has recently been proposed.

TRANSFER RNA plays a central part in protein biosynthesis. Each molecule carries an amino acid to the ribosome and decodes the genetic information in messenger RNA, to put the sequence of amino acids in order. Since tRNA was first crystallised¹ we have looked² for a species which would give crystals suitable for high resolution X-ray analysis. Two years ago we reported³ the results of systematic crystallisation studies of yeast phenylalanine tRNA which yielded such crystals. Here we report the solution of the structure to 3 Å resolution using the method of isomorphous replacement. The map obtained is of sufficient quality to trace the ribose-phosphate chain over most of its course. In addition to the base pairs of the clover leaf formula (Fig. 1), we have found several interactions which help to maintain the tertiary structure. These are additional base pairs or triples, mostly not of the Watson-Crick type, and also a variety of stacking interactions. Most of these interactions can be related to invariant features of the nucleotide sequence of tRNA. The model is in accord with the chemical reactivities of different tRNAs.

The tRNA species studied here is the same as that for which a model has been recently proposed by a group at MIT⁴. Although the crystal forms are different, the molecular structure is expected to be the same. The two models differ, however, in certain major respects. Most of the nucleotide residues are differently placed in the two models, and none of the essential tertiary interactions are present in the MIT model.

X-ray analysis

The unit cell⁵ is monoclinic, space group P2₁ with $a=56.0$ Å, $b=33.4$ Å, $c=63.0$ Å and $\beta=90.4^\circ$. All the heavy atom derivatives were made by soaking the crystals in solutions containing various cations or complex ions. The addition of almost any heavy atom produced changes in the angle β , but this change could be minimised by keeping the crystals in a stream of air at 4° C (ref. 5). Table 1 lists the heavy atoms which gave good isomorphous derivatives, together with their occupancies and coordinates. The data were collected on a diffractometer. No significant deterioration (<15% in the intensity of monitored reflections) was observed during data collection, so that a single crystal could be used for each derivative. The quality of the crystal data is only moderate compared with that of some proteins of similar size. We attribute this not to intrinsic disorder in the crystal, but to the difficulty of handling the thin plate-

like crystals which easily crack or buckle. Heavy atom refinements were carried out by least squares (Table 1). The absolute hand was determined from the anomalous scattering of the samarium derivative. The electron density map was computed on a 1 Å grid. Sections perpendicular to x were assembled at a scale of 2 cm per Å in a Richards box⁶, and Kendrew skeletal parts were used to build the model.

Electron density map

As with a protein at the same resolution, the electron density map by itself does not suffice to solve the structure, but when combined with the nucleotide sequence and the known stereochemistry of the residues, it does provide enough information for the construction of an atomic model. In a protein map the polypeptide backbone is however visible as a continuous chain of high density and the side chains as bulges, many of which are sufficiently characteristic in

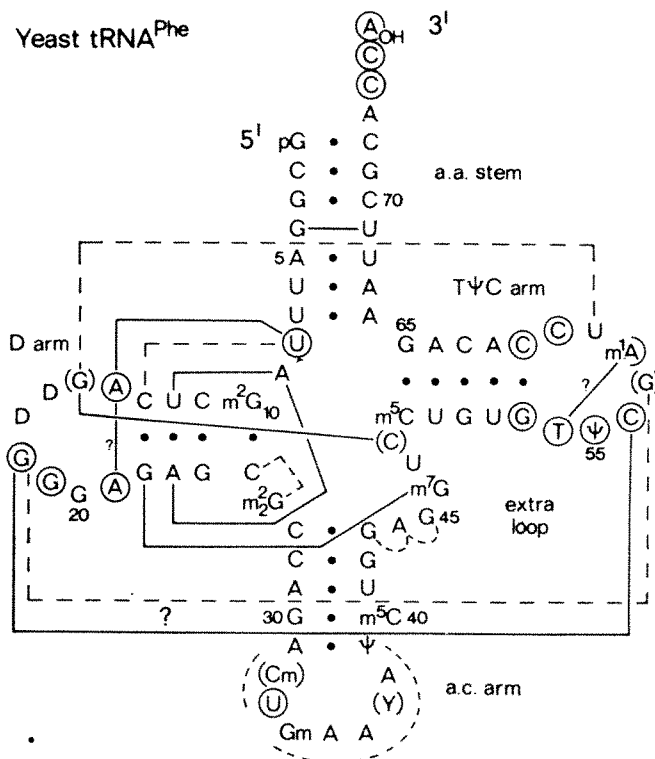


Fig. 1 The sequence of yeast tRNA^{Phe} arranged in the clover leaf formula²¹. An arm of the clover leaf is made up of a double helical stem and a single stranded loop. Bases which are invariant in all tRNA sequences²² are circled; semi-invariants, that is, purines or pyrimidines exclusively, are bracketed. Nucleotides which are base paired in the tertiary structure are joined by solid lines, and those which stack on each other by dashed lines.

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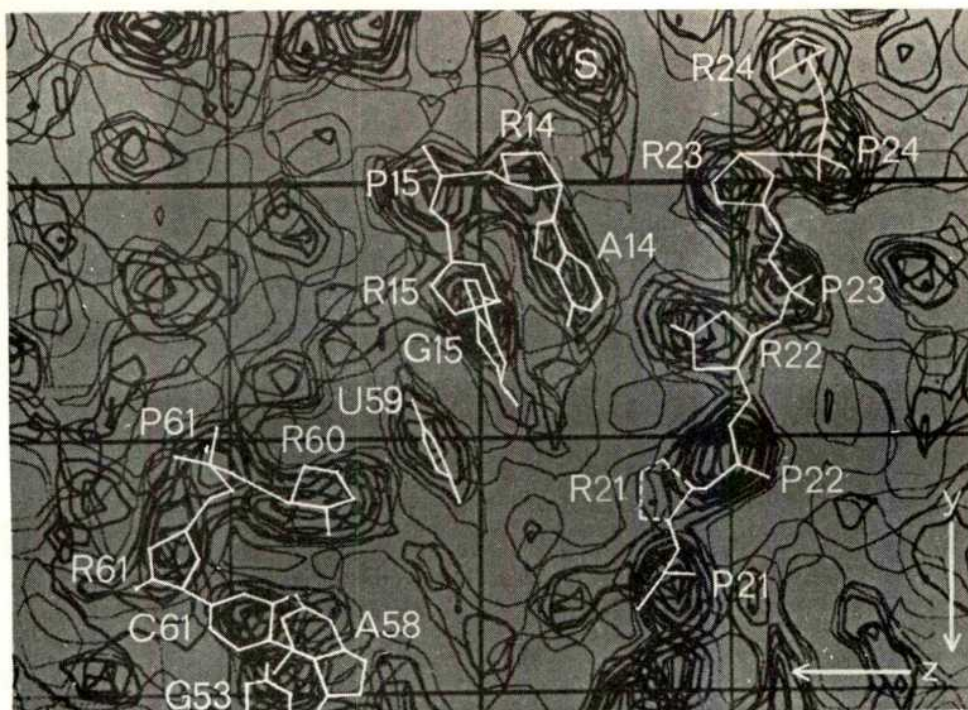


Fig. 2 A composite of four electron density map sections at $x=48-51$ Å. This includes a strand of the D stem. Most of the electron density not identified belongs to adjacent molecules or to cations. The heavy white lines represent the skeleton of the molecule. R21 lies just outside the sections shown. P: phosphate group; R: ribose; S: probably spermine. The grid squares are of side 10 Å. This region corresponds to part of Fig. 4 of the MIT paper⁴.

size and shape to be identified and hence related to the amino acid sequence. In contrast, in RNA there are essentially only two types of residue, purine and pyrimidine, not easily distinguishable from each other. Moreover the bases have a total mass similar to the phosphates and riboses, and, when base-paired to bridge two pieces of backbone not in a double helical region, they give that part of the map more the character of a network than of a dominant single chain standing out from a lower landscape. The situation is further complicated by the presence of numerous cations, Mg^{2+} , Na^+ or spermine, many hydrated and held in fixed positions. In tracing the ribose phosphate chain from the electron density map, the most important constraint comes from the expectation of finding the four double helical stems of the clover leaf formula and the necessity of joining them correctly. The electron density corresponding to the chain was generally clear in the double helical regions. In most areas clearly resolved peaks could be seen for each ribose and for each phosphate, which could usually be distinguished by their shape and by the connectivity of the density: the phosphate groups tend to be spherical while ribose density is more disk-like. The angles made between the groups are also very characteristic, giving a distinct sugar-phosphate stagger which is difficult to misinterpret (Fig. 2). Fitting the model in such regions fixed the polarity of the chain unambiguously.

The assignment of the helical stems could be checked because in some base pairs the purine can be distinguished from the pyrimidine, and wherever a decision can be made, the result agrees with the sequence (Fig. 3a). Confirmation of the helix assignment comes from chemical work on the platinum derivative. Fingerprints of various enzyme digests of the tRNA reacted with the platinum compound showed that the metal was bound in the fragment 34 to 39, agreeing with the position found for it in the model.

In regions of the map which do not contain double helical segments, the connectivity and polarity of the chain is more difficult to determine from electron density alone. In some places the thermal motion or disorder smears out the phosphate or ribose peaks. Here it is important to use conformational information^{7,8} obtained from single crystal structure analysis of oligonucleotides. In the end we were able to trace the whole chain except for several nucleotides

in the D and T ψ C loops. These lie close together in one corner of the molecule and are also in tight contact with the same loops of two other symmetry-related molecules. Here the density is strong but broken up and we have not been able to arrive at an unambiguous chain tracing. The stretches in question are however confined to a small region of the map so that, whatever the final details, their structure will be constrained within the bounds proposed in our model.

Table 1 Final heavy atom parameters and refinement statistics to 3 Å

Derivative	Site	Z	x	y	z	B	n	r.m.s. f_H	r.m.s. E	P
<i>trans</i> Cl ₂ (NH ₃) ₂ Pt	1	76.8	0.179	0.0	0.145	24.6	4,411	69.9	40.8	1.72
	1	58.7	0.629	0.147	0.453	17.4	2,090	86.9	59.5	1.46
	2	44.0	0.098	0.089	0.594	23.8				
	3	29.4	0.769	0.001	0.548	21.4				
Sm ³⁺	4	16.7	0.825	0.238	0.264	20.8				
	1	47.7	0.626	0.145	0.454	18.3	4,627	55.4	39.7	1.40
	2	31.7	0.098	0.091	0.594	26.9				
	3	9.7	0.768	-0.008	0.554	19.3				
Os-ATP complex ^{23,24}	4	9.1	0.828	0.243	0.264	25.5				
	1	32.7	0.236	0.446	0.974	36.2	4,338	42.9	37.9	1.14
	2	29.1	0.177	-0.001	0.146	14.2				
	3	21.6	0.626	0.362	0.735	34.4				
Pt + Lu	Pt1	62.8	0.180	0.0	0.146	25.5	4,184	80.0	43.5	1.84
	Lu1	41.3	0.629	0.144	0.453	15.6				
	2	32.5	0.099	0.090	0.595	24.8				
	3	26.2	0.771	-0.005	0.548	35.9				

Z is the occupancy. B is the isotropic temperature factor. n is the number of reflections used in the refinement. f_H is the calculated heavy atom contribution to the structure factor. E is the closure error, $|f_H(\text{observed}) - f_H(\text{calculated})|$. P is the phasing power, the ratio of the two previous columns. The Os derivative was refined in three shells⁹ because of changing occupancy; the Z quoted is the mean. The mean figure of merit for 4,838 reflections = 0.67. The mean standard deviation in $|F|$ of the reflections measured more than once during data collection was 6% of the mean $|F|$ for the native crystal, and somewhat higher for the derivatives. Structure factors and intensities were calculated using hand-measured coordinates of residues 1-17, 21-54 and 58-76. The classical R-factor on $|F|$ s is 0.468 and the correlation coefficient between calculated and observed intensities is 0.567.

Since we were not always able to distinguish purines from pyrimidines even within helical regions, the sequence data were initially of little help in solving the atomic structure, but as the helical regions and sequence numbering became established, features of density corresponding to the bases became interpretable. For example, the electron density at base position 26 was of a shape consistent with a twice

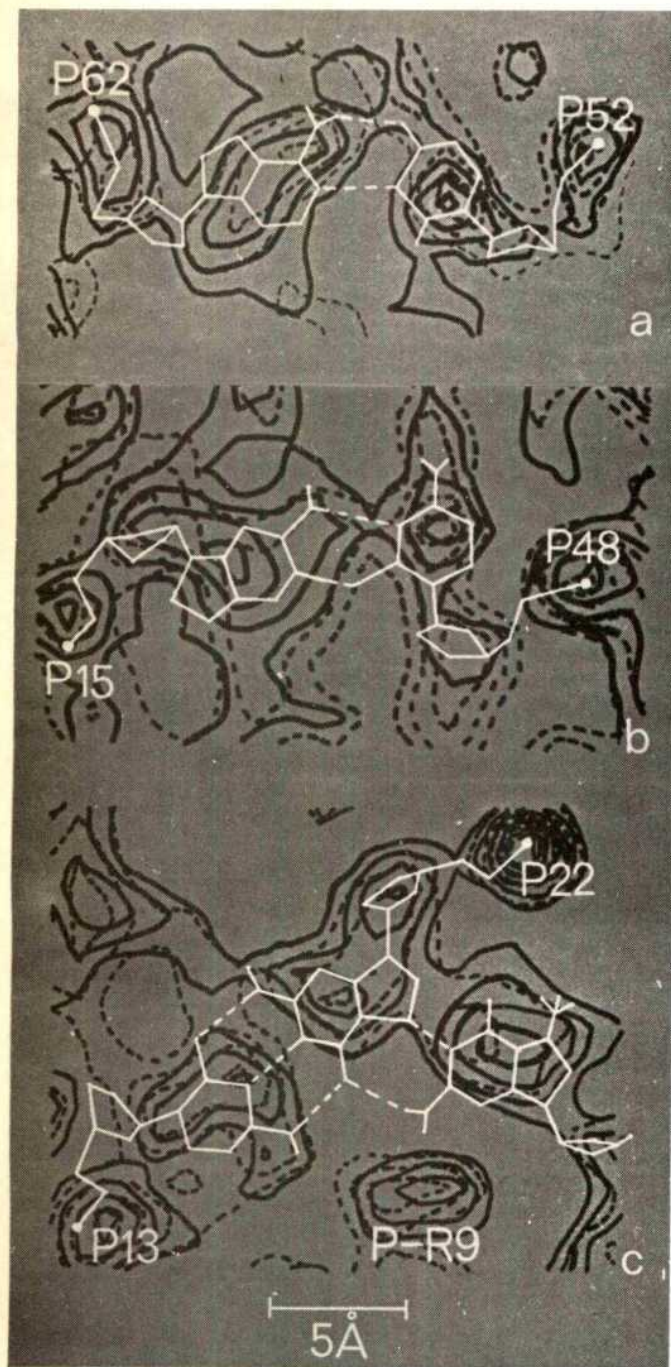


Fig. 3 Parts of skew slices of the electron density map showing the density distribution in the planes of certain base pairs or base triples. Each slice comprises two sections 1 Å apart (full and dotted contours). The superposed skeleton is taken from the coordinates of the unrefined model. The ribose groups generally lie out of the planes of the sections. *a*, A Watson-Crick base pair from the TψC stem. Additional peaks can be seen in the neighbourhood of non-bonded polar groups, probably water molecules or cations. *b*, The unusual base pair G15-C48 with the glycosyl bonds in the *trans* position. The peak above the amino group of C48 may be a cation or water molecule. *c*, A base triple from the augmented D helix, 13-22-46.

methylated G, and so provided a valuable marker in the model building. Also, the shape of the electron density for the G15 base (Fig. 3*b*) gave us confidence that the base pair 15-48 was of the unorthodox type to be discussed below.

Some features of the cation distribution also began to emerge. An interesting example is an ion found chelated between the amino groups of G25 and G45 and the oxygens of phosphates 23 and 24. This may be one of the tightly bound magnesium ions which seem to be an integral part of the native structure⁹.

Molecular structure and tertiary interactions

Figures 4 and 5 are photographs of the molecular model. The anticodon is at the upper right, the amino acid stem at the upper left, with the TψC loop in the bottom left corner. The relative arrangement of the double helical segments is indicated schematically in Fig. 6 which also shows the topology of the connections made in the tertiary structure. (These tertiary interactions are also indicated in Fig. 1.) All but seven of the bases are represented in the diagram. Base pairs additional to those in the stems are shown as dotted lines. As might be expected¹⁰, they are protected from water by stacking or intercalation. The stacking and intercalation of unpaired bases is also indicated. The region shown dashed is the TψC loop-D loop corner where we have not been able to determine the structure unambiguously.

The amino acid stem is stacked on the TψC stem in such a way that a long double helix of 12 base pairs is formed in which one of the ribose phosphate chains (72-61) is not interrupted. In the companion strand there is a break between nucleotides 7 and 49, though this does not seem to disturb the overall perfection of the long double helix. But the sharp turn made at the 50-49-48 corner involves the close approach of phosphates (~5 Å), and the details are subject to revision at higher resolution. The G-U base pair 4-69 also seems to be made without any major disturbance to the helix, at least at this resolution. The long double helix is right handed, has its bases tilted to the axis, and the axial distance between residues 61 and 72 is 30 Å so that the repeat per residue along the helix axis is 2.8 Å. The helix is therefore of the A form. At one end of the helix are stacked the unpaired bases A73 and C74, and at the other end, T54 which is also paired to another base in the TψC loop.

In contrast to the amino acid and TψC stems, the D stem and the anticodon stems are only approximately collinear, lying 20° from each other. The two together, with nucleotide 26 in between, form a long imperfect double helix which lies roughly at right angles to the first long helix, and meets it at its middle, so that the two are arranged rather as the letter T.

The D stem is guyed at the T join to the long double helix by part of the variable loop (residues 44-48) and by the stretch 8-9 which connects one strand of the amino acid stem to one strand of the D stem. This stretch, which is two nucleotides long in all tRNAs, is fully extended in the model, allowing it to span the length of the D stem. A9 makes a base pair, of the type found in poly(A)¹², with A23, which is paired to U12 in the D stem, so that all three together form a base triple. A base pair of the reverse Hoogsteen type¹³ is formed between U8 and A14, both of which are invariant nucleotides. U8 is then stacked on C13 and in the right orientation to allow the photoreaction observed by Yaniv¹⁴. On the pair 8-14 is stacked an unusual base pair formed between G15 of the D loop and C48 of the extra loop (Fig. 3*b*). These two bases form the correlated pair noticed by Levitt¹⁵ and might have been expected to form a Watson-Crick pair. We discuss this question below.

Thus the D stem has been augmented along its length by the non-standard base pairs 8-14 and 15-48. On the end of

this again there is stacked a single base for which there is strong density coming up from the T ψ C loop. This is either U59 or A58 depending on the resolution of the ambiguity discussed below. This base seems to mediate the abutting of the augmented D stem on to the long double helix formed by the amino acid and T ψ C stems, by fitting into the large groove and acting as a sort of peg to help hold the two perpendicular helices together. Apart from the connection 15–48, the D arm is also connected to the extra arm at a further point: G46 is base paired to G22, so that there is a second base triple 46–22–13 (Fig. 3c) in the structure. At the other end of the D stem is stacked the unpaired base G26, so that finally the augmented D helix contains a stack eight base spacings long. The translation per residue is about 3 Å so that this helix is closer to the A' form of RNA¹¹.

Continuing around the D loop, nucleotides D16 and D17 arch outwards from the body of the molecule. The invariant pair of residues G18 and G19 come into close contact with the T ψ C loop in a manner to be described,

with the base of 26 lying in the bearing formed by 44 and 45. A result of the tilt is that the major groove of the long, imperfect double helical segment formed by the anticodon and D stems is now some 5 Å narrower than for the more perfect double helix formed by the amino acid and T ψ C stems.

The anticodon loop itself is stacked on the 3' side roughly in the manner suggested by Fuller and Hodgson¹⁷. The density is weak in this region so we cannot be confident of the position of the bases but it seems that base 38 points inwards, 37 more outwards and finally the three bases of the anticodon protrude outwards from a quasihelical backbone. Here O2' of ribose 35 comes close to O1' of ribose 36, suggesting a hydrogen bond, and there is a similar linkage between 36 and 37. We can see no special structural rôle for the Y base 37. On the 5' side of the loop the bases 32 and 33 are also stacked but seem to face inwards rather than outwards⁷. This is made possible by kinks in the backbone at the two residues 32 and 34 methylated in the O2' position.

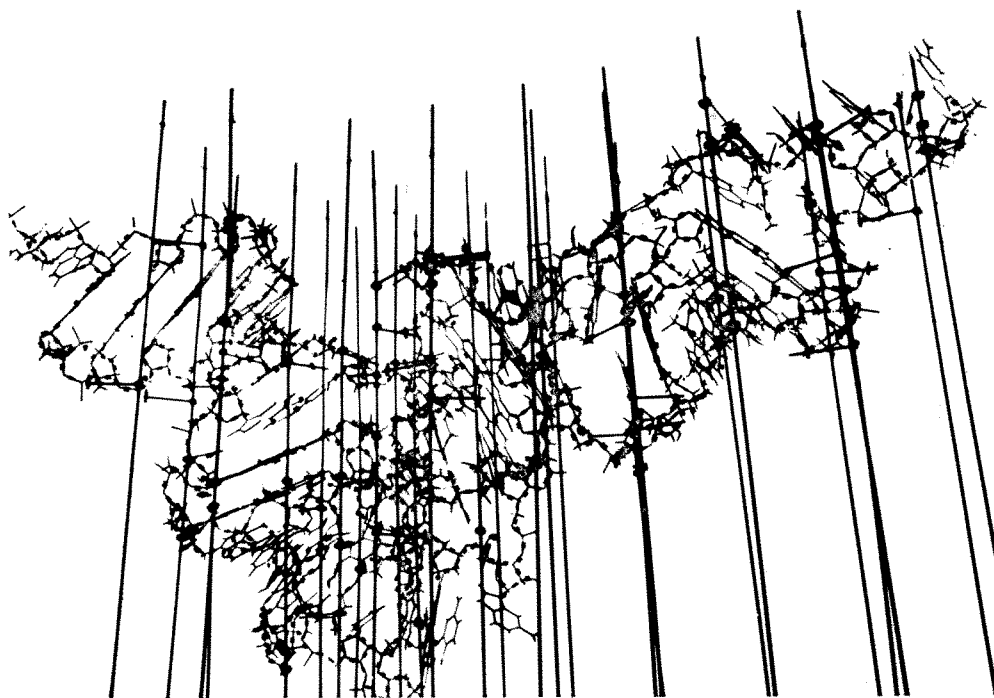


Fig. 4 A photograph of the model taken in a direction roughly perpendicular to the plane of the T made by the two long double helical segments (see Fig. 6). The geometrical shape of the T is somewhat obscured by the chains of the T ψ C and extra loops.

whereas G20, which is variable, comes up again close to the other variable nucleotides 16 and 17 and points outwards. The exposed position of these three residues is consistent with their chemical reactivity¹⁸. A21, which is invariant, stacks and intercalates between the base pairs 13–22 and 15–48. It lies in the plane of the base pair 8–14 but the density does not seem to permit another base triple here, tantalisingly close as it is. Possibly A21 forms a hydrogen bond with the ribose of 8. It may be that in other species of tRNA this would be a base triple formed by the three invariant bases, 8, 14 and 21.

The axis of the anticodon stem is not only tilted by about 20° to the D stem, but is also dislocated by about 5 Å from the line of the D stem. Thus the bases A44 and G45 are stacked on the last base pair 27–43 of the anticodon stem, but lie below the right hand end of the augmented D helix, with G26 partially intercalating between them. All tRNAs have a single unpaired base in position 26 and the structure suggests that this feature is associated with the tilting of the anticodon stem relative to the D helix. Indeed, the structure here presents the appearance of a hinge,

We have not been able to make an unambiguous tracing of the chain in the T ψ C loop region, but there are some features of which we are reasonably certain. T54 is paired to another base in the same loop, a base from this loop stacks on the end of the augmented double helix and G57 lies close to G18 and G19 of the D loop. We have found two alternative configurations, both of which are stereochemically acceptable. In the first version, T54 is Hoogsteen paired with A58, U59 stacks on G15, G57 intercalates between the bases of G18 and G19, and C56 is close enough to G18 or G19 to form a base pair. In the second version, the base which pairs with T54 is C60 and that which stacks on the augmented D helix is A58. The three bases G18, G19 and G57 still form a stack, but the order is now 18–19–57 rather than 18–57–19. This stacking proposed for the three invariant Gs is a novel and certainly unexpected tertiary interaction, but it remains to be proved correct. In both versions G20 is in the accessible part of the D loop, in the neighbourhood of D16 and D17.

At the 3' end, A73 and C74 are stacked on the end of the amino acid stem but the final ACCA stretch swings

across the unit cell and packs between the anticodon stems of two other symmetry related molecules. These latter stems are 33 Å apart in the *y* direction and the gap is nicely filled by this ACCA chain from the first molecule.

Relation to the MIT model

The relationship between the monoclinic form and the orthorhombic form studied⁴ at MIT is *a, b, c* (MRC) → *b, -a, c* (MIT). In view of the similarity of X-ray intensities³ the contents of half the orthorhombic unit cell should superpose on the whole monoclinic cell when aligned on the common 2₁ axes. We have found the relative translation from the lanthanide positions common to the two crystals, and the map sections published by the MIT group⁴ are generally similar to the corresponding sections in our map, but there are everywhere significant differences in detail and major differences in interpretation.

The overall shape of our model is similar to that proposed by the MIT group and we seem to agree about the relative positions of the anticodon, amino acid and TψC stems, but there is complete disagreement about the orientation of the central D stem. This difference means that almost all the connections to the remainder of the molecule must be radically different in the two models. We have been able to define a number of tertiary interactions in the molecular centre holding it rigid, whereas the only tertiary interaction proposed in the MIT model is that between the correlated base pair 15-48 which is, by implication, of the Watson-Crick type, contrary to our findings. In our model the D helix lies approximately normal to the TψC stem, that is, parallel to the long *c* axis, and the base pairs lie in planes roughly perpendicular to *c* (Fig. 4). The density corresponding to the bases is quite unequivocal in this respect. But in

(MIT Fig. 5) the sugar-phosphate stagger and the bases have not been fitted correctly to the density. It is presumably these misfittings which have led the MIT workers to state⁴ that the double helices in the stems are not regular and cannot be assigned to one of the standard classes. We find helices which are regular at the resolution of the map.

There seem to be many other differences between the two structures, though they mostly involve the loop regions, where the MIT chain tracing is presented as tentative. Thus, in the MIT model the large Y base 37 extends across the major groove between the anticodon and D double helices to touch a phosphate on the D stem, whereas we have found the Y base positioned inside the anticodon loop. The structure of the anticodon loop is not described in the MIT paper, but from the picture of their model it is clearly not the ordered structure which we have built. Likewise, the interactions formed by the extra loop in our model do not seem to be made in the MIT model, and no others are described. In the MIT model, many of the bases in the TψC loop and the distal part of the D loop seem to be exposed, whereas in our model, despite the ambiguity mentioned earlier, they are stacked or otherwise not accessible to chemical attack, as is observed¹⁰.

Conclusions

The molecule can be described as consisting of three major substructures (Fig. 6). These are: (1) the long double helix formed by the amino acid and TψC stems stacking on top of each other; (2) the augmented D helix, forming the "thorax" of the molecule: this consists of the D stem augmented by part of its own loop and interlocked with both the stretch 8-9 and with part of the extra loop; (3) the anticodon stem, tilted off by about 20° at the other end

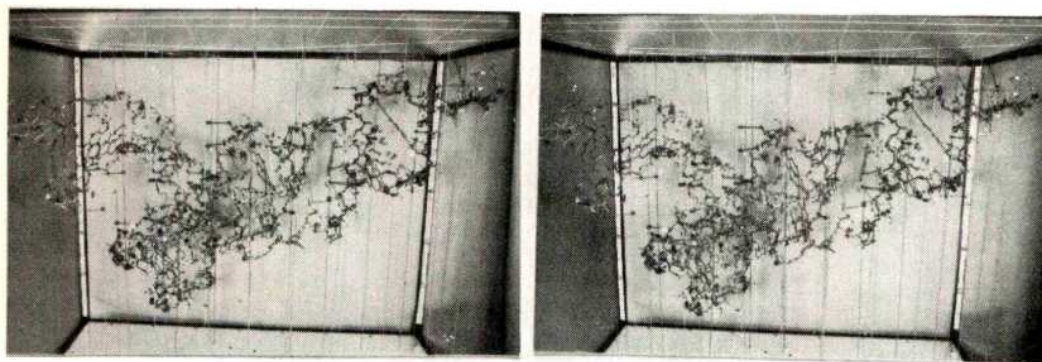


Fig. 5 A stereophoto-graph of a Kendrew skeletal model (2 cm per Å) of yeast tRNA^{Phe} built to fit the 3 Å map. The amino acid stem is at the upper left, and the anticodon at upper right. The TψC loop and distal part of the D loop are at the bottom left. The model is viewed approximately in the +*x* direction with +*z* running approximately from right to left. The whole CCA end at upper left is not in the frame of the photograph.

the MIT model the direction of the D stem is roughly perpendicular to *c*, as can be seen in the MIT Fig. 2 and from the disposition of the base pair 13-22 in their Fig. 4. Likewise the connecting sequence 8 and 9 runs horizontally in our model and more or less vertically in the MIT model.

Comparing our Fig. 4 with the corresponding area in Fig. 4 of the MIT paper, one sees that the only assignment here common to the two models is ribose 61. The MIT model has the base pair 13-22 placed in a way quite inconsistent with our density map and in effect forces a connection between residues 15 and 23. The assignment of the nucleotide sequence on the D stem is thus incorrect by two residues on one strand and by one on the other. We note that the other double helical stems have also not been fitted correctly in the MIT model²⁵. Thus, the MIT workers have placed R26 (their Fig. 3) where we have R27, so that the phase of the nucleotide sequence is incorrect and the base pair 42-28 is wrongly fitted to the density. This means that the tilt of all the bases in the anticodon helix must be wrong. Likewise we find that in the TψC helical stem

of the D stem and possibly hinged to it. These three main helical directions correspond well to the three largest peaks in Levitt's search of the X-ray intensities of the native crystal¹⁸: (1) corresponds to the peak at (−60°, −35°), (2) to (−15°, 0°), and (3) to (−35°, 0°), where the first value gives the rotation angle about *a*, and the second about *b*. The angle between (1) and (2) is then 103°.

To these substructures are adjoined other parts with clear functional rôles: (4) the ACCA sequence, at one end of (1), to carry the amino acid; (5) at the other end, the almost invariant TψC loop tightly folded and connected to the two invariant Gs 18 and 19 of the D loop, probably forming a ribosomal recognition site common to all tRNAs. The most variable nucleotides in the D loop, 16, 17 and 20, form (6) a patch flanking the augmented D helix (on the far side in Fig. 5); this may be an enzyme discrimination site, different for different tRNAs. There is also the region (7), on the other side of the D helix here consisting of a few variable bases; this can be assigned to the large extra arm which is present in other classes of tRNA, presumably for discrimi-

nation. Finally there is (8), the anticodon loop carrying the anticodon itself. This loop is stacked on the 3' side of the anticodon stem, but it has been suggested to us by Dr F. H. C. Crick that, if indeed the join between the anticodon stem and the augmented D helix functions as a hinge, then the conformation of the loop might possibly alter during protein synthesis, in the manner proposed by Woese¹⁹.

The unusual base pair 15-48 requires some discussion. Levitt¹⁵ noticed that 15 is always either G or A with 48 respectively C or U. There is only one exception²⁰ to this rule so far observed, in glycine tRNA where the bases are A and C. It is at first sight rather surprising that the nucleotides in these positions do not form a standard base pair. But if they did, one might expect the purine and pyrimidine to be exchanged between the two positions in some tRNAs. The fact is that the purine is always in position 15 and the pyrimidine always in 48. This speaks

example of a set of correlated base changes. Most class 1 sequences also have U12, A23 and A9 and can make the second triple base described above. There is a subclass which has G12, C23 and G9, but G9 can make only one hydrogen bond to C23, unless there is a different tautomeric form or adopts a syn conformation. There is always the possibility of a third triple interaction using the invariants U8-A14-A21 as mentioned above.

We should like to emphasise that the model is in good accord with the results of a companion study of the chemical reactivity of yeast tRNA^{Phe} as already reported by us²⁶. The model is stereochemically sound within the physical limits of building with skeletal wire components, and is now being refined. Data to higher resolution are also being collected.

We thank Dr M. J. Cleare of Johnson, Matthey & Co. Ltd. for the gift of *trans*-dichlorodiammine platinum and him and Dr B. Johnson for advice. We thank Dr M. Levitt for help and for his earlier model building, and Mrs S. Porter for help. J. D. R. held a National Institutes of Health post-doctoral fellowship.

Note added in proof: The MIT workers have now revised their model (Rich, personal communication).

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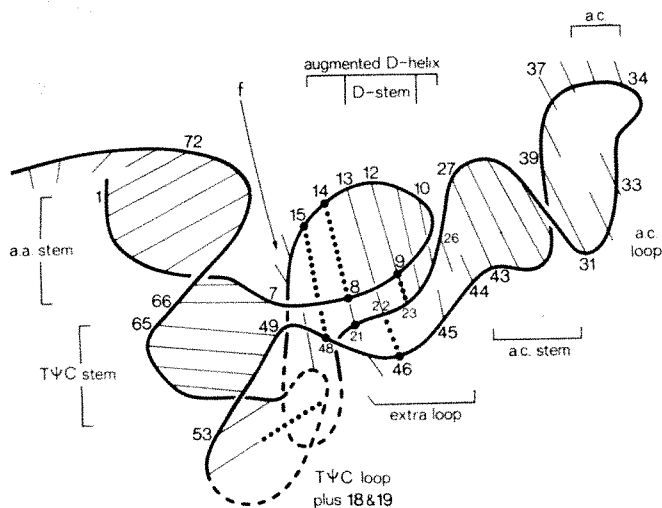


Fig. 6 A schematic diagram of the tertiary structure of yeast tRNA^{Phe}. The ribose-phosphate backbone is represented by a continuous line, except where there is ambiguity when it is shown dashed. Base pairs in the double helical stems are represented by long light lines, and non-paired bases by shorter lines. Many of the latter stack as indicated; those which do not are drawn at an angle, for example 16, 17 and 47. Base pairs additional to those in the clover leaf formula are indicated by dotted lines. The region marked f is explained in the text.

for an asymmetry in the bonding between the two chains, such as we have described. It is not possible to interchange the purine and pyrimidine and maintain the same disposition of the glycosyl bonds. Moreover, a 'reverse Watson-Crick' A-U pair can be accommodated here with almost the same *trans* arrangement of glycosyl bonds. It would seem that this nonsymmetrical pair of glycosyl bonds is required by the particular demand of connecting two locally parallel chains not related by any kind of dyad. Models of all ten combinations of base pairs in which the glycosyl bonds are *trans* to each other show that the two pairs G-C and A-U are the most similar, distinct from all the other possibilities.

We believe that our model can accommodate all tRNA sequences of the class 1 type¹⁵, and that many of its features will be retained in the other two classes. Class 1 has four bases in the D stem and five nucleotides in the extra loop, and all sequences can be fitted into the model without rearrangement. Most sequences have C13, G22 and G46 and hence can make the base triple 13-22-46. One of the remaining species has U13-A22, but 46 is now an A, and this combination can form a base triple with the three glycosyl bonds in the same positions. We thus have another

- ¹ Clark, B. F. C., Doctor, B. P., Holmes, K. C., Klug, A., Marcker, K. A., Morris, S. J., and Paradies, H. H., *Nature*, **219**, 1222-1224 (1968).
- ² Brown, R. S., Clark, B. F. C., Coulson, R. R., Finch, J. T., Klug, A., and Rhodes, D., *Eur. J. Biochem.*, **31**, 130-134 (1972).
- ³ Ladner, J. E., Finch, J. T., Klug, A., and Clark, B. F. C., *J. molec. Biol.*, **72**, 99-101 (1972).
- ⁴ Suddath, F. L., Quigley, G. J., McPherson, A., Sneden, D., Kim, J. J., Kim, S. H., and Rich, A., *Nature*, **248**, 20-24 (1974).
- ⁵ Marsh, D. J., and Petsko, G. A., *J. Appl. Cryst.*, **6**, 76-80 (1973).
- ⁶ Richards, F. M., *J. molec. Biol.*, **37**, 225-230 (1968).
- ⁷ Rubin, J., Brennan, T., and Sundaralingam, M., *Biochemistry*, **11**, 3112-3128 (1972).
- ⁸ Kim, S. H., Berman, H. M., Seeman, N. C., and Newton, M. D., *Acta Cryst.*, **B29**, 703-710 (1973).
- ⁹ Fresco, J. R., Adams, A., Ascione, R., Henley, D., and Lindahl, T., *Cold Spring Harb. Symp. quant. Biol.*, **31**, 527-537 (1966).
- ¹⁰ Levitt, M., in *Polymerisation in Biological Systems* (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 147-171 (Elsevier, Amsterdam, 1972).
- ¹¹ Arnott, S., *Progress in Biophysics and Molecular Biology*, **21** (edit. by Butler, J. A. V., and Noble, D.), 265-319 (Pergamon Press, Oxford, 1970).
- ¹² Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D., *J. molec. Biol.*, **3**, 71-86 (1961).
- ¹³ Hoogsteen, K., *Acta Cryst.*, **12**, 822-823 (1959).
- ¹⁴ Yaniv, M., Favre, A., and Barrell, B. G., *Nature*, **223**, 1331-1333 (1969).
- ¹⁵ Levitt, M., *Nature*, **224**, 759-763 (1969).
- ¹⁶ Brown, D. M., in *Basic Principles in Nucleic Acid Chemistry* (edit. by Ts'O, P. O. P.), **2**, chapter 1 (Academic Press, London, 1974).
- ¹⁷ Fuller, W., and Hodgson, A., *Nature*, **215**, 817-821 (1967).
- ¹⁸ Levitt, M., *J. molec. Biol.*, **80**, 255-263 (1973).
- ¹⁹ Woese, C., *Nature*, **226**, 817-820 (1970).
- ²⁰ Hill, C. W., Combriato, G., Steinhart, W., Riddle, D. L., and Carbon, J., *J. biol. Chem.*, **248**, 4252-4262 (1973).
- ²¹ Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462-1465 (1965).
- ²² Barrell, B. G., and Clark, B. F. C., *Handbook of Nucleic Acid Sequences* (Joynson-Bravvvers, Oxford, 1974).
- ²³ Schevitz, R. W., Navia, M. A., Bantz, D. A., Cornick, G., Rosa, J. J., Rosa, M. D. H., and Sigler, P. B., *Science*, **177**, 429-431 (1972).
- ²⁴ Kim, S. H., Quigley, G., Suddath, F. L., McPherson, A., Sneden, D., Kim, J. J., Weinzierl, J., Blattmann, P., and Rich, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3746-3750 (1972).
- ²⁵ Klug, A., Robertus, J. D., Ladner, J. E., Brown, R. S., Finch, J. T., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ²⁶ Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C., and Klug, A., *Nucleic Acids Res.*, **1**, 927-932 (1974).

letters to nature

A model of the magnetospheric substorm

It is generally recognised that the Earth's magnetic tail and the plasma sheet play a vital role in the processes responsible for the observational phenomenon known as the magnetospheric substorm. Many different models¹⁻⁵ of substorm behaviour have been described with differing degrees of success, and to propose another requires some justification. A new model, if it is to be useful, must do two things. It must be specific enough to make quantitative theoretical analysis possible, and it must fit the morphological features more completely or in a more elegant way than other models. In addition, it should be distinctive enough from other models to be separated from them by observational tests. I believe the model presented here has those properties.

The main features of the model are that the plasma sheet is formed on closed field lines and moves inward during the growth phase; the magnetic field line reconnection across the neutral sheet occurs on the lobe field lines, is essentially continuous but is not directly responsible for the expansion phase; and the expansion phase is the result of an interchange type of instability with the plasma sheet expanding on to empty, closed field lines.

The two principal regions in the tail—the plasma sheet and the lobes of the tail connected to the polar caps—are easily distinguished by their particle populations. The plasma sheet is denser and hotter than the lobe plasma⁶ and its energy density is of the same order as its magnetic energy density. The boundary between the two is normally not more than $1 R_e$ thick^{7,8}. One possible reason for the well defined boundary is that there is a source mechanism which only injects particles on plasma sheet field lines⁵. Alternatively, lobe field lines can be open allowing

plasma to escape down the tail, with the plasma sheet trapped on closed field lines⁹. I shall discuss the consequences of the latter.

A convenient starting point for the model substorm sequence is illustrated in Fig. 1a. The plasma sheet occupies all the closed field lines and at its end there is a neutral sheet which extends out of the plane of the paper on both sides. Suppose that the plasma flows towards the neutral sheet under the influence of an electric field (Fig. 1b). The open magnetic field lines of the tail would be reconnected at the neutral sheet at a steady rate. On the Earthward side of the neutral sheet the electric field would convect the plasma inward and accelerate the particles¹⁰. On the far side of the neutral sheet the plasma would be accelerated away from the Earth and gets lost down the tail. The important feature is that lobe field lines thus become reconnected and form closed field lines outside the plasma sheet (Fig. 1b). The plasma sheet, which contains relatively hot, dense plasma, becomes surrounded by closed field lines almost devoid of plasma in comparison. The magnetic field strength outside the plasma sheet would have to be stronger than inside in order to maintain a pressure balance across the boundary. The inward convection would continue (forming the growth phase of the substorm) until the plasma sheet boundary became unstable to a form of interchange instability. As a result the plasma would expand outwards to fill all the closed ex-lobe field lines and the magnetic field strength inside the plasma sheet would increase by a compressional wave propagating inwards from the position of the boundary. This, of course, is the expansion phase of the substorm (Fig. 1c). It leaves a region of turbulent plasma which would eventually thermalise during the recovery phase (Fig. 1d), returning the plasma to the starting point. The inward convection would continue during the recovery phase and may generate another substorm before the original sequence is complete.

The most important magnetospheric feature in this model is the outer boundary of the plasma sheet which initially convects inward stably and later becomes grossly unstable. There are plausible reasons why this should occur. If the model is to make any observational sense the quiet auroral arcs of the growth phase must connect to this boundary. Thus, there is high conductivity strip through the ionosphere on the field lines that pass through the boundary. That helps to stabilise the boundary by short circuiting the electric field of the developing instability. The expansion phase is triggered by the current, which is aligned with the field, becoming too large for the plasma to carry in a stable manner¹¹.

The outer boundary of the plasma sheet is likely to have a complex structure during any magnetospheric convection. The convective velocity, $\vec{V} = -(\vec{E} \times \vec{B})/B^2$, normal to the boundary must be continuous; therefore, because B changes, there must also be a change in the electric field as the boundary is crossed. The simple fluid concept of convective flow is quite inadequate to analyse that situation. It is necessary to take into account the three-dimensional nature of the boundary, currents aligned with the field, and the possibility of electric fields parallel to the magnetic field. It is thus not yet possible to project the electric field discontinuity at the boundary of the plasma sheet in the equatorial region down the field lines to the ionosphere to see whether it matches the electric field reversal region observed by low altitude satellites¹². A detailed theoretical analysis of the boundary is of crucial importance.

The source of the electric field must be considered. The field—

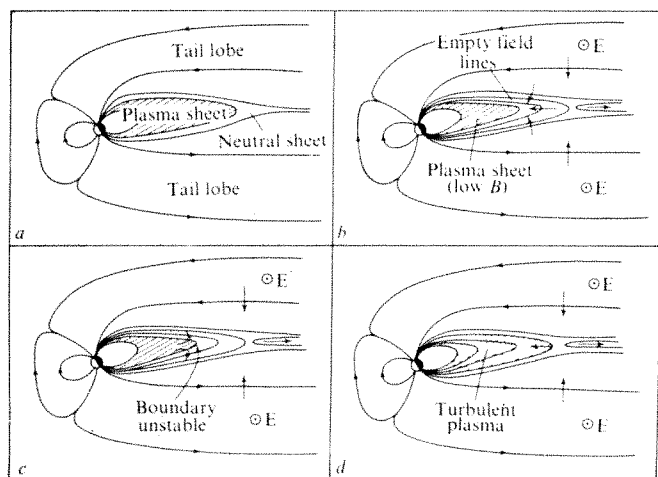


Fig. 1 *a* Basic configuration of the magnetosphere; *b*, the growth phase of the substorm, showing inward convection and reconnection; *c*, the expansion phase, caused by an interchange instability at the outer boundary of the plasma sheet; *d*, the recovery phase during which the turbulent plasma gradually thermalises. Arrows indicate plasma flow and the field strength in the lobes is not intended to be represented by the density of field lines; E , electric field, directed perpendicular to the plane of the page.

line reconnection takes place on lobe field lines and so the following equation, derived by Alfvén¹³⁻¹⁵ on the basis of self consistency between fields and currents, should be at least approximately true. It relates the total electric potential ϕ across the tail, to the field strength B_x and particle density N .

$$\phi = B_x^2 / 4\pi Ne$$

Thus the crosstail electric field is determined by the field strength, B_x , in the tail. B_x is controlled by the total magnetic flux in the tail and the configuration of the magnetopause¹⁹. There should be a direct relationship between energy input to the magnetosphere in the form of substorm activity and the magnetic field strength in the tail¹⁶.

The model obviously satisfies many of the gross morphological features of the magnetospheric substorm. The inward movement of the plasma sheet and the motion of quiet auroral arcs towards the equator are natural features. The outward movement of the plasma sheet and the inward magnetic field compression wave during the substorm expansion phase are directly related through the interchange nature of the instability. The poleward expansion of the aurora during breakup is also explained. It can easily accommodate such features as a multiple substorm onset¹⁷ because the boundary could become unstable over a range of longitudes. There is one auroral feature which does not seem to fit easily into the model—the multiple arc system. It has been reported¹⁸ that the most vigorous breakups occur when they are initiated on the arc nearest to the equator, and not on that furthest from the equator, as may be expected in the model.

There is one morphological feature of this model which differentiates it from most other models¹⁻⁵. If the model is correct it should be possible to cross the neutral sheet, or the region where the magnetic field along the tail changes sign, without detecting the plasma sheet before the onset of a substorm.

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- ¹ Russell, C. T., and McPherron R. L., *Space Sci. Rev.*, **15**, 250 (1973).
- ² Axford, W. I., Petschek, H. E., and Siscoe, G. L., *J. geophys. Res.*, **70**, 1231 (1965).
- ³ Atkinson, G., *J. geophys. Res.*, **71**, 5157 (1966).
- ⁴ Parks, G. K., Laval, G., and Pellat, R., *Planet. Space Sci.*, **20**, 1391 (1972).
- ⁵ Hill, T. W., and Dessler, A. J., *Planet. Space Sci.*, **19**, 1275 (1971).
- ⁶ Akasofu, S.-I., Hones, E. W., Bame, S. J., Asbridge, J. R., and Lui, A. T. Y., *J. geophys. Res.*, **78**, 7257 (1973).
- ⁷ Hones, E. W., Asbridge, J. R., and Bame, S. J., *J. geophys. Res.*, **76**, 4402 (1971).
- ⁸ Buck, R. M., West, H. I., and D'Arcy, R. G., *J. geophys. Res.*, **78**, 3103 (1973).
- ⁹ Axford, W. I., *Rev. Geophys.*, **7**, 421 (1969).
- ¹⁰ Axford, W. I., and Hines, C. O., *Can. J. Phys.*, **39**, 1433 (1961).
- ¹¹ Swift, D. W., *Planet. Space Sci.*, **15**, 1225 (1967).
- ¹² Swift, D. W., and Gurnett, D. A., *J. geophys. Res.*, **78**, 7306 (1973).
- ¹³ Cowley, S. W. H., *Cosmic Electrodynamics*, **3**, 448 (1973).
- ¹⁴ Bornatici, M., and Schindler, K., *J. geophys. Res.*, **79**, 529 (1974).
- ¹⁵ Alfvén, H., *J. geophys. Res.*, **73**, 4379 (1968).
- ¹⁶ Fairfield, D. H., *Planet. Space Sci.*, **20**, 1455 (1972).
- ¹⁷ McPherron, R. L., Russell, C. T., and Ajubry, M. P., *J. geophys. Res.*, **78**, 3131 (1973).
- ¹⁸ Akasofu, S.-I., *Polar and magnetospheric substorms* (Reidel, Dordrecht, 1968).
- ¹⁹ Coroniti, F. V., and Kennel, C. F., *J. geophys. Res.*, **77**, 3367 (1972).

Lunar electrical conductivity

THE lunar magnetometer experiment¹ has made important contributions to studies of the lunar interior. The magnetic fluctuation data can be used to estimate the structure of lunar electrical conductivity because it allows comparisons of predicted and observed lunar electromagnetic responses. There are many complications in the correct computation of predicted responses, and approximations are always used. There has been a gradual evolution of models, each improvement including more and more complications, with resultant changes in the estimated conductivities. The first models² had a solar wind incident on the Moon from all sides, in order to approximate the front side lunar response. The backside data was modelled by a Moon in a vacuum¹. Improvements in the front side response were made by incorporating the effects of a finite solar wind velocity³. Previous calculations on a cylindrical model⁴ indicated that the presence of the void region behind the Moon could also modify the sunlit side response. Formal solutions to the electromagnetic problem in such a geometry have been presented⁵.

We have carried out numerical inversions of the lunar electromagnetic response, incorporating in the calculations a void region behind the Moon. We used two different numerical methods. They both gave similar results, although one method is definitely superior in its ability to satisfy the boundary conditions near the limb of the void region. This method consists of using a series of electric and magnetic multipoles, situated at the centre of the Moon, in order to express the field in the void region, which is caused by currents and charges inside the Moon and on its sunlit surface. By using dual Fourier-Bessel series⁶, we can associate to each multipole a series of spherical TE and TM modes that satisfy the boundary conditions on the surface of the cylinder. The amplitude of each multipole is then determined by applying the boundary conditions on the lunar surface.

The inclusion of the void region behind the Moon substantially lessens the predicted lunar response on the sunlit

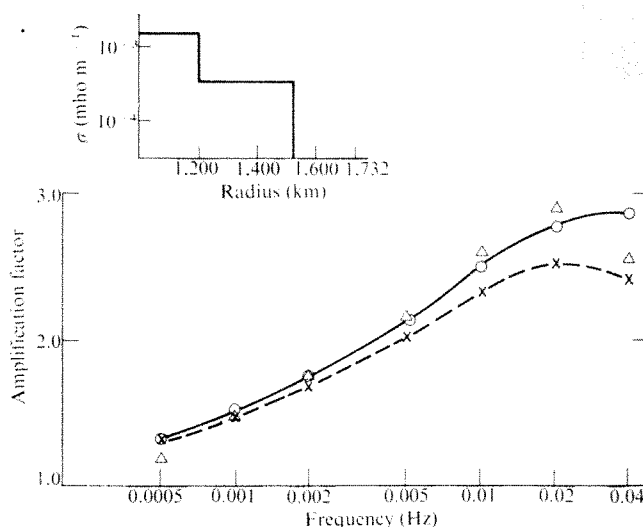


Fig. 1 The theoretical amplitude of the transfer function obtained by considering the void region behind the moon (x), and that obtained by assuming the void to be filled with plasma (O). Δ , Data values.

side, especially at shorter periods (Fig. 1). This effect occurs because some of the internal field is escaping out into the void region. In order to build up the response again, conductivities are required near the surface. Figure 2 shows a model which gives a good fit to the observed amplification on the front side and which also agrees well with the backside

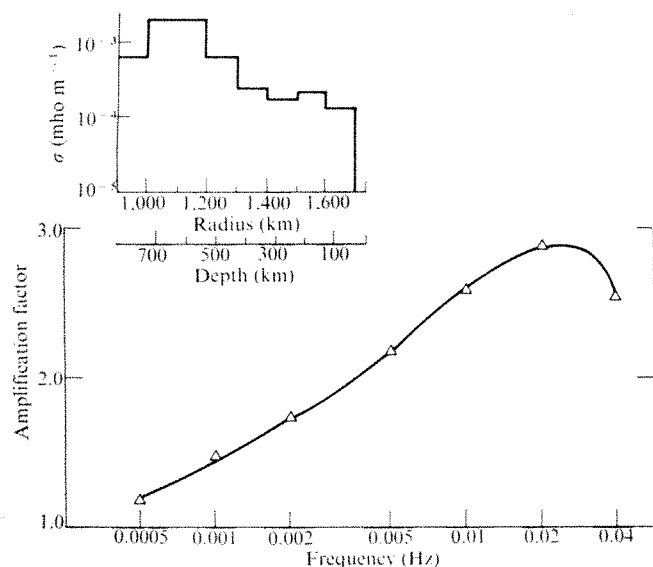


Fig. 2 Amplitude of the transfer function of an 8 layer model, calculated by including the void region behind the moon.

response⁷. There is a remarkable similarity between the conductivity values in the top 200 km of the Moon and those in the Earth's crust⁸. The temperature and pressure conditions in these regions of the Moon are probably quite similar to terrestrial crustal conditions, but in the Earth's crust the conductivity is probably associated with the presence of free water in the rocks. This possibility seems most unlikely for the Moon. A water-saturated lunar model, in fact, does not give a good fit to the electromagnetic response data, as it does not provide the increase conductivity-depth relationship that is needed because of the decrease of porosity with pressure. If free water is not present, then a semiconduction type of mechanism is required in order to explain the degree of conductivity observed at low temperatures.

In order to examine this more closely, various models of lunar temperature were assumed, and the electromagnetic response data were inverted to find the parameter of a semi-

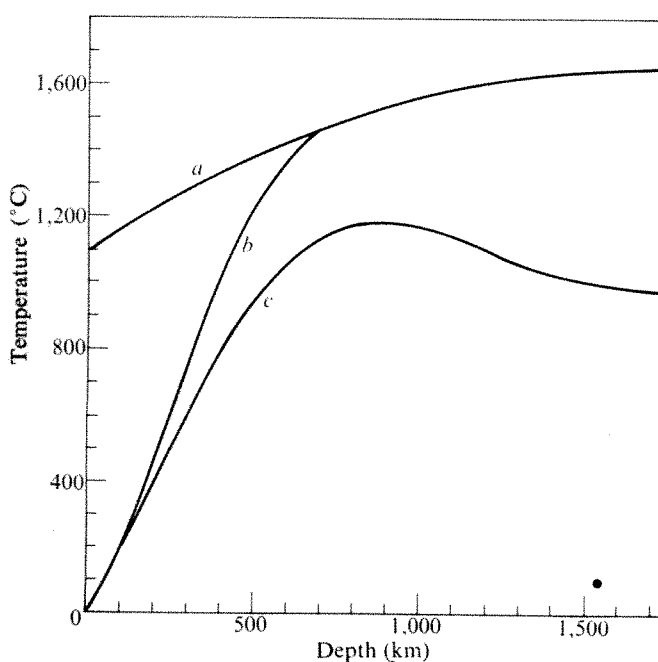


Fig. 3 Model of the temperature distribution inside the moon. a, Solidus (anhydrous basalt); b, uranium concentration = $2.3 \times 10^{-8} \text{ g g}^{-1}$; c, $U = 1.1 \times 10^{-8} \text{ g g}^{-1}$.

conductor characterised by a conductivity prefactor, σ_0 , and activation energy, E_0 . The temperature models used are those of Toksöz *et al.*⁹ (Fig. 3). A very good fit to the data was obtained using this two-parameter model (Fig. 4). The parameters were not very sensitive to the temperature models (Table 1).

Table 1 Parameters of lunar semiconductor model

High temperature model	Low temperature model
$\sigma_0 \text{ mho m}^{-1}$ 2.1×10^{-3}	3.1×10^{-3}
$E_0 \text{ eV}$ 0.13	0.14

This low activation energy results from the fact that the electromagnetic data can accommodate only a moderate increase in conductivity at depths characterised by a sharp thermal gradient. Figure 5 shows a comparison of the conductivity values with laboratory measurements of terrestrial and lunar rocks. Low activation energy conduction has been observed in lunar rocks but the conductivity level is much lower than that inferred for the Moon¹⁰. These experiments were, however, plagued by thermal hysteresis. This was subsequently attributed to the experimental procedure¹¹. We are not yet able to draw definite conclusions on the lunar composition.

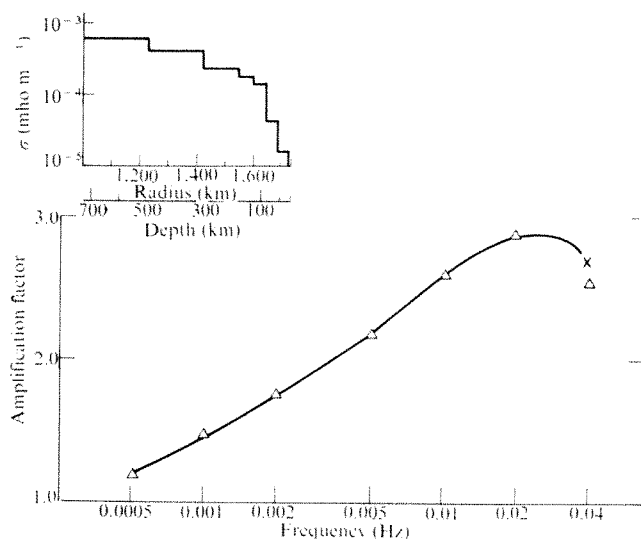


Fig. 4 Amplitude of the transfer function of a semiconductor lunar model which satisfies the high temperature curve in Fig. 3. The model values, x, agree with the data, Δ , except at 0.04 Hz

There is very little resolution in the present electromagnetic data for the electrical properties at depths greater than 700 km, and, therefore, another conduction mechanism cannot be identified. The shallower conductivity parameters were so insensitive to the temperature model variations, that temperature inferences could not be made from these results.

The model results were calculated by assuming the lunar surface magnetometer to be at the subsolar point, and the source field to propagate parallel to the downstream cylindrical cavity. The magnetic amplification depends, however, on detailed considerations of the location of the sensor relative to the subsolar point, and on the orientation of the magnetic field of the solar wind. Moreover, the data are sensitive to certain parameters of the solar wind. The results shown in Table 1 and Fig. 5 were computed using a solar wind velocity of 300 km s^{-1} . If 400 km s^{-1} is used, the conductivity parameters increase by 50% in σ_0 , and by 25% in E_0 . In this case the conductivity value of $10^{-4} \Omega^{-1} \text{ m}^{-1}$ is not reached until a depth of 150 km. The fluctuation of plasma pressure of the solar wind, which accompanies the magnetic fluctuations, is another parameter that can influence the results. An empirical correc-

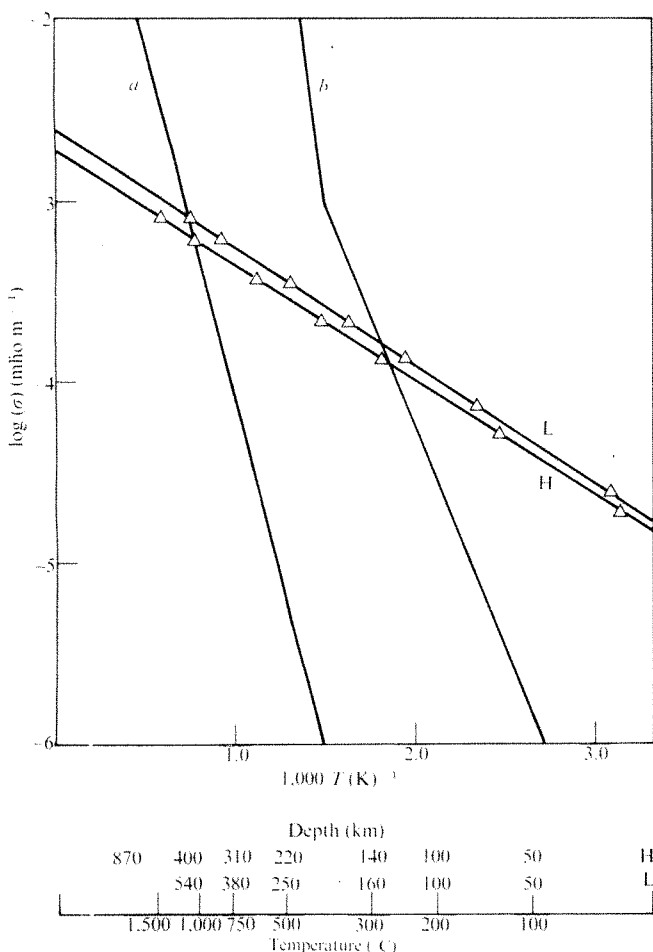


Fig. 5 Log σ against $10^{-3} T^{-1}$ for a semiconductor lunar model with the high (H), and low (L) temperature distribution shown in Fig. 3. Also shown are the laboratory data for a, terrestrial olivine sample (10.4% fayalite); b, lunar basalt sample 10024.22.).

tion for the interaction of the plasma with the remanent field at the Apollo 12 site has already been applied to the data³ and the resulting set of data agrees fairly well with that obtained at the Apollo 15 site¹². We have not included in our calculation the possible effect that these fluctuations might have on the diamagnetic signature usually observed in the downstream cylindrical cavity. We do not know to what extent these parameters can be reconstructed from the original observations.

These factors render our conclusion somewhat tentative but we believe, nevertheless, that the results suggest rather strongly that a conduction mechanism with a low activation energy plays an important role in determining the conductivity at shallow and moderate depths inside the Moon.

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- ⁵ Schwartz, K., and Schubert, G., *J. geophys. Res.*, **78**, 6496 (1973).
- ⁶ Smythe, W. R., *Static and Dynamic Electricity*, third ed., 211 (1968).
- ⁷ Schubert, G., Smith, B. F., Sonett, C. P., Colburn, D. S., and Schwartz, K., *J. geophys. Res.*, **78**, 3688 (1973).
- ⁸ Brace, W. F., *Geophys. Monogr.*, **14**, 243 (1971).
- ⁹ Toksoz, M. N., Solomon, S. C., Minear, J. W., and Johnston, D. H., *The Moon*, **4**, 190 (1972).
- ¹⁰ Schwerer, F. C., Nagata, T., Fisher, R. M., *The Moon*, **2**, 408 (1971).
- ¹¹ Schwerer, F. C., Huffman, G. P., Fisher, R. M., and Nagata, T., *Fourth Lunar Sci. Conf.*, 663 (1973).
- ¹² Smith, B. F., Schubert, G., Colburn, D. S., Sonett, C. P., and Schwartz, K., *Fourth Lunar Sci. Conf.*, 683 (1973).

Tungus event was not caused by a black hole

JACKSON and Ryan¹ suggested that a black hole may have caused the Tunguska catastrophe in 1908, in which an explosion occurred in a remote part of Siberia. About 10^{23} erg (equivalent to 2 Mton of TNT) of energy was released. Several lines of evidence render the black hole hypothesis extremely unlikely.

The explosion blew down trees in open places within a radius of 30–40 km and seared them within a radius of 15–18 km. The exposed roots were directed towards the centre of the area². In ravines, partially protected trees remained standing, but many had had their tops broken.

These and other characteristics of the event indicated that the main part of the energy went into an explosion in the air. By contrast, a typical, solid nickel-iron meteorite first buries itself below the surface and then dissipates its energy in a subsurface explosion. The Barringer crater in Arizona, which is 200 m deep, and 1 km in diameter, was produced in this way. No significant excavation was caused by the Tunguska explosion.

It has been suggested that the event was caused by the impact of a small cometary nucleus, consisting of a mass of frozen gases mixed in with nickel-iron and silicate particles^{3–5}. The whole body would then have had a low degree of cohesion, so that it would have fragmented in the air and dissipated most of its kinetic energy before it reached the surface.

The air blast could also have resulted from the impact of a small black hole with a diameter of the order of angstroms and an asteroidal mass. The black hole would, however, have passed through the Earth in 10–15 min and caused a similar explosion at the point of exit, which would have occurred in the North Atlantic between 30°–40° W and 40°–50° N (ref. 1).

Microbarographs in the London and Cambridge areas recorded infrasound from the explosion⁶. The waves arrived at the centroid of the stations—51°30'N, 0°2'W—at approximately 0515 UT, on June 30, 1908. The impact itself occurred at 60°55'N, 101°57'E at approximately at 0017 UT (ref. 2). The approximate great circle distance from the point of impact to the centroid of the stations is 5,720 km, so the average speed of the waves was about 320 m s⁻¹, which is about the usual value for this type of wave.

It is clear that the recorded waves were travelling from Siberia, and not from the North Atlantic, because the microbarograph at Cambridge recorded the wave 10 min or so before the time that it was recorded at Petersfield. Infrasound waves from the site of the suggested exit explosion should however, have arrived in the London area between about 0215 UT and 0330 UT—about three hours before the arrival of the Siberian wave—assuming that any waves from either source travelled with the same velocity. We have examined copies of the English microbarograph records, but have been unable to find any sign of waves from the suggested exit explosion.

In the London area, the maximum recorded amplitude

¹ Dyal, P., and Parkin, C. W., *Proc. second Lunar Sci. Conf.*, **3**, 2391 (1971).

² Sill, W. R., and Blank, J. L., *J. geophys. Res.*, **75**, 201 (1970).

³ Sonett, C. P., Smith, B. F., Colburn, D. S., Schubert, G., and Schwartz, K., *Proc. third Lunar Sci. Conf.*, **3**, 2309 (1972).

⁴ Reisz, A. C., Paul, D. L., and Madden, T. R., *The Moon*, **4**, 134 (1972).

of the Siberian waves was about 150 μ bar. It is possible to resolve amplitudes which are as low as 20–30 μ bar, so that any waves not observed must have had amplitudes which were less than about 20% of those of the Siberian waves. The suggested exit site is, however, closer to London than is the Tunguska region, so the recorded amplitudes of exit waves at London should have been greater, than those of the waves from Siberia in the absence of unusual propagation effects. We have considered whether strong zonal winds to the west could have caused ducting effects which reduced the amplitude of the eastward travelling exit wave to below 20–30 μ bar. Ducting between the mesosphere and the Earth's surface reduces greatly the amplitudes of waves with periods shorter than a few tens of seconds when they are travelling upwind, but it reduces the amplitudes of waves with periods of 1–2 min, such as those recorded from the explosion, by only a factor of two or three. If such ducting had been in effect, any waves from the exit explosion should still have had amplitudes of about 100 μ bar in the London area. In addition, the eastward travelling reverse wave from the explosion was observed on a Fuess barograph at the Potsdam Geophysical Observatory about 26 h after the direct waves². If the eastward wave from the entrance explosion could propagate against the zonal winds it seems unlikely that eastward waves from an exit explosion could not do so as well.

Other arguments against the black hole hypothesis have been given by Wick and Isaacs⁵. The spatial pattern of numerous small magnetic globules with high nickel content found in the Tunguska region indicates that the source of these extraterrestrial particles was the impact explosion. Eyewitness accounts noted a thick dust train along the path of the fireball immediately after its passage, which is consistent with the deposition of material in the atmosphere, rather than loss of air into a black hole. The first night after the fall was exceptionally bright everywhere in Europe and western Siberia. In the Caucasus it was possible to read a newspaper at midnight without artificial light. That implies deposition of extraterrestrial material in the upper atmosphere simultaneously with the impact. The wide area of atmospheric deposition is comparable to the dimensions of a cometary tail, and is not compatible with the idea of slow transport of dust vertically and horizontally from a ground level explosion. The deposition in the upper atmosphere of both fine cometary dust and icy material could give rise, at high latitudes and at that time of year, to a phenomenon like noctilucent clouds. That could account for the bright nights.

Fessenkov⁶ investigated the measurements of the transparency of the atmosphere made in California in 1908, and found that from approximately the middle of July to the second half of August 1908, a noticeable decrease in the coefficient of transparency occurred. Fessenkov concluded that it was caused by the dissipation in the atmosphere of material from the Tunguska object. He estimated the total mass of dissipated material to be several million tons.

All the evidence favours the idea that the impact which caused the Tunguska catastrophe involved a body with characteristics like a cometary nucleus, rather than a black hole.

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- ¹ Jackson, A. A., and Ryan, M. P., *Nature*, **245**, 88 (1973).
- ² Krinov, E. L., in *The Moon Meteorites and Comets* (edit. by Middlehurst, B. M., and Kuiper, G. P.), (Univ. Chicago Press, Chicago, 1963).
- ³ Whipple, F. J. W., *Q. Jl R. Met. Soc.*, **16**, 287 (1930).
- ⁴ Astapovich, I. S., *Soviet Astron.*, **10**, 465 (1933).
- ⁵ Wick, G. L., and Isaacs, J. D., *Nature*, **247**, 139 (1974).
- ⁶ Fessenkov, V. G., *Meteoritika*, **6**, 8 (1949).

The formation of the Earth

THREE recent hypotheses for the origin of the inner planets are based on chemical data obtained from meteorites or from solar observation^{1–5}. The chondritic earth model of Ringwood¹ or the cold accretion model (see summary in ref. 2) require partial melting of meteorite material and segregation of metal and sulphide to form a core, but the fractionation must have been a rapid, disequilibrium process to leave 2,000 p.p.m. Ni in the silicate mantle. The heterogeneous accretion model^{3,4} overcomes the problem of high Ni in the mantle by having accretion of the metal core first, followed by silicate mantle and finally by volatile-bearing material which formed the crust, no two fractions having been in equilibrium. Lewis⁵ maintains that condensation from a homogeneous nebula under differing physical conditions can account for the density variation between the inner planets without invoking the metal-silicate fractionation suggested by Urey⁶. Compared to the above hypotheses based on meteorite composition, the following approach is more direct as it is based on a reliable estimate of modern upper mantle composition.

The best estimate of undepleted upper mantle composition obtained from a study of some 200 spinel-lherzolite xenoliths in basalt, is as follows: SiO₂ 45.0, TiO₂ 0.09, Al₂O₃ 3.5, Cr₂O₃ 0.41, total Fe as FeO 8.0, MnO 0.11, NiO 0.25, MgO 39.0, CaO 3.25, Na₂O 0.28, K₂O 0.035; sum 99.92 (weight). (R. H., A. L. Chambers, D. K. Paul, and P. G. Harris, unpublished). This composition is close to that of three undepleted garnet-lherzolite xenoliths from the Bultfontein kimberlite pipe, but it has much lower TiO₂, Na₂O and K₂O contents than Ringwood's pyrolite¹.

In meteorites, Ni is always concentrated in the metal relative to the silicate. For example, the metal of pallasites has about 12% Ni (weight), but coexisting olivine has only some 20 p.p.m.⁷. If an Fe–Ni metal fraction had been segregated from silicate mantle to form or to enlarge the core, then Ni should have been almost quantitatively removed from the upper mantle. The Ni:Fe ratio of modern upper mantle, about 0.03, is much lower than that in meteorites but higher than that of the silicate of meteorites. One possibility put forward here is that Ni was almost totally removed from the mantle during early segregation of metal and sulphide into the core, then partially replenished by the addition of further meteorite material. If this assumption is made, the composition of pre-replenishment, Ni-free upper mantle can be calculated by subtracting a suitable amount of Ni carrier from modern upper mantle.

The most Ni-rich carrier, ataxitic iron meteorite, would require about 0.15 lunar mass to be mixed into the Ni-free upper mantle and oxidised. In addition to providing the 2,000 p.p.m. Ni, about 1% FeO (weight) would also have been introduced. At the other extreme, the maximum mass of material capable of providing the necessary amount of Ni would be just under three lunar masses of type 1 carbonaceous chondrite (C1). C1 fragments have been identified in a variety of meteorite types^{8,9}. In addition, C1 material has probably been incorporated into lunar regolith^{10,11}, and so seems a better candidate as Ni carrier than the rare ataxites. Although other types of chondrite are also potential Ni carriers, some C1 is necessary to provide the earth's volatiles and so the model presented here will consider C1 only.

Table 1 Calculation of lithophile element content of primitive Ni-free mantle

Atomic	*	†	‡	§	
Si	10,000	10,000	2,000	8,000	10,000
Ti	118	15	5	10	12.5
Al	923	915	174	741	926
Cr	75	72	25	47	59
Mg	12,359	12,900	2,100	10,800	13,500
Ca	731	773	140	633	791

* 'Pyrolite'¹

† Modern upper mantle from composition given in text

‡ 20% carbonaceous chondrite, from ref. 12

§ † minus ‡

|| §, recalculated to 10,000 Si atoms – primitive Ni-free (and Na-free) upper mantle

To allow comparison between the upper mantle and meteorite groups, the method used for calculating the composition of hypothetical, Ni-free upper mantle is that of Larimer and Anders¹². In Table 1 the lithophile element contents of 'pyrolite'¹ and upper mantle as given above are expressed in atoms per 10,000 Si atoms. By subtracting 20% C1 from the modern upper mantle composition and recalculating to 10,000 Si atoms, primitive, Ni-free upper mantle composition is obtained. This has a 100 FeO:(FeO + MgO) molecular ratio of 5. In Table 2, the data of Table 1 are expressed relative to carbonaceous chondrite composition; ordinary and enstatite (E) chondrite compositions are also included.

Table 2 Lithophile element fractionation relative to carbonaceous chondrites

	*	†	‡	§		¶
Si	1.0	1.0	1.0	1.0	1.0	1.0
Ti	0.46	0.55	4.3	1.0	0.74	0.55
Al	1.06	1.05	1.06	1.0	0.71	0.55
Cr	0.47	0.58	0.59	1.0	0.82	0.77
Mg	1.29	1.23	1.18	1.0	0.90	0.74
Ca	1.13	1.10	1.04	1.0	0.67	0.53

* Recalculated Ni-free primitive upper mantle, from Table 1

† Modern upper mantle from composition given in text

‡ 'Pyrolite'¹

§ Carbonaceous chondrite

|| Ordinary chondrite¹²¶ Enstatite chondrite¹²

From Table 2 it is evident that fractionation of the lithophile elements has been different on Earth from that in meteorites. The sequence carbonaceous to ordinary to E chondrites has fairly regular depletion in all five elements, whereas in the upper mantle (column †) Al, Mg and Ca are enriched relative to carbonaceous chondrites, but Ti and Cr are depleted. 'Pyrolite'¹ shows the depletion in Cr, but not in Ti; this is probably due to a failing in Ringwood's model. The recalculated, Ni-free upper mantle composition shows the same trends as modern upper mantle, but accentuated by the subtraction of unfractionated material. Lack of coherence within the lithophile element group requires a mechanism different from any responsible for fractionation between the different chondrite groups, as does enrichment in Mg over Al and Ca. Gain by the Earth of the Mg, Ca, Al-rich fraction preferentially lost by ordinary and E chondrites¹² would have resulted in an impoverishment in Mg over Al and Ca in the upper mantle. These two problems are dealt with separately below.

Depletion of the upper mantle in Ti and Cr is best explained by their partial removal into the core as sulphides. In the highly reduced E chondrites, virtually all Cr occurs as the sulphide daubréelite¹³, and 75% of the Ti is present in troilite¹⁴. The figures in Table 2, column*, indicate that, in the upper mantle, conditions were less strongly reducing than those which obtained during the formation of the E chondrites, for, in the

former, a much higher proportion of the Ti and Cr remained as oxides. This is borne out by the presence of Fe in the upper mantle, there being virtually no oxidised Fe in E chondrites. Reducing conditions on Earth probably were not strong enough for reduction of significant SiO₂ to the metal, which occurred to limited extent in E chondrites. It is therefore unlikely that the light element in the Earth's core is Si. Removal of over half the Ti and Cr from the upper mantle probably took place during the early differentiation which also removed the Ni. The depletion in Ti and Cr and enrichment in Al, Mg and Ca cannot be accounted for by heterogeneous accretion.

Mg enrichment over Al and Ca could be the result of the Earth having gained pure forsterite (Mg₂SiO₄). I think, however, that the following two possibilities are more probable. Each necessitates SiO₂ loss from the upper mantle. The first and perhaps simpler explanation is that the mantle differentiated into upper and lower portions, with preferential enrichment of the latter in FeO and SiO₂ (refs 2, 15). This would be acceptable to geophysicists, whose models for lower mantle composition range from pyroxenite to harzburgite with molecular 100 FeO:(FeO + MgO) ratios from 36 to 20 (ref. 2). But this mechanism requires greater stability in the lower mantle of Ca and Al relative to Mg, a property which seems difficult to explain. The second alternative is that loss of almost three lunar masses of SiO₂ by evaporation from the upper mantle could have produced the observed Mg, Al and Ca enrichment, if primitive mantle had ordinary chondritic Mg/Si, Al/Si and Ca/Si ratios. The 35% SiO₂ loss necessary would have been accompanied by total depletion in alkalis and volatiles, but the addition of 20% C1 Ni carrier would have replenished the upper mantle in these; if the Na content of C1 of Schmitt *et al.*¹⁶ is used, the 0.28% Na₂O in the upper mantle is exactly accounted for. Abundances of other trace elements and alkalis in the upper mantle are not sufficiently well known for rigorous testing of this hypothesis. This second alternative is attractive in that an early period of surface heating might have radically altered the pattern of convection in the earth to ultimately bring about the distinction between upper and lower mantle. The second alternative could also apply to a homogeneous mantle, but in this case over ten lunar masses of SiO₂ would have had to have been lost.

The chemical composition of the upper mantle is incompatible with a single model based on heterogeneous accretion or cold accretion. Geophysical evidence for lower mantle composition² combined with the upper mantle composition given above suggest that the bulk mantle has a 100 FeO:(FeO + MgO) molecular ratio of about 20, in the range of the ordinary chondrites. Ordinary chondrite parent is also required to explain the enrichment in the upper mantle of Mg over Al and Ca. Although Anders¹⁷ maintains that the five components of meteorites (lithophile elements, metal, silicate, sulphide and volatiles) should each be considered independently in modelling planetary composition, the evidence put forward here argues that this might not be necessary.

My preferred model for the formation of the Earth is as follows: accretion began either as a metal-rich core followed by ordinary chondrite material, or as iron-rich ordinary chondrite-like material alone. Partial melting resulted in loss of siderophile and chalcophile elements from the silicate mantle to the core. Surface heating by a superluminous sun drove off volatiles, alkalis and about 35% of the SiO₂ from the upper part of the primitive mantle. Addition of C1 material during protracted cooling of the earth added Ni, alkalis, and, finally, volatiles. That accretion took place on an extended time scale is evidenced by meteorites, some of which have grains irradiated by solar flares in their interiors; such irradiation must have occurred after the dissipation of the bulk of the nebular gas¹⁸.

Along the midplane, pressure in the solar nebula increased towards the centre¹⁹. The series carbonaceous to ordinary to E chondrites represents material accreted under increasing

nebular gas pressure²⁰⁻²². Because the oxidation state of the Earth is close to that of ordinary chondrites, the more highly reduced E chondrites probably originated within the orbit of the Earth. This possibility is currently being considered.

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- ¹ Ringwood, A. E., *Geochim. cosmochim. Acta.*, **30**, 41-104, (1966).
- ² Anderson, D. L., Sammis, C., and Jordan, T., *Science*, **171**, 1103-1112 (1971).
- ³ Turekian, K. K., and Clark, S. P., jun., *Earth planet. Sci. Lett.*, **6**, 346-348 (1969).
- ⁴ Grossman, L., *Geochim. cosmochim. Acta*, **36**, 597-619 (1972).
- ⁵ Lewis, J. S., *Earth planet. Sci. Lett.*, **15**, 286-290 (1972).
- ⁶ Urey, H. C., *Geochim. cosmochim. Acta.*, **1**, 209-277 (1951).
- ⁷ Buseck, P. R., and Goldstein, J. I., *Science*, **159**, 300-302 (1968).
- ⁸ Fodor, R. V., and Keil, K., *Abs. in Meteoritics*, **8**, 366-367 (1973).
- ⁹ Wilkening, L. L., *Geochim. cosmochim. Acta*, **37**, 1985-1989 (1973).
- ¹⁰ Baedeker, P. A., Chou, C.-L., Sundberg, L. L., and Wasson, J. T., *Earth planet. Sci. Lett.*, **17**, 79-83 (1972).
- ¹¹ Krähenbuhl, U., Ganapathy, R., Morgan, J. W., and Anders, E., *Geochim. cosmochim. Acta, Suppl.*, **4**, 1325-1348 (1973).
- ¹² Larimer, J. W., and Anders, E., *Geochim. cosmochim. Acta*, **34**, 367-388 (1970).
- ¹³ Mason, B., *Geochim. cosmochim. Acta*, **30**, 23-39 (1966).
- ¹⁴ Keil, K., *J. geophys. Res.*, **73**, 6945-6976 (1968).
- ¹⁵ Kumazawa, M., Sawamoto, H., Ohtani, E., and Masaki, K., *Nature*, **247**, 356-358 (1974).
- ¹⁶ Schmitt, R. A., Goles, G. G., Smith, R. H., and Osborn, T. W., *Meteoritics*, **7**, 131-213 (1972).
- ¹⁷ Anders, E., *A. Rev. Astron. Astrophys.*, **9**, 1-34 (1971).
- ¹⁸ Pellas, P., Poupeau, G., Lorin, J. C., Reeves, H., and Audouze, J., *Nature*, **223**, 272-274 (1969).
- ¹⁹ Cameron, A. G. W., and Pine, M. R., *Icarus*, **18**, 377-406 (1973).
- ²⁰ Larimer, J. W., and Anders, E., *Geochim. cosmochim. Acta*, **31**, 1239-1270 (1967).
- ²¹ Blander, M., *Geochim. cosmochim. Acta*, **34**, 61-76 (1971).
- ²² Larimer, J. W., *Geochim. cosmochim. Acta*, **37**, 1603-1623 (1973).

Inner floor of the Rift Valley: first submersible study

THE Rift Valley of the Mid-Atlantic Ridge¹, within which lies a segment of the accreting plate boundary between Africa and North America, is well defined between 36° 40' N and 36° 55' N. It is about 30 km wide and 1.5 km deep in that area (Fig. 1). This small portion of the rift, WSW of the Azores, was chosen as the primary target of the French-American Mid-Oceanic Undersea Survey (FAMOUS) programme, and many surface ship studies have been conducted already. We report here preliminary results of seven dives into the deepest part of the Rift Valley that were made by the bathyscaphe *Archimède* during the summer of 1973.

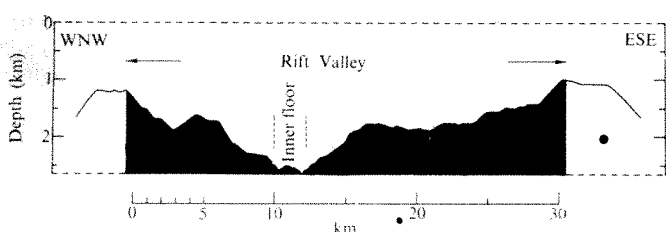


Fig. 1 Topographic cross section of the Rift Valley near 36° 50' N, based on surface-ship data², showing the setting of the Inner Floor in the area of the *Archimède* dives (Fig. 2).

Needham and Francheteau² showed that, in the area visited by *Archimède*, steep, inward facing slopes of the deepest outward tilting blocks of the Rift Valley walls bound an uneven, regionally flattish Inner Floor. The central part of this is occupied by a ridge, 200 m high and asymmetric along at least part of its length. The ridge, or Central High², which we have named Mont de Vénus, is about 4 km long and 1 km wide. Two marginal topographic highs, or platforms, mapped during the course of a detailed narrow beam echo-sounding survey made by the French naval vessel *d'Entrecasteaux*, lie along the bottom of the bordering scarps of the Inner Floor (cv. Schrumph, cc. Caillaud, and V. Renard, personal communication). The marginal highs extend further south than Mont de Vénus and may be more rectilinear in gross shape.

The width between the major bordering scarps of the Inner Floor is 3.3 km. Assuming a mean opening rate of about 2.2 cm yr⁻¹, the Inner Floor may be entirely younger than 1.5 × 10⁶ yr, an inference supported by the occurrence here of very fresh lavas². All seven dives of *Archimède* took place within the Inner Floor, and all were concentrated in an area of less than 5 km² near 36° 50' N (Fig. 2).

The major purpose of the dives was to show that useful, detailed geological mapping of very rough deep sea terrain, such as the Inner Floor, can be undertaken from a manned submersible. It was further hoped that new evidence could be gathered, which would help to answer two important questions. First, is the surface expression of the boundary between the two diverging lithospheric plates confined to one of the highs or deeps within the Inner Floor, or is the locus of emplacement of new crust spread over a wider zone? And second, is the structural pattern of the Inner Floor steady state or transient? With these problems in mind, an effort was made to search for evidence of tectonic activity and to establish specific associations between microtopography and lava morphologies. A close, precisely controlled look was taken at a considerable part of Mont de Vénus, and at the eastern deep and adjacent marginal high (Fig. 2).

About 9 km was covered by the dives, in visual contact with the ocean floor. The position of the *Archimède* was known within 10-200 m, depending on her distance from the nearest of the acoustic transponders that were moored to the bottom and used for navigation. The transponder network for positioning *Archimède* was tied, with an accuracy of about 50 m, to the networks used by the *d'Entrecasteaux* in the making of the detailed surface-ship map that was used as a base for the dives. The scale of features that could be resolved (0-20 m) is clearly beyond the reach of surface-ship, or even deep-towed, instrumentation. A downward looking sonar and a pressure gauge were used to measure the altitude and relative depth of *Archimède* respectively. These were recorded every 10 s on digital printouts, and the precision was about 1 m. Useful complementary information was provided by a panoramic sonar device with a maximum range of 1,400 m. A dual system of still and television cameras (which kept film and videotape recordings) gave good images of the sea floor, whether it was flattish or very steep; the usual distance of observation was 3-5 m, and the maximum distance about 10-15 m. A telemanipulated grab was used for sampling and four pillow fragments, together with small quantities of sediment, were collected during the course of the dives.

Mont de Vénus is largely covered by recent lava flows. It is a lenticular structure with apparent dextral offsets. Above a water depth of 2,560 m its summit is less than about 500 m wide and deepens progressively northwards (Fig. 2). The surface of the summit is characterised by large (approximately 1 m) globular (dome like) lava forms (Fig. 3a) partially immersed in pale, carbonate-rich sediment. The globular structures may represent the surface ex-

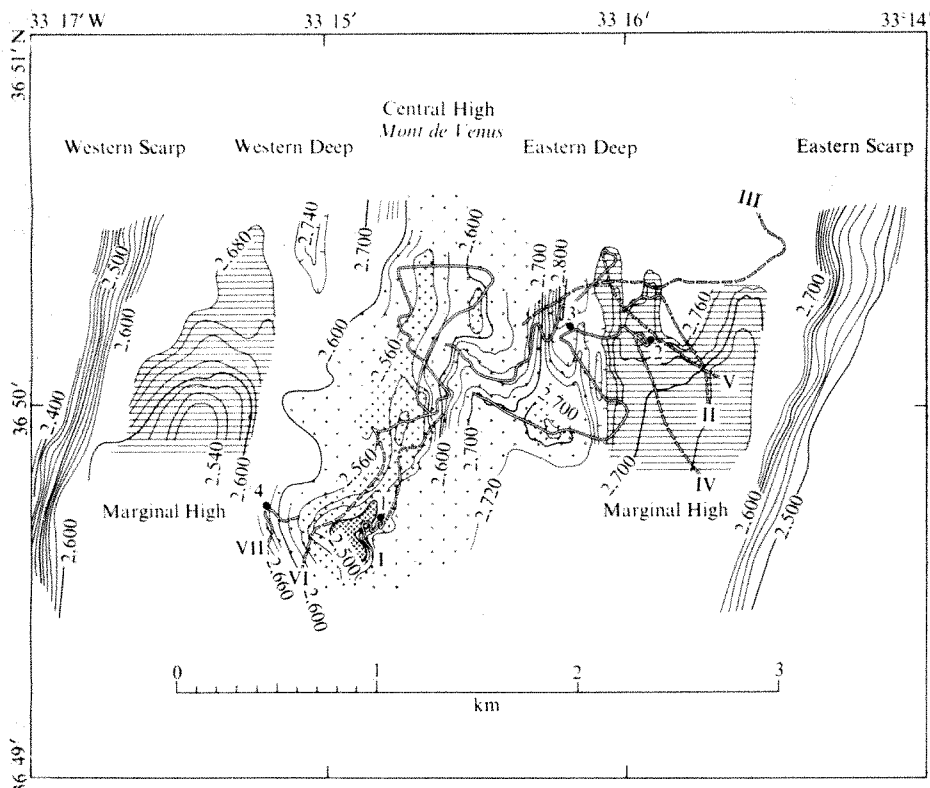


Fig. 2 Map of the Inner Floor of the Rift Valley of the Mid-Atlantic Ridge (Fig. 1), showing tracks of Archimède and positions of rock samples taken from Archimède. Roman numerals, sequences of dives 1-7; ●, rock samples. Different ornamentation for Central High and marginal highs helps to distinguish these features. The map is based on Archimède data along tracks and other contours are sketched from the d'Entrecasteaux map. Depths shown are from pressure gauge and downward looking sonar measurements made from Archimède, or are normalised to this base; corrected depths recorded by precision echo-sounders from surface ships are approximately 30 m smaller. The average cruising speed of the bathyscaphe was less than 1 knot when in visual contact with seafloor. The map is tied to Europe 50 coordinates. Depths are in metres. Track of Archimède: continuous double lines, visual contact with ocean floor; broken double lines, sonar contact with ocean floor.

pression of the top of a gently dipping, coherent lava flow unit (J. G. Moore, personal communication). The sediment may be thin, but it occupies up to 80% of the fields of view from the bathyscaphe. No large open fissures, nor any definite indications of small fissures, were seen. On the crest of the summit, relatively gentle slopes are distributed over a width of 50 m, and the organisation of lava flows shows that the crest coincides with the dividing line between eastern and western sets of flows, both directed downslope. In addition, mapping from Archimède demonstrated that the steep south-eastern slopes of the summit of Mont de Vénus are lobate in character. The wavelength of the lobes was typically 50-100 m.

The topography on the eastern side of Mont de Vénus down to the bordering deep is of a staircase type, with precipitous, north-south scarps, free of sediment, separating relatively flattish areas where the sediment cover is sporadic. One of the scarps is about 100 m high and has a slope of about 80° (Fig. 2). There is also a prominent east-west trending slope near 36° 50'N (Fig. 2). Cylindrical lava forms several metres long, which we call bolsters (Fig. 3b), are present on all of the traversed scarps. The bolsters are commonly associated with shorter, phallus shaped forms which we call phalli (Fig. 3b), and with lava forms resembling entrails which we call tripe (Fig. 3c). Some of the lavas associated with the scarps suggest regular stacking. Tali of lava fragments (Fig. 3d) occur typically at the feet of the scarps; and domed or globular, and commonly striated forms, similar to those seen on the summit, are a feature of the flatter areas between scarps. Collapsed or broken pillows (Fig. 3e) and bolsters occur on the scarps and on the flatter areas.

The short section of slope crossed by the bathyscaphe on the western side of Mont de Vénus has a relatively uniform gradient and is less steep than scarps crossed on the eastern side, but it is similarly typified by bolsters which were directed downslope. Some of these reach lengths of as much as 7 m, and many collapsed forms are present. No fields of angular boulders were seen, and sediment cover of up to 15-20% was observed.

The scarps bordering the deep to the east of Mont de

Vénus follow north-south directions rather than the dominant NE-SW trend of the summit. Isolated boulders lie on a blanket of sediment on the flattish floor of the deep, and some of them are obviously detached from adjacent flow fronts. The floor of the deep is represented, at least locally, by an extremely narrow (15-20 m) corridor (Fig. 2).

East of the deep, a series of ridges, with a relief of about 50 m or more, represent northward deepening fingers of the marginal high that lies between Mont de Vénus and the major eastern scarp of the Inner Floor. The topographic grain of the eastern region seems to be more linear than that near the summit of Mont de Vénus. Slopes of the eastern ridges, however, are similarly covered by coherent lavas, including elongated forms which are directed downslope; the measured flow directions are predominantly north-west to west on the westward facing slopes, and mainly east on the eastward facing slopes. Sediment cover, locally prominent in the flatter areas, is sparse on the slopes which represent the inner edge of the eastern marginal high. There is appealing local evidence, along these slopes, of a north-south, possibly normal, fault with a 2-3 m throw (Fig. 3d).

The four pillow fragments collected by Archimède come from very well known positions (Fig. 2) and, although not sampled from outcrops, they are evidently derived from adjacent flows. The rocks vary in size from 20-40 cm in diameter, and weight 15-47 kg. They are well jointed, have glassy selvages in various states of preservation and are similar, in mineralogy and chemistry, to other rocks from the Mid-Atlantic Rift Valley³.

Samples 1 and 4 from Mont de Vénus are olivine tholeiites with olivine crystals set in a matrix containing olivine, plagioclase and traces of spinel. Sample 2, an aphanitic pyroxene tholeiite from east of the eastern deep, is made up of equal amounts of plagioclase and clinopyroxene. Sample 3, a picritic basalt from the eastern depression itself, contains abundant, large olivine crystals set in a groundmass of plagioclase laths, olivine and a dark mesostasis; minor spinel is present both within the olivine, or as isolated crystals. The two Mont de Vénus tholeiites are more

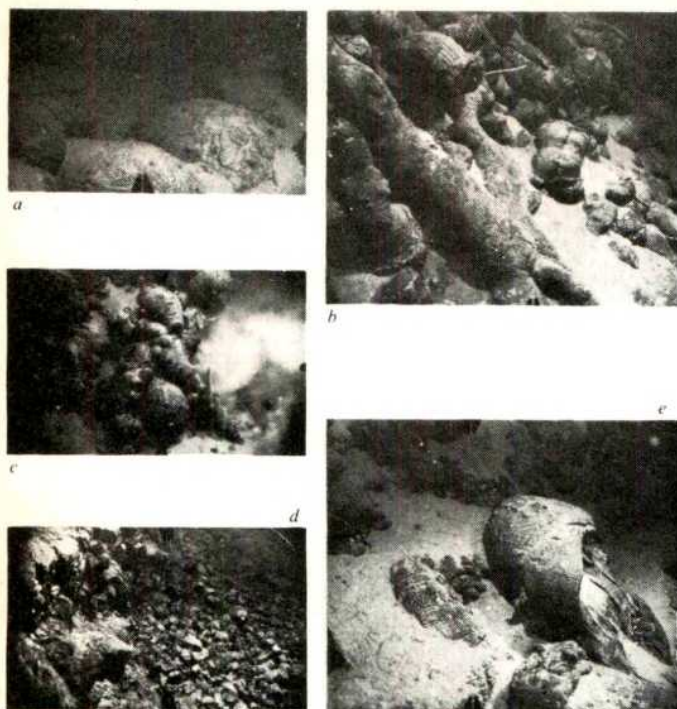


Fig. 3 Different types of lava morphologies on the Inner Floor of the Rift Valley, mapped from the bathyscaphe. *a*, Globules, partially immersed in sediment; particularly characteristic of flat and gently dipping areas. Photograph taken on summit of Central High. *b*, Bolsters and shorter blunt ended forms (phalli); directed downslope; typically found on several steep and very steep slopes where sediment cover is absent or sporadic; note cracked glassy selvages and longitudinal striae. Photograph taken on western slope of eastern marginal high. *c*, Tripe, frequently associated with, or occurring on, other lava forms found on slopes. Photograph taken on western part of eastern marginal high. *d*, Talus of well jointed lava fragments at foot of steep, probably faulted scarp; in general, sediment-free talus is typical of feet of steep slopes including those covered by lava forms which are directed downslope. Photograph taken on western slope of eastern marginal high. *e*, Pillow, broken egg structure and frozen lava flow; similar isolated lava forms lie commonly on less steep parts of slopes and on flattish areas where sediment cover is relatively abundant. Photograph taken on western edge of eastern marginal high.

mafic, with higher MgO (10%), and lower TiO₂ (0.8–0.9%) and K₂O (0.15–0.2%) content than the tholeiite sampled further east (6.4% MgO; 1.6% TiO₂; and 0.4% K₂O). The most mafic of all four sediments is the picritic basalt (sample 3) which contains 22.6% MgO, 0.5% TiO₂ and 0.06% K₂O (P. Cambon, personal communication).

The four specimens show different degrees of surface weathering. Using the thickness of manganese⁴ and palagonite⁵ as indicators, and assuming a mean manganese accumulation rate of 3 μm per 10³ yr, suggests that all of the samples are younger than about 10⁵ yr. The youngest rocks are the two samples from Mont de Vénus; these are younger than 10⁴ yr. Sample 3 from the eastern depression is at least twice as old and sample 2 at least three times as old. The data thus suggest the possibility that the age of the Inner Floor increases eastwards from Mont de Vénus.

The results of the dives, taken together, lead to some general conclusions. Steep, and very steep (80–90°), major slopes of the Inner Floor near 36° 50'N are associated with bolsters which are in a downslope direction and other elongated and blunt ended lava forms. Lava talus is clearly associated with the feet of the scarps. Globule (dome) shaped, and commonly striated pillows lie more

or less buried in sediments on flattish areas between scarps. Incipient break-up of many of the intact lava forms is indicated by jointing and cracking.

The lobate structures and the distribution pattern of lava forms on scarps and slope surfaces indicate that Mont de Vénus is a constructional volcanic feature, with the corollary that its major scarps are the fronts of one or more flow units. It is, nevertheless, possible that flows which are directed downslope mask evidence of vertical movements that have contributed to the process which formed the scarp. Such an interpretation would be consistent with the steepness of the scarps and with the partially demonstrated bilateral asymmetry of Mont de Vénus. The apparently straighter trend of structures east of the eastern deep which flanks Mont de Vénus, and the local evidence of faulting along the inward facing slopes of the eastern deep, point rather more persuasively to some tectonic influence between the faulted scarps that border the Inner Floor. Indeed, the mapped part of the eastern deep near 36° 50'N may be a tensional fissure and the volcanic activity reflected by the downslope flows east of the eastern deep may be associated with dilation and small scale vertical movements.

The observation that the two rocks sampled from Mont de Vénus are younger than those sampled further east suggests, in a tentative way, that Mont de Vénus is the youngest part of the Inner Floor. The dominant, and approximately central position of Mont de Vénus within the Inner Floor adds some weight to this inference, as does the limited evidence that the relief of the marginal highs has been more subject to modification by vertical movements than that of Mont de Vénus itself. It seems, therefore, quite possible, although young lavas are found over a considerable part of the width of the Inner Floor, that Mont de Vénus represents the most recent surface expression of new crust along the plate boundary.

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¹ Heezen, B. C., Tharp, M., and Ewing, M., *Spec. Pap. geol. Soc. Am.*, **65**, 122 (1959).

² Needham, H. D., and Francheteau, J., *Earth planet. Sci. Lett.*, **22**, 29–43 (1974).

³ Muir, I. D., Tilley, C. E., and Scoon, J. S., *J. Petrology*, **7**, 193–201 (1966).

⁴ Bender, M. L., Ku, T.-L., and Broecker, W. S., *Science*, **151**, 325–328 (1966); *Earth planet. Sci. Lett.*, **8**, 143–148 (1970).

⁵ Moore, J. G., *Prof. Pap. US geol. Surv.*, **550**, 163–171 (1966).

Negative magnetic anomaly associated with Mount Kenya

MOUNT Kenya is a large central volcano just south of the equator in central Kenya. It rises to a height of 5,199 m above sea level. The base of the volcano covers an area of approximately 7,000 sq km, and is roughly circular in plan with an average diameter of 105 km. A detailed geological study¹ indicated that some 1,500 m of the upper part of the mountain has been removed by erosion since the volcano was formed in the Plio-Pleistocene.

The highest peaks of the mountain are formed from the eroded central plug which is about 2.2 km in diameter. There are a number of small glaciers in the peak area, the

intensity, associated with the peak area and displayed by all the observations.

All field measurements were made with a proton precession magnetometer and have been reduced to a common epoch time of 1430 UT on March 16, 1974, by using the continuous variometer records from the Nairobi geomagnetic observatory. The correlations for epoch were small and less than 60 γ in all cases ($1\gamma \equiv 10^{-9}T$). In addition to the field measurements on the volcanic plug, measurements were taken at six sites along the main approach track from the township of Naro Moru which is situated 32 km due west of the peak of Mount Kenya. (Fig. 1 and Table 1).

Corrected values of total intensity, F , have been plotted as a function of the distance of each measuring site from

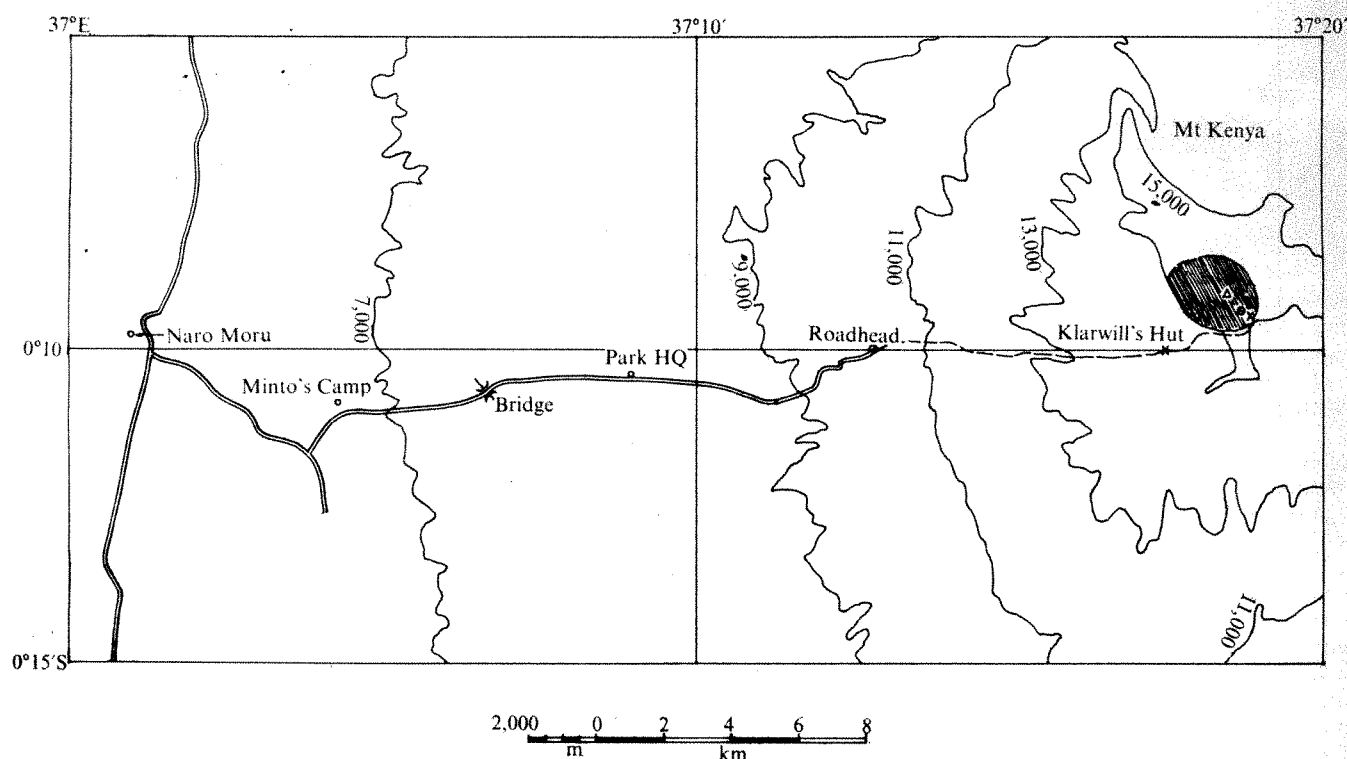


Fig. 1 Sketch map of the positions of the measuring sites along the track from Naro Moru to Batian. Contours are in feet. Shaded area, volcanic plug; \odot , Lewis Glacier; Δ , Batian; \times , Top Hut.

largest of which, the Lewis glacier, is being studied in detail by one of us (S.H.). As part of this study, with a view to determining the thickness of the ice and the shape of the underlying topography, close-spaced measurements were taken of the total intensity of the geomagnetic field along two profiles across the Lewis glacier at altitudes near 4,785 m. The glaciological interpretation of these observations will be reported in detail elsewhere. We here draw attention to a large negative anomaly in the total field

Batian, the highest peak of Mount Kenya (Fig. 2). The expected regional field value for the area (34,440 γ) obtained from a recent magnetic survey of the whole of Kenya (N.J.S.) is also shown (Fig. 2). A shallow negative anomaly of up to -400 γ exists over the whole traverse (all of which is underlain by volcanics from Mount Kenya), and the anomaly is sharply accentuated to greater than -1,300 γ on the volcanic plug. A somewhat similar negative anomaly of even larger amplitude has been reported on the volcano

Table 1 Data from the measurement sites

Map No.	Measuring site	Latitude	Longitude	Altitude (m)	Distance from Batian (km)	Total field F , corrected to common epoch (γ)
1	Naro Moru	0°09.7'S	37°00.9'E	1,975	32.5	34,283
2	Minto's Camp	0°10.8'S	37°04.3'E	2,100	26.4	34,283
3	Bridge (Naro Moru River)	0°10.9'S	37°06.5'E	2,195	22.4	34,334
4	Near Park HQ	0°10.4'S	37°09.0'E	2,440	17.7	33,957
5	Roadhead	0°10.1'S	37°12.8'E	3,050	11.0	34,073
6	Klarwill's Hut	0°10.0'S	37°17.5'E	4,145	2.5	34,057
7	Top Hut	0°09.6'S	37°18.9'E	4,785	1.2	33,118
8	Lewis Glacier (1)	0°09.5'S	37°18.8'E	4,725	0.7	33,610
9	Lewis Glacier (2)				0.9	< 33,350

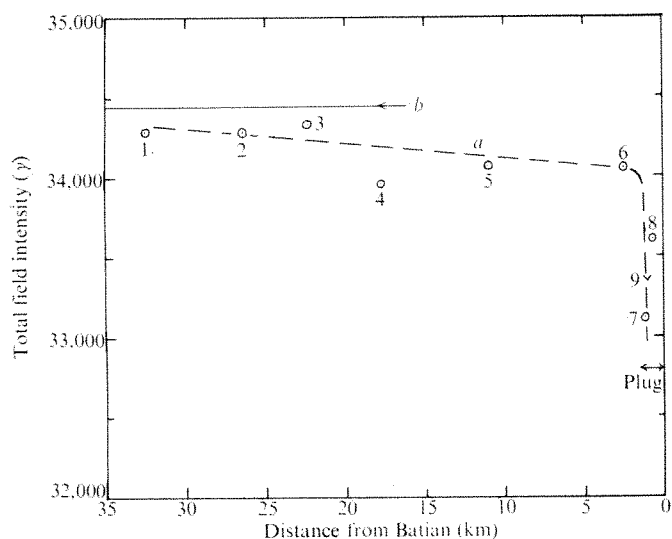


Fig. 2 a, Total field intensity as a function of distance from Batian. (Table 1 gives a key to the numbered sites); b, expected regional field.

Kilimanjaro in northern Tanzania², and in this case also the anomaly increases sharply near the peak (Kibo) of the mountain.

The plug of Mount Kenya is composed of a central core of nepheline syenite with outer partial sheaths of fine grained phonolite which in places is penetrated and locally altered by the syenite. Baker¹ considers that the main inner syenite body is younger than the adjacent phonolite but it is not clear whether all the syenite was emplaced in a single phase of volcanic activity. Evernden and Curtis³ have given isotopic ages of 2.64 Myr for a specimen of nepheline syenite taken from the plug and of 3.1 Myr for phonolite from the main eruptive phase collected near Embu, 40 km south-east of the peaks. The intense local negative field anomaly associated with the plug suggests that the rock of which it is composed possesses strong 'normal' remanent magnetisation, that is, it was emplaced at a time when the Earth's magnetic field was in the same direction as at present. Furthermore, an oriented specimen of phonolite collected at the edge of the Lewis glacier, near Top Hut, was strongly magnetised in a 'normal' sense. The present magnetic field measurements and the isotopic date of 2.64 Myr for the syenite suggest that the last eruptive phase through the main vent of Mount Kenya occurred during the Gauss normal epoch. The high degree of erosion of the peak area certainly precludes eruption during the present Brunhes epoch. On the other hand, for Kilimanjaro, the relatively slight degree of erosion of the Kibo crater, and the large negative magnetic anomaly², point to a much younger main eruptive phase within the Brunhes normal epoch, and this is confirmed by the isotopic ages in the range 0.365–0.514 Myr for basalts from Kilimanjaro³.

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¹ Baker, B. H., *Rep. geol. Surv. Kenya*, **79** (1967).

² Emeleus, T. G., and Osborne, D. G., *Ann. Géophys.*, **24**, 715–718 (1968).

³ Evernden, J. F., and Curtis, G. H., *Curr. Anthropol.*, **6**, 343–384 (1965).

⁴ Cox, A., *Science*, **163**, 237–245 (1969).

The sub-Palaeozoic basement in central Ireland

THERE is no direct evidence to indicate the nature of the sub-Palaeozoic basement beneath central Ireland. The recognition of gneissic xenoliths in Viséan volcanics in this region is therefore significant. They have been located at Croghan Hill in County Offaly (National grid ref. N4833; Fig. 1), Clare Castle (N2447), and the Dungolman River (N1851) in County Westmeath.

At the first locality one specimen, 30 cm³ in volume, was found in agglomerates cropping out to the west of Croghan village, and a second specimen of 1,600 cm³ in basalt a little further south. From agglomerates near Clare Castle 17 specimens were obtained, ranging from 10–200 cm³. The agglomerates from this latter locality occur as loose

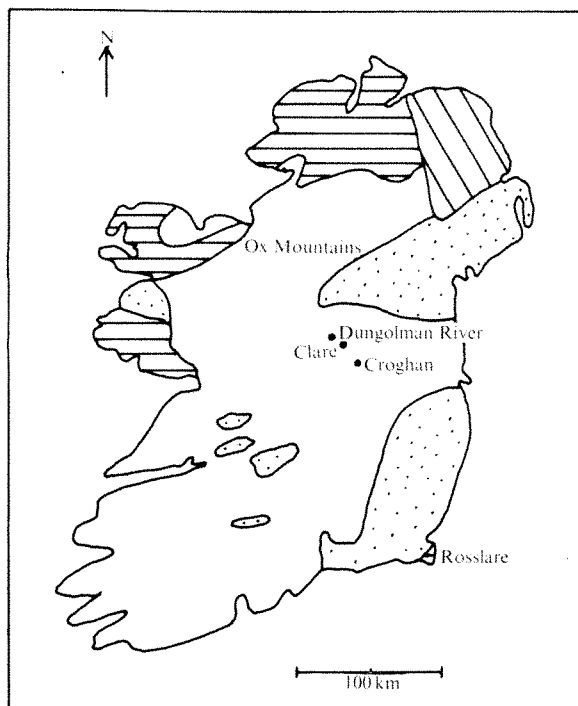


Fig. 1 Localities referred to in the text and areas of Dalradian or older rocks (horizontal ruling), Lower Palaeozoic rocks (shading), Upper Palaeozoic rocks (blank) and Mesozoic and Tertiary rocks (diagonal ruling).

boulders, often of great size, and they are certainly of local origin. They predominate in the boulder train and their lithic content, of Upper Palaeozoic age, closely matches that from beneath the area, which is known from boreholes (K. Cullen, personal communication). Similar agglomerates outcrop 11 km to the north-west in the bed of the Dungolman River. Material dredged from the bed of the river includes basalt fragments containing a further 10 xenoliths ranging from 10–30 cm³.

The Clare Castle gneisses are coarse grained and weakly banded, and contain the mineral assemblage: garnet–sillimanite–potash feldspar–plagioclase–quartz–rutile. Euhedral garnet, up to 2 mm across, has a composition close to the pyrope–almandine join at about 56% almandine. Sillimanite prisms up to 10 mm long define a strong lineation. Both feldspars are quite fresh. The plagioclase composition is An_{45–50}, and it is unzoned; the potash feldspar is a string perthite. Modes vary within the range: garnet 20–30%, sillimanite 5–20%, plagioclase 25–30%, potash feldspar 10–20% and quartz 10–40%. Other phases occur only in small amounts. Books of graphite up to 2 mm long occur in most specimens and there are traces of phlogopite in a

few cases. The assemblage belongs to a high amphibolite or granulite facies.

The gneiss from the agglomerate at Croghan is very similar, though it lacks potash feldspar. That from the basalt is very altered; only coarse sillimanite and quartz remain in a matrix of secondary chlorite, fibrous amphibole and altered glass. Abundant pseudomorphs after garnet can be seen readily, however, and Watts¹ has observed garnet in xenoliths from the area.

The gneisses from the Dungolman River are undoubtedly of granulite grade: they contain the critical assemblage hypersthene-garnet. They are rich in basic plagioclase (about An₆₀), hypersthene, garnet and quartz, and are medium grained and weakly banded. There is some alteration to anthophyllite, chlorite and carbonates.

Other lithic fragments are abundant in the agglomerates. Many compare with phyllites and slates of currently accepted Ordovician age which are exposed in the surrounding Lower Palaeozoic massifs. These rocks are of an extremely low metamorphic grade. That is in strong contrast to the gneisses which are therefore likely to be Dalradian or older. Indeed, the only rocks of similar type in Ireland are pre-Dalradian. The Dalradian everywhere differs in lithology²⁻⁴ and is generally of much lower grade, though it reaches middle amphibolite facies in places. The older Deer Park Schists⁴ are also unlike the xenoliths. Gneissic rocks from north-western Mayo⁵ and the Rosslare Complex^{6,7}, which may be Lewisian, are mica bearing and poor in garnet, and of a distinctly lower grade. The only comparable rocks are those from the Precambrian of the Ox Mountains, which Lemon⁸ has tentatively referred to the Moinian. These differ in that they contain kyanite.

The gneisses are clearly much older than the Lower Palaeozoic sediments. It is most unlikely that the gneiss has been derived indirectly, from the Old Red Sandstone (ORS) for example. None of the 29 xenoliths has adhering sediment, and gneissic clasts do not occur in the ORS exposed in nearby areas, or in ORS blocks within the agglomerates themselves. The gneiss must have been derived from the sub-Palaeozoic basement.

That is of interest in view of recently proposed plate tectonic models of Precambrian and Lower Palaeozoic history. Most of those require oceanic crust to lie beneath the Southern Uplands and its structural continuation into central Ireland⁹⁻¹³. Though some are not clear on the point, others clearly show this to be present even after the final Caledonian deformation. Powell¹⁴ has pointed out that the geophysical evidence conflicts with that idea, and proposed that there is a sialic basement "of Lewisian-type rocks" beneath the Southern Uplands. Jeans¹⁵ has accepted this, and his model shows oceanic crust lying, virtually pinched out, beneath the Midland Valley. The evidence presented here strongly support the latter hypotheses.

It is improbable that the gneiss was derived from a detached slice of sialic crust thrust over the oceanic basement. The localities discussed here are separated by 35 km, and the furthest is 70 km from Strokestown, the nearest point on the northern subduction zone proposed by Dewey^{6,7}. It is far more likely that beneath this area there is a true basement of high grade metamorphic rocks. It seems probable that this basement extends beneath the Down-Longford Massif and into the Southern Uplands.

I would like to thank Mr K. Cullen of Irish Base Metals Ltd, who brought my attention to the Clare agglomerates, and IBM who supplied drilling logs from the area. I also thank Drs J. R. Andrews and P. S. Kennan for discussion and criticism.

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- ¹ Watts, W. W., *Guide to the collection of rocks and fossils of the Geological Survey of Ireland* (H.M.S.O. Dublin 1895).
- ² Cambray, F. W., *Am. Ass. Petrol. Geol., Memoir* 12, 181 (1969).
- ³ Hartley, J. J., *Proc. R. Ir. Acad.*, **41**, 218 (1933).
- ⁴ Phillips, W. E. A., Kennedy, M. J., and Dunlop, G. M., *Am. Ass. Petrol. Geol., Memoir* 12, 194 (1969).
- ⁵ Sutton, J. S., and Max, M. D., *Geol. Mag.*, **106**, 284 (1969).
- ⁶ Baker, J. W., *Proc. R. Ir. Acad.*, **69**, 1 (1970).
- ⁷ Max, M. D., and Dhonau, N. B., *Scient. Proc. R. Dubl. Soc.*, **4**, 103 (1971).
- ⁸ Lemon, G. G., *Geol. Mag.*, **108**, 193 (1971).
- ⁹ Dewey, J. F., *Nature*, **222**, 124 (1969).
- ¹⁰ Dewey, J. F., *Scott. J. Geol.*, **7**, 219 (1971).
- ¹¹ Bird, J. M., Dewey, J. F., and Kidd, W. S. F., *Nature*, **231**, 28 (1971).
- ¹² Fitton, J. G., and Hughes, D. J., *Earth planet. Sci. Lett.*, **8**, 223 (1970).
- ¹³ Garson, M. S., and Plant, J., *Nature phys. Sci.*, **242**, 34 (1973).
- ¹⁴ Powell, D. W., *Scott. J. Geol.*, **7**, 369 (1971).
- ¹⁵ Jeans, P. J. F., *Nature phys. Sci.*, **245**, 120 (1973).

Reversals of the Earth's magnetic field and climatic changes

It has been suggested that evolution may be influenced by reversals of the Earth's magnetic field¹⁻³. It was observed that there were correlations between discontinuities in microfossil assemblages (or evolutionary discontinuities) and reversals of the Earth's magnetic field in deep sea sediment cores⁴⁻⁶. This observation was put on a firm base when Hays⁷ showed statistically that reversals directly or indirectly exert a selective influence on radiolaria. The explanation offered by Uffen^{1,2} and Simpson³ for the connection between evolution and reversals was that during reversals of the Earth's magnetic field, the intensity of the field would be reduced to a very low value, allowing organisms at the surface of the Earth to be bombarded by increased cosmic radiation normally shielded by the field. This increased radiation should then cause an enhanced mutation rate and hence produce an evolutionary discontinuity. It was, however, shown later that the estimated increase in the mutation rate would be small and unlikely to cause evolutionary discontinuities⁸⁻¹⁰.

Two other ways have been suggested in which reversals of the Earth's magnetic field could influence evolution. One way is by the direct biological effect of a magnetic field on organisms; there is a short discussion of this by Crain¹¹. The second mechanism proposes that a reversal of the Earth's magnetic field could cause a change in the climate of the Earth and hence indirectly produce faunal extinctions¹⁰.

Here, we shall discuss the second proposed mechanism and speculate on a possible causal relationship between reversals and climate. Evidence has been previously presented (for example refs 12, 13) suggesting a connection between the Earth's magnetic field and climate. Wollin *et al.*¹² showed that the record of mean annual temperature (averaged over 10-yr periods) was inversely correlated with the magnetic field intensity at nearby magnetic observatories. They were, however, unable to suggest what the causal relationship between these two parameters was. King¹³ has discussed the possibility that the Earth's field controls in some unknown way the variations in pressure to be found at high latitudes in the troposphere. King has also suggested¹⁴ that solar ionising particles are responsible for some of the correlations to be seen between the yearly mean sunspot number and various meteorological phenomena.

Roberts and Olson¹⁵ have demonstrated a relationship between one climatic parameter and geomagnetic disturbances and have also offered an explanation for this relationship. They have

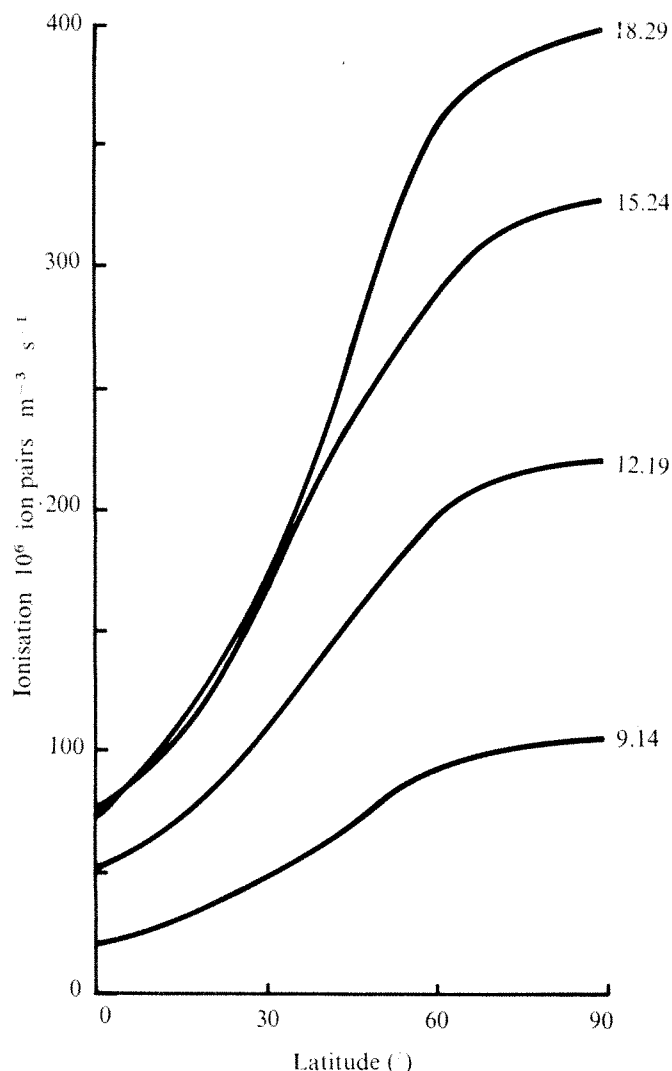


Fig. 1 Ionisation as a function of latitude. Curves are shown for four altitudes (measured in km).

shown¹⁵ that troughs at the 300 mbar level are influenced by geomagnetic disturbances. They found that, during the winter, troughs which moved into the Gulf of Alaska between 2 and 4 d after a geomagnetic storm underwent a greater degree of intensification than did troughs which appeared at the same place and during the same season but not within the 2–4-d time lapse after a geomagnetic storm. They suggest that this intensification may be caused by modification of the black body radiation from the relatively warm North Pacific as a result of increased cirrus cloud cover. It is thought by Roberts and Olson that increased ionisation at the tropopause level, caused by the ionising particles associated with the geomagnetic disturbances, could cause ion-induced nucleation and, hence, enhanced formation of clouds; the increased cirrus cloud cover at high latitudes during the winter results in a decrease in cooling rate which, in turn, leads to the increased vorticity.

A reversal of the Earth's magnetic field must be classified as a major geomagnetic disturbance. It is believed that the period of reduced field intensity during a reversal lasts between 1,000 and 10,000 yr^{16,17}. During the time of very low magnetic field intensity, the whole Earth would receive the present-day polar cosmic ray ionisation rate, all other factors being assumed to remain constant. We can estimate this rate by using the data of Saylor *et al.*¹⁸ for latitudinal variations in cosmic-ray ionisation rates as a function of altitude.

Table 1 gives ionisation rates at three different altitudes and at the equator and pole, taken from ref. 18. The top figure in each category gives the rate during a solar activity maximum, and the lower figure gives the rate during a solar activity minimum. Means

of the two values are given, and the polar/equatorial ratios of the means are shown in the last column.

Figure 1 shows the mean ionisation rate plotted as a function of latitude for four different altitudes. It can be seen that half or more of the Earth's area at these altitudes would have the ionisation rate increased by a factor of two or more during a reversal, if it is assumed that the polar ionisation rate is applicable to the whole Earth during a reversal. Using these curves and performing a simple numerical integration to determine average ionisation rates over the Earth's surface, it can be shown that the average ionisation increases by factors of 3.03 (for the lowest altitude curve), 2.87, 2.84 and 3.11 (for the highest altitude curve). If Roberts and Olson¹⁵ are correct in assuming that ionisation at such levels in the atmosphere is important for the production of cirrus clouds, then we should expect profound changes in the Earth's climate during a reversal, due to increased cloud cover at lower latitudes.

Table 1 Ionisation rate, (10^6 ion pairs $m^{-3} s^{-1}$) (measured at a pressure of 101.3 kN m^{-2})

Altitude (km)	Equator	Pole	Ratio
9.144	14.3 } 21.4 28.6 }	102.4 } 104.7 107.1 }	4.9
12.192	33.3 } 52.3 71.4 }	211.9 } 219.0 226.2 }	4.2
15.240	69.0 } 77.3 85.7 }	285.7 } 326.2 366.7 }	4.2

The specific mechanism which Roberts and Olson¹⁵ suggest as being responsible for the formation of cirrus, ion-induced nucleation, is highly speculative. Castleman¹⁹ presents evidence that ions can promote water vapour nucleation at supersaturation ratios considerably below that required for homogeneous nucleation; he suggests, for example, that at the summer mesopause (130 K, water concentration 10^9 cm^{-3}) sufficient supersaturation exists for this nucleation process to be operative. But Castleman does not state if this process would be significant lower in the atmosphere. Montefinale *et al.*²⁰, in a review of recent advances in the chemistry and properties of atmospheric nucleants, decline to discuss the role of ions because of the lack of information on the primary chemical nature of the ions and, hence, on their modes of action; they exclude ions from the population of active condensation nuclei on the basis of the high water supersaturations necessary for the ions to become active. On the other hand, Montefinale *et al.*²⁰, suggest that ions, because they electrostrict conspicuous amounts of water molecules, may be responsible for the vertical transport of water under the influence of the electric gradients in the atmosphere. This transport may, in itself, have a significant role in effecting weather changes in response to geomagnetic events.

Because of the general lack of knowledge about the physical and chemical properties of the upper atmosphere, the questions concerning the nature of the mechanism linking geomagnetic disturbances and weather will most probably remain unanswered for some time. But we submit that the relationship between geomagnetic disturbances and weather warrants further investigation not only because of the short term implications but also because such studies may lead to an explanation of climatic changes on the geological time scale.

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¹ Uffen, R. J., *Nature*, **198**, 143 (1963).

² Uffen, R. J., *Proc. 22nd int. Geol. Congr., New Delhi* (1964).

³ Simpson, J. F., *Bull. geol. Soc. Am.*, **77**, 197 (1966).

- ⁴ Harrison, C. G. A., and Funnell, B. M., *Nature*, **204**, 566 (1964).
⁵ Opdyke, N. D., Glass, B., Hays, J. D., and Foster, J., *Science*, **154**, 349 (1966).
⁶ Watkins, N. D., and Goodell, H. G., *Science*, **156**, 1083 (1967).
⁷ Hays, J. D., *Bull. geol. Soc. Am.*, **82**, 2433 (1971).
⁸ Black, D. I., *Earth Planet. scient. Lett.*, **3**, 225 (1967).
⁹ Waddington, C. J., *Science*, **158**, 913 (1967).
¹⁰ Harrison, C. G. A., *Nature*, **217**, 46 (1968).
¹¹ Crain, I. K., *Bull. geol. Soc. Am.*, **82**, 2603 (1971).
¹² Wollin, G., Kukla, G. J., Ericson, D. B., Ryan, W. B. F., and Wollin, J., *Nature*, **242**, 34 (1973).
¹³ King, J. W., *Nature*, **247**, 131 (1974).
¹⁴ King, J. W., *Nature*, **245**, 443 (1973).
¹⁵ Roberts, W. O., and Olson, R. H., *J. atmos. Sci.*, **30**, 135 (1973).
¹⁶ Ninkovich, D., Opdyke, N., Heezen, B. C., and Foster, J. H., *Earth Planet. scient. Lett.*, **1**, 476 (1966).
¹⁷ Harrison, C. G. A., and Somayajulu, B. L. K., *Nature*, **212**, 5067 (1966).
¹⁸ Saylor, W. P., Winer, D. E., Eiwen, C. J., and Carriker, A. W., *Space Radiation Guide*, (Technical Documentary Report AMRL-TDR-62-86, 1962).
¹⁹ Castleman, A. W., jun., *Space Sci. Rev.* (in the press).
²⁰ Montefinale, A. C., Montefinale, T., and Papée, H. M., *Pure appl. Geophys.*, **91**, 171 (1971).

Equatorial undercurrent and climate in the Galapagos Islands

PALYNOLOGICAL data from sediment cores taken from El Junco crater lake on San Cristobal Island led to the recognition of stratigraphical fluctuations resulting from the water budget in the crater¹. The results show that the crater was dry between at least 34,000 and 10,000 yr BP. Colinvaux¹ suggested that the Galapagos Islands had a dry climate during that period, because the intertropical convergence zone (ITCZ) remained north of the geographical equator. On the other hand, Newell² showed that the ITCZ probably existed south of the equator at about that time (20,000 yr BP).

I demonstrate here that another mechanism could explain this general dryness.

The climate in the Galapagos Islands is influenced by regional and local hydrographic conditions which regulate the annual course of the temperature in the superficial waters. The most prominent features of the thermal properties in the Eastern Tropical Pacific are the upwellings: those which occur along the coast of the South American mainland, and those which are connected to the divergence along the equator. I have already shown³ that the local upwelling west of Isabela is a fluctuating but permanent feature which influences the temperature distribution throughout the archipelago. The annual thermal regime in the superficial waters of the islands is balanced between the warm season, when warm waters are drifting from the north and solar heating takes place, and a

cold season, when upwelling reaches maximum development.

Year to year comparisons of meteorological data and surface sea temperature, taken at the Charles Darwin Research Station (Bahia Academia, Santa Cruz Island)^{4,5}, have revealed that interannual variations in the thermal regime are similar to those recorded further east in the coastal waters along the Peruvian^{6,7} and Ecuadorian coast (G. Houvenaghel, unpublished). There is also a good correlation between the annual rainfall values from some localities on Santa Cruz Island, and the annual mean sea temperature measured in the surface waters at Bahia Academia (Fig. 1). The climate may be characterised using the pluviothermic quotient of Emberger. Calvet⁸ gives a formula for the computation of a pluviothermic quotient from the annual rainfall, R , the mean air temperature of the hottest, T , and the coldest, t , month, and the evaporation:

$$Q = 1,000 R / 0.5 (T + t) (P_T - P_t)$$

where P_T and P_t are the water vapour pressure at temperatures T and t , respectively. Plotted against the mean annual sea temperature (Fig. 2), the computed values for Q also correlate well. Figure 2 shows that only a small decrease

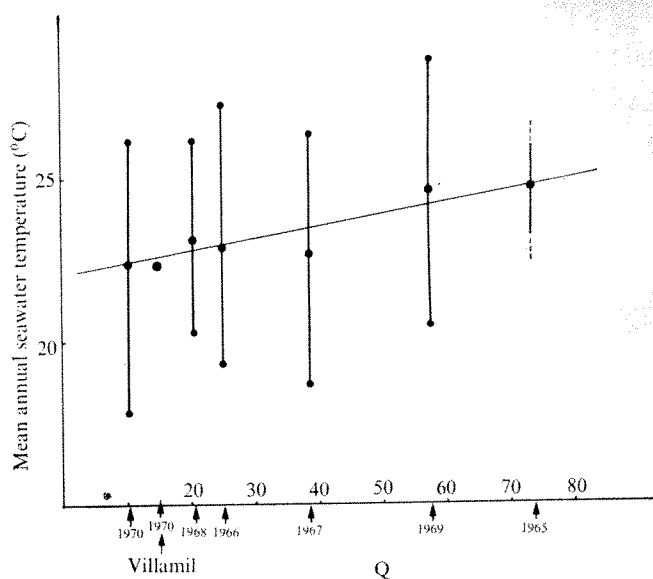


Fig. 2 The relationship between the mean annual seawater temperature and the annual pluviothermic quotient, Q , at Bahia Academia: $r = 0.91$ ($r = 0.87$, $P = 0.01$).

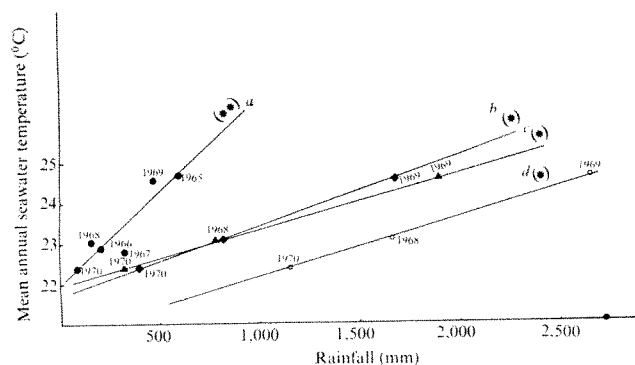


Fig. 1 The relationship between the mean annual seawater temperature, taken at Bahia Academia (Santa Cruz), and the rainfall collected in the same year at selected stations on Santa Cruz Island: ●, Bahia Academia (altitude, 6 m); ▲, Divine (320 m); ○, Media Luna (620 m); solid diamonds (200 m). a, $r = 0.903$; b, $r = 0.995$; c, $r = 0.996$; d, $r = 0.996$. (••, $r = 0.875$, $P = 0.01$; (*), $r = 0.990$, $P = 0.1$).

in the mean annual sea temperature is required to provide an arid or desert climate in the highlands of Santa Cruz. El Junco crater is located at an altitude of 650 m (ref. 1) which is intermediate between the highest two recording stations on Santa Cruz, and so the climate conditions prevailing on San Cristobal Island, 100 km ESE of Santa Cruz, must be similar to those recorded here. The dry climate which according to Colinvaux¹ existed in the past, could well have developed if a superficial cooling of sea water by about 1°C occurred at that time. Cooling can be expected to follow only a slight increase in the rate and duration of the upwelling (such as occurred, for example, during 1967) and it could last throughout the entire dry epoch. According to Bjerknes's model of equatorial circulation⁹, increases in both rate and duration of upwelling depend on the oceanwide stress of trade winds on the equator. Cooling in the Galapagos Islands may thus occur if the intensity of the field of the SE trade winds is increased. This could have been the case in the past: Newell² reports that the temperature gradient in both hemispheres was higher at 20,000 yr BP, and that the intensity of the Hadley cell circulation increased as well.

A dry climate in the Galapagos Islands could thus result

from changes in the intensity of the southern trade wind rather than from the shifting and continuing existence of the ITCZ in the Northern Hemisphere.

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¹ Colinvaux, P. A., *Nature*, **240**, 17-20 (1972).

² Newell, R. E., *Nature*, **245**, 91-92 (1973).

³ Houvenaghel, G., thesis, Univ. Libre de Bruxelles (1974).

⁴ *Meteorological Data*—Archives at the Charles Darwin Research Station, Bahia Academia, Santa Cruz.

⁵ Houvenaghel, G., *Mem. Acad. r. Sci. d'outre-mer Cl. Sci. nat. méd.* **49** **14** (1), 1-102 (1973).

⁶ Zuta, S., and Guillen, O., *Bol. Inst. Mar Peru*, **2** (5), 157-324 (1970).

⁷ Zuta, S., and Urquiza, W., *Bol. Inst. Mar Peru* **2** (8), 459-520 (1972).

⁸ Calvet, C., *Bull. Soc. Sci. nat. phys. Maroc*, **46**, 1-7 (1966).

⁹ Bjerknes, J., *Second Int. Oceanogr. Congr. Moscow*, 11-20 (1966).

Relative sizes of high and low spin states of atoms

STRUCTURAL studies with X rays^{1,2} of five and six-coordinated iron porphyrin complexes have shown that the distance from the iron atom to the nitrogen atoms of the porphyrin rings is less for low spin Fe(III) than for high spin Fe(III). As a result, it is often assumed that the Fe(III) atom is larger in its high spin state than in its low spin state. Similar size differences for the Fe(II) system form the basis of the Perutz model³ for the cooperativity of oxygen binding in haemoglobin. I discuss here the relative sizes of the high and low spin states of atoms in terms of the results of quantum mechanical calculations on isolated atoms. Contrary to earlier interpretations of structural studies, I have found that in the atoms I have studied the lowest lying high spin state of an atom in a given configuration is smaller than any state of lower spin.

Any theoretical discussion of the size of atoms must be concerned, in one way or another, with the radial density function $D(r_1)$ which is a measure of the probability of finding an electron at a distance r_1 from the nucleus. Unfortunately there is no unique definition of the size of an atom. In fact, many indices may be considered to be of atomic size. For example, the mean value of r_1 , denoted by $\langle r_1 \rangle$, is proportional to the average radial distance of an electron from the nucleus. Comparison of $\langle r_1 \rangle$ for two or more states of a given atom should indicate their relative radii. Also, as it is well known that the size of an atom is essentially determined by the outer shell⁴, the value of r_1 at the outer shell maximum of $D(r_1)$ could be taken as a measure of the size of an atom. Finally, the radius of a sphere containing a specified percentage of the total electronic charge is taken as a third index.

Consider the simple case of two electrons in a particular configuration in the field of a single nucleus. Figure 1 illustrates $D(r_1)$ for the singlet and triplet states arising from the $1s2s$ configuration of helium (the Pauli principle forbids a triplet state for the $1s^2$ configuration). The curves have been calculated for the moderately accurate correlated wave functions of Perkins⁵. The use of more accurate wave functions, such as those of Accad, Pekeris and Schiff⁶, which are nearly exact, would not alter the conclusions. On the basis of the indices listed in Table 1, it is clear that the helium atom is distinctly smaller by as much as 19% in the high spin 2^3S state than in the 2^1S state.

Similar calculations with the Perkins wave functions for Be^{2+} show that the 2^3S state is smaller than the 2^1S state by as much as 10%. (One curious result should be noted: in both He

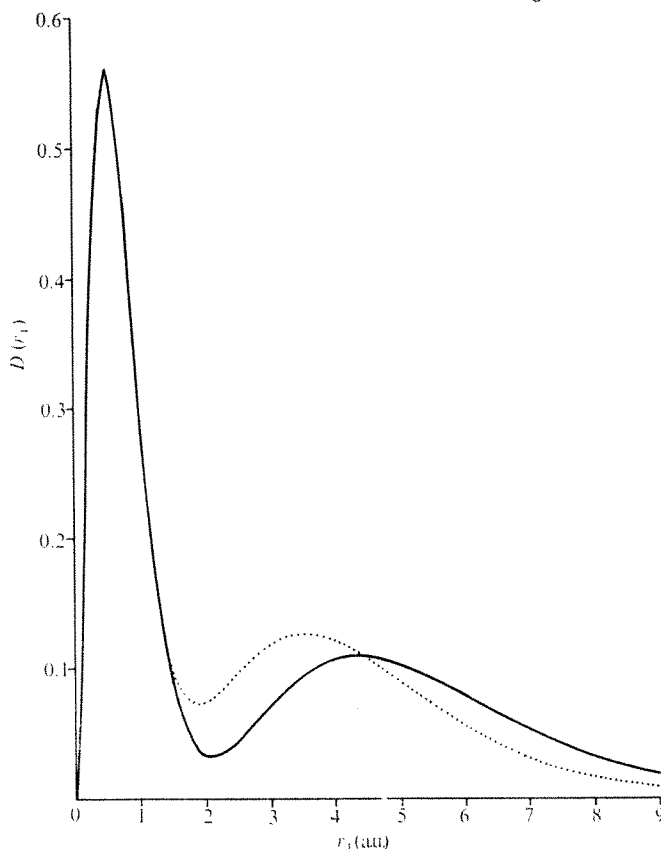


Fig. 1 Radial density function $D(r_1)$ in the 2^3S (dotted line) and 2^1S (solid line) states of helium.

and Be^{2+} the value of r_1 at the L shell maximum of $D(r_1)$ corresponds to the radius of a sphere containing ~69% of the electronic charge in both the 2^3S and 2^1S states.) Very accurate values of $\langle r_1 \rangle$ have been reported⁶ for the singlet and triplet states of the $1sns$ and $1snp$ ($n = 2-5$) configurations of the helium isoelectronic sequence up to $Z = 10$. In every case, the triplet state $\langle r_1 \rangle$ is greater than the corresponding singlet value and, therefore, it must be concluded that for atoms with two electrons a high spin atom is smaller than a low spin atom with the same configuration and nuclear charge.

For atoms with many electrons, only a few wave functions have been reported with an accuracy approaching that obtained with atoms which have only two electrons. It is, however, well known that the Hartree-Fock method gives good charge distributions. Also, because the size of an atom is essentially determined by the outer shell, the size of atoms with many electrons can be discussed in terms of the radial density associated with the outermost orbital, in much the same way as atoms with two electrons have been discussed in terms of $D(r_1)$. Assuming that, and using Clementi's Hartree-Fock wave functions⁷, a high spin atom is found to be smaller than a low spin atom with the same configuration and nuclear charge, for all the states arising from the p^2 , p^3 and p^4 configurations of the partially filled L and M shells. The conclusion is valid for large atomic numbers. For example, qualitatively identical results are obtained for the 4S and 3P states of the $\text{K}(2) \text{L}(8) 3s^2 3p^3$ configuration of both P and Kr^{21+} .

Unfortunately, Clementi's tables⁷ include only the lowest state of the $\text{K}(2) \text{L}(8) \text{M}(8) 3d^n$ configurations and, therefore,

Table 1 Relative radii of the 2^3S and 2^1S states of helium (a.u.)

Index	2^3S	2^1S
$\langle r_1 \rangle$	2.55	2.97
L shell maximum	3.54	4.38
99% charge	8.75	10.23
95% charge	6.78	7.90
90% charge	5.67	6.75
80% charge	4.49	5.42
65% charge	3.24	3.98

cannot be used to discuss the spin states of biological interest. It would, however, be very surprising if results similar to those observed for p^n configurations were not also found for d^n configurations. This conjecture is supported by some results⁸ for the $K(2) L(8) 3s^2 3p^6 3d^1 4d^1$ configuration of Ca. The 4d radial densities indicate that Ca is smaller in the high spin 3F state than in the low spin 1S , 1D and 1G states. In this case, unlike the p^n configurations, there is a second allowed state which has the maximum multiplicity. Thus, the 3P state lies above the 3F and 1D states and leads to a correspondingly larger atomic radius.

Although it has long been realised that the effective radius of an atom depends on its electronic structure and environment⁹, it seems that the observations of free atoms discussed here have not been reported previously. Of course, environmental effects can be large and can lead to results opposite to those found in free atoms; a detailed discussion of environmental effects is, however, beyond the scope of this article.

Although these results are likely to be contrary to the 'chemical intuition' of many scientists, it should be noted that electron repulsion is greater in the triplet state than in the singlet state of some two-electron systems¹⁰ contrary to the usual interpretation of Hund's rule.

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- ¹ Hoard, J. L., in *Structural Chemistry and Molecular Biology*, (edit. by Rich, A., and Davidson, N.), (Freeman, San Francisco, 1968).
- ² Countryman, R., Collins, D. M., and Hoard, J. L., *J. Am. chem. Soc.*, **91**, 5166-5167 (1969).
- ³ Perutz, M. F., *Nature*, **228**, 726-734 (1970).
- ⁴ Coulson, C. A., *Valence*, second ed., 39 (Oxford University Press, London, 1961).
- ⁵ Perkins, J. F., *J. chem. Phys.*, **39**, 687-693 (1963).
- ⁶ Accad, Y., Pekeris, C. L., and Schiff, B., *Phys. Rev.*, **A4**, 516-536 (1971).
- ⁷ Clementi, E., *IBM JI Res. Dev., Suppl.* 9 (1965).
- ⁸ Froese Fischer, C., *J. Phys.*, **B5**, 1302-1307 (1972).
- ⁹ Pauling, L., *Nature of the Chemical Bond*, third ed., 224 (Cornell University Press, Ithaca, 1960).
- ¹⁰ Boyd, R. J., and Coulson, C. A., *J. Phys., B (GB)*, **6**, 782-793 (1973), and references therein.

Significance of the even-carbon n -paraffin preference of a Spanish crude oil

It has been suggested that processes other than the accumulation of biogenic hydrocarbons play an important role in the organic formation of petroleum. That can be inferred from a comparison of the different types of organic compounds found in sediments and petroleum^{1,2}.

Those studies dealt mainly with saturated hydrocarbons, among which n -paraffins have a special geochemical significance. That is because their distribution depends on the degree of maturity and the nature of the source material.

Most recent sediments exhibit an n -paraffin distribution concentrated between C_{18} and C_{32} , characterised by a marked predominance of odd numbers of carbon atoms ($CPI > 1$), (refs 3 and 4). As a result of physicochemical maturation processes, this predominance diminishes in the oldest sediments, and disappears in petroleum in which there is a shift in the maximum of the n -paraffin distribution curve towards lower carbon numbers. Thus, the CPI of a sediment is considered as an indication of its capacity for generating hydrocarbons³, although both the extent of generation of hydrocarbons and the type of source material, influence the distribution of the n -alkanes found in ancient sediments⁵.

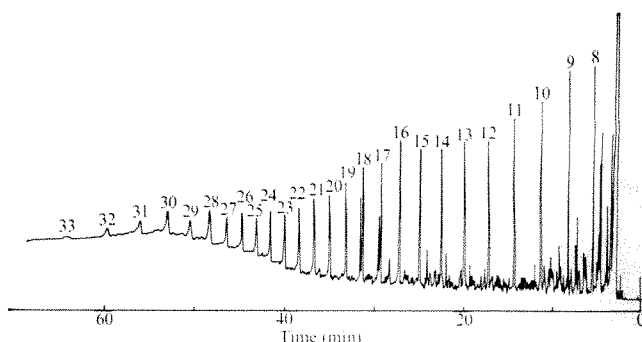


Fig. 1 Gas chromatogram of the Amposta-marino crude oil (OV-101 capillary column).

Smooth n -paraffin distributions ($CPI \approx 1$) are typical of marine organic facies. A relative predominance of high molecular weight n -paraffins, with possibly an odd carbon preference in the C_{20} - C_{30} range has, however, been invoked as evidence of non-marine origin in some sediments^{4,6} and crude oils⁷⁻⁹. A slight predominance of odd number n -paraffin is not definite proof of continental origin, because it may have arisen by diagenetic processes¹⁰. The predominance of n -paraffins with odd numbers of carbon atoms has also been claimed to support the hypothesis of fatty acid decarboxylation in the generation of n -paraffins.

In our work on the chemical characterisation of the crude oils from the first oil field in production in the Mediterranean (near the mouth of the Spanish Ebro River), we have found that the Amposta-marino crude (Mesozoic, 16.6 API) apparently exhibits a marked predominance of n -paraffins with even numbers of carbon atoms within the C_{20} - C_{34} range (Fig. 1). That is interesting not only geochemically but also because it provides a means for the characterisation of accidental spillage of the crude oil in the sea¹¹.

We isolated the n -paraffins by separate treatment of the light distillates ($< 200^\circ C$) and of the desasphalted residue with 5 Å molecular sieves. Analysis by gas-liquid chromatography provided the real distribution of n -paraffins in the crude oil (Fig. 2). A maximum appeared at $n=8$, and a CPI value of 0.74 was observed in the range C_{24} - C_{34} .

Slight predominances of sedimentary n -paraffins with even number of carbon atoms have been reported^{4,12-14}. Considering the n -paraffin distributions found in living organisms (odd number preference), the even number predominances should be a result of post-depositional reductive processes acting on the organic matter, that is, hydrogenolysis of fatty acids^{4,12} and alcohols¹⁵. There is experimental evidence of such geochemical transformations¹⁶⁻¹⁸.

Even-carbon n -paraffins can also be formed by the condensation of two fragments originating from fatty acid decarboxylation or from n -paraffin thermal degradation^{19,20}. For thermodynamic reasons, however, these processes should be of secondary importance in comparison with true degradation.

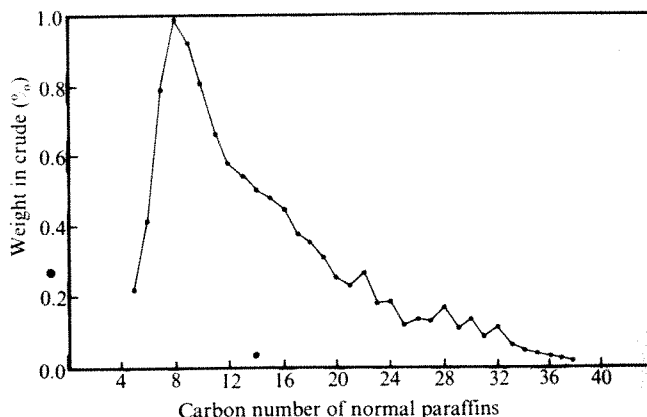


Fig. 2 Distribution of n -paraffin in the Amposta-marino crude.

As far as petroleum is concerned only a very few cases of even-carbon predominances have been reported²¹. The most remarkable was found in a Nigerian crude oil within the C₄₀–C₄₆ range²². Its origin is very difficult to explain by the processes already mentioned.

To the best of our knowledge, there are no previously described examples of such a clear even-carbon number predominance within the C₂₀–C₃₄ range in crudes, as that exhibited by the Amposta-marino oil. The type of distribution shown in Fig. 2 leads to the assumption that the process of formation is not secondary but rather that the even-carbon number predominance is produced by the reduction of organic material containing large amounts of long chain fatty acids or alcohols. This could indicate a continental character in the crude oil, as would be expected for a deltaic environment. As long as no definite preference is observed in the C₁₂–C₁₈ region, the reduction of alcohols seems to be the more probable process.

Such a reduction has already been invoked, for example, to explain the formation of steranes from sterols in petroleum¹². Furthermore, the *n*-C₂₂ and *n*-C₂₃ paraffins, the principal hydrocarbons of the C₂₀–C₃₄ range studied, have been considered to be related geochemically to *n*-docosanol² and *n*-octacosanol¹⁵, the main components of some continental plants and sediments. On the other hand, the formation of even-number *n*-paraffins by the reduction of waxy alcohols could explain, in some cases²⁴, the balanced distribution of C₂₀–C₃₀ *n*-paraffins in old sediments and crude oils: it may result from dilution of the pre-existing odd-number compounds.

It is worth pointing out, in connection with these reduction processes, that phytane is more abundant than pristane in this crude (phytane/pristane=1.4), although it does not seem necessary to invoke a reduction process in order to explain this predominance, because of the existence of isoprenoid alkanes in living organisms.

In short, chemical and physical environments must be as important as time in establishing differences in the *n*-paraffin distributions. In particular, the manifest existence of reduction processes during the formation of the Amposta-marino crude affords direct evidence of the need for a reducing condition in the sediments for the formation of petroleum. This idea has been supported until now by the absence of olefins and other easily reducible substances in crudes. In any case, the mechanism of such processes has not yet been established.

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- ¹ Philippi, G. T., *Adv. org. Geochem.*, **25** (1968).
- ² Ourisson, H., and Albrecht, P., *Angew. Chem., int. Ed. Engl.*, **10**, 209 (1971).
- ³ Bray, E. E., and Evans, E. D., *Bull. am. Ass. Petrol. Geol.*, **49**, 248 (1965).
- ⁴ Kvenvolden, K. A., *Adv. org. Geochem.*, 335 (1966).
- ⁵ Koons, C. B., Jamieson, G. W., and Ciereszko, L. S., *Bull. Am. Ass. Petrol. Geol.*, **49**, 301 (1965).
- ⁶ Rogers, M. A., and Koons, C. B., *Adv. Chem. Ser.*, **103**, 67 (1971).
- ⁷ Martin, R. L., Winters, J. C., and Williams, J. A., *Nature*, **199**, 110 (1963).
- ⁸ Didyk, B. M., and McCarthy, E. D., *Nature*, **232**, 103 (1971).
- ⁹ Reed, K. J., *Bull. Am. Ass. Petrol. Geol.*, **53**, 1502 (1969).
- ¹⁰ Albrecht, P., thesis, Univ. Strasbourg (1969).
- ¹¹ Albaigés, J., *Oilgas*, **75**, 73 (1974).
- ¹² Welte, D. H., and Ebbardt, G., *Geochim. cosmochim. Acta*, **32**, 465 (1968).
- ¹³ Han, J., McCarthy, D., Van Hoeven, W., Calvin, M., and Bradley, W. H., *Proc. Natn. Acad. Sci., U.S.A.*, **59**, 29 (1968).
- ¹⁴ Schenk, P. A., *Adv. org. Geochem.*, 261 (1968).
- ¹⁵ Knoche, H., Albrecht, P., and Ourisson, G., *Angew. Chem., int. Ed. Engl.*, **7**, 631 (1968).
- ¹⁶ Blumer, M., *Science*, **149**, 722 (1965).
- ¹⁷ Mitterer, R. M., and Hoering, T. G., *Y6. Carnegie Instn Wash.*, 510 (1966).

- ¹⁸ Bayliss, G. S., *Am. chem. Soc. Div. Petrol. Chem.*, **13**, F117 (1968).
- ¹⁹ Robinson, R., *Nature*, **212**, 1291 (1966).
- ²⁰ Jürg, J. W., and Eisma, E., *Science*, **144**, 1451 (1964).
- ²¹ Henderson, W., Eglinton, G., Simmonds, P., and Lovelock, J. E., *Nature*, **219**, 1012 (1968).
- ²² Brunnock, J. V., *Nature*, **212**, 385 (1966).
- ²³ Speers, G. C., and Whitehead, E. V., *Adv. org. Geochem.*, 638 (1968).
- ²⁴ Oró, J., Noonan, D. W., and Wihström, S. A., *Science*, **147**, 870 (1965).

Age of valley deposits in Périgord

IN Périgord, as in other parts of Europe, climatic and ecological variations inferred from Pleistocene sediments and organic remains have long provided the basis for correlating stratified Palaeolithic artefacts¹ and for subdividing the geological record^{2,3}. Radiocarbon dating now makes it possible to establish independent environmental, archaeological and palaeontological sequences and thus to investigate the links between them. I report here the results of work on valley deposits undertaken with this end in view; their archaeological and palaeoclimatic implications will be considered elsewhere.

Near their junction, the Vézère and Dordogne rivers and their larger tributaries (Fig. 1) display traces of three post-Tertiary depositional episodes separated by periods of erosion. Names are proposed for the resulting rock units (Fig. 2) to simplify discussion.

The 'Condat Tufa' is a localised deposit consisting of marls and tufas. Five ¹⁴C dates were obtained at various levels in the type section (section I), where the formation attains a maximum observed thickness of 8.2 m and overlies Jurassic limestones. (To judge from the published description this is the tufa at Coly from which Bourdier⁴ reported a temperate molluscan fauna.) The dates (Table 1) are consistent with their relative stratigraphical position and when plotted against depth indicate a uniform depositional rate of about 21 cm per 1,000 yr; but being on tufa they are automatically suspect. Variations in δ¹³C do not necessarily provide a good measure of the limestone dilution effect⁵ and in the present case yield no consistent pattern; and, as tufa is not forming today, a correction based on current ¹⁴C:¹³C ratios could not be introduced. As regards contamination by younger carbon, pretreatment with acid washes was supplemented by various analyses of the three uppermost samples. Although thin sections reveal no obvious evidence of recrystallisation, and X-ray diffraction shows that all three samples consist of calcite with less than 4 mol% MgCO₃, JD-22 displays some scattered rhombs of dolomite and signs of secondary calcitic cementation (P. Wigley, personal communication). This supports the inference that the high porosity (15–30% void ratio) of JD-21 and 22 coupled with their position at the surface of the tufa would have exposed them to ¹⁴C exchange, and thus argues for their rejection. Sample JD-2, being compact and rich in algal material, is more likely to yield a date which refers to the time of deposition.

The 'Montignac Formation' includes scree, colluvium and alluvium, and ranges in lithology from gravel to clay. Its surface slope attains 30° at some valley margins and decreases to form horizontal terraces along the larger streams, such as the Vézère at Montignac; its thickness commonly exceeds 25 m. The deposit is separated from the Condat Tufa by a weak unconformity; elsewhere it rests on Tertiary or older rocks. Although some aggradation may have taken place before the end of tufa deposition (unlike JD-2, samples JD-21 and JD-22 contain quartz grains), the bulk of the Montignac phase presumably postdates 21,730 BP. Derived Middle and Advanced Palaeolithic artefacts were found in the Montignac deposits at various exposures; at the Magdalenian open site of Solvieux, on the

Table 1

Rock unit	Sample No.	Laboratory No.	Grid reference ¹	Location	Age (y BP)	¹³ C: ¹² C Rel PDB	Material	Remarks
Condat Tufa	JD-21	I-6945	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	12,890 ± 175	—	Porous Tufa	At contact with Montignac Fm
Condat Tufa	JD-22	I-6946	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	12,320 ± 175	-8.4	Nodular Tufa	At contact with Montignac Fm
Condat Tufa	JD-2	I-6795	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	21,730 ± 410	-10.3	Algal Tufa	2 m below contact
Condat Tufa	JD-93	I-7472	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	25,880 ± 650	-9.2	Dendritic Tufa	3 m below contact
Condat Tufa	JD-94	I-7473	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	31,050 ± 1500	-11.7	Marl	4 m below contact
Limeuil Alluvium	DP-5	I-6794	494.5 × 296.3 (Section V)	1°0'5"E 44°57'21"N	1,335 ± 90	-22.2	Charcoal	Age normalised for fractionation 1,375 ± 90

¹Grid references refer to Carte de France (1/25,000).

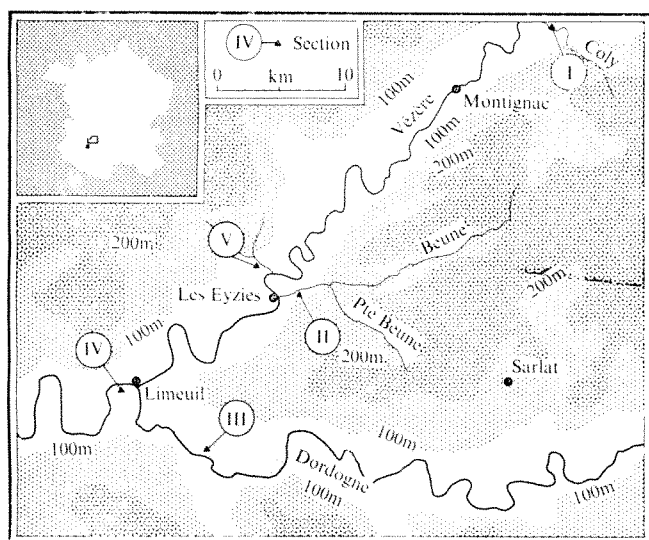


Fig. 1 Location map of places mentioned in text.

Isle (which joins the Dordogne west of the main area of study), deposition was apparently still in progress during the period of occupation. Radiocarbon dates for comparable material from the caves and shelters of Périgord⁶ suggest that deposition of the Montignac Formation continued until about 10,000 yr ago. It is worth noting that in the Beune valley, where the Montignac deposits are coarse in texture and slope down beneath the fine-grained material in which the river is now incised, a ¹⁴C date of 9,040 ± 60 yr BP (Grn-4492) has been obtained at a depth of about 13.7 m slightly above the contact between silty muds and the underlying sands and gravels⁷.

The 'Limeuil Alluvium' generally underlies the plains that border the larger streams, although it may be concealed by modern flood deposits. It consists principally of silty sand (whereas the flood material is dominated by coarse sand with abundant pebbles), and attains a maximum observed thickness of 7.5 m at Limeuil (section IV). Roman potsherds have been found within the deposit here as well as in a quarry west of Coux-et-Bigaroque (section III) and in the Beune opposite the cave of Font de Gaume (section II). Radiocarbon dating of the Limeuil Alluvium in the Manaurie valley (section V; see Table 1) has confirmed its historical age.

The resulting sequence may be summarised as follows. Localised tufa deposition came to an end about 21,000 yr ago. It was closely followed by a phase of scree formation, slope wash and valley filling. About 10,000 yr ago stream incision supervened. Renewed aggradation took place during or after Roman times, to be supplanted by the current erosional episode at some stage after AD 575.

Field mapping shows that the Montignac Formation of the present account includes most of the deposits attributed on the geological sheets of the area⁸ to the lower terrace of the *alluvions anciennes* as well as part of the *alluvions modernes*; the rest of the *alluvions modernes* correspond to our Limeuil Alluvium. Comparison with the accounts of Fénélon² and Bourgon³ shows similar discordances.

Chaput⁹ has described a deposit in the Garonne near its junction with the Dordogne similar in morphology and archaeological content to the Montignac Formation and linked to moraines at the foot of the Pyrenees which he ascribes to the Würm glaciation; the deposition of the Montignac Formation during glacial times, already suggested by its wealth in presumably frost-riven scree material, is further supported by the ¹⁴C dates of 17,500 and 9,700 BP obtained for peats laid down behind two of the outermost Pyrenean moraines¹⁰. The apparent absence in the area under review of valley deposits demonstrably older than the Condat Tufa, though surprising in view of the very long and complex sequence of glacial episodes that has been identified in the caves of Périgord¹¹, accords with the dearth of evidence for pre-Würm glaciations in the Pyrenees¹⁰ and with the finding that only one, 'rather recent', glacial phase is represented in the upper Dordogne¹². Moreover, neither Fénélon nor Bourgon succeeded in identifying slope deposits older than the Würm^{2,3}. As in the Mediterranean Basin¹³, we can either postulate that evidence for earlier glacial episodes has been destroyed¹⁴ or take the field record at its face value when attempting climatic reconstructions.

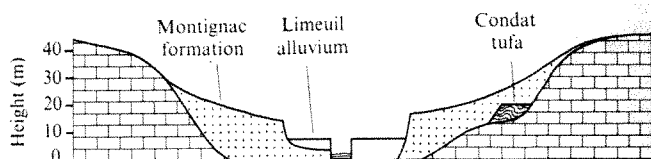


Fig. 2 Schematic section of valley fills.

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¹ Bordes, F., in *The Explanation of Culture Change* (edit. by Renfrew, C.), 217-226 (Duckworth, London, 1973).

- ² Fénelon, P., *Le Périgord. Étude morphologique*, 526 (Lahure, Paris, 1951).
- ³ Bourgon, M., *Archives Inst. Paléont. Hum., Paris, Mém.* 27, 141 (1957).
- ⁴ Bourdier, F., *Soc. préhist. Fr.*, **42**, 173 (1945).
- ⁵ Bowler, J. M., and Polach, H. A., in *Paleopedology* (edit. by Yaalon, D. H.), 97–108 (International Society of Soil Science and Israel Universities Press, Jerusalem, 1971).
- ⁶ Coles, J. M., and Higgs, E. S., *The Archaeology of Early Man*, 454 (Faber and Faber, London, 1969).
- ⁷ Donner, J. J., *Pollen et Spores*, **11**, 97–116 (1969).
- ⁸ *Carte Géologique de la France (1/80,000)*, Sheets 182 and 183.
- ⁹ Chaput, E., *Bull. Soc. géol. Fr.*, **24**, 449–461 (1924).
- ¹⁰ Taillefer, F., *Bull. Ass. Fr. Ét. Quat., Suppl.*, 19–32 (1969).
- ¹¹ Laville, H., *Bull. Ass. Fr. Ét. Quat., Suppl.*, 77–80 (1969).
- ¹² Veyret, Y., *Bull. Ass. Fr. Ét. Quat.*, **9**, 21–30 (1972).
- ¹³ Vita-Finzi, C., *Nature*, **224**, 173 (1969).
- ¹⁴ Fairbridge, R. W., in *The Mediterranean Sea* (edit. by Stanley, D. J.), 99–113 (Dowden, Hutchinson and Ross, Stroudsburg, 1971).

Grassland species can influence the abundance of microbes on each other's roots

NEIGHBOURING plants usually influence each other's growth. These interactions are very important in determining the composition and structure of plant communities, but much remains to be discovered about the mechanisms by which plants influence each other. Often plants compete for requirements such as light and mineral nutrients. Some plants exude substances which are toxic to others¹⁻³. Another possibility, as yet unexplored, is that interactions occur through changes in the microbial populations on the root surface or in the rhizosphere (the soil near the root surface). It is known that rhizosphere and root surface microorganisms can affect the plant's nutrient uptake and growth^{4,5}, but up to now there has been no evidence on whether plant species which naturally grow together can influence each other's microbial populations. We present here such evidence for two species common in British lowland grassland, *Lolium perenne* L. (perennial ryegrass) and *Plantago lanceolata* L. (ribwort plantain, a dicotyledonous species).

Seeds of the two species were collected from a permanent grassland site near Bristol. Soil was collected from a neighbouring field where both species also occur. The soil was mixed and placed in pots 13 cm in diameter; it was not sterilised, nor was fertiliser added. Seeds were germinated in vermiculite, and four seedlings were planted in each pot, either four *Lolium*, four *Plantago*, or two of each species.

Two similar experiments were performed. In the first there were nine replicate pots per treatment, and in the second ten. In the first experiment the plants grew from July until September 1973, and in the second from November 1973 to February 1974. The plants grew in a glasshouse for 2–3 months, and the roots were then washed free of soil. Randomly selected root segments were stained with phenyl acetic aniline blue and the abundance of bacteria and fungi on the root surface was determined by direct observation under a microscope: the percentage of the root area covered by bacteria was estimated in numerous randomly-positioned squares, and the length of fungal mycelium per unit root surface area was determined by the line intersection method⁶. The techniques are described in more detail elsewhere⁷. In the second experiment only fungi were determined.

Table 1 summarises the results. In every case the microbial abundance was greater when the two species were growing together than when they were separate, and by analysis of variance this main effect (separate/together) was always statistically significant. It is true that by Duncan's multiple range test only *Lolium* showed a significant increase in microorganisms in experiment 1, and only *Plantago* did so in experiment 2. Nevertheless, both species always showed the trend in the same direction.

We have no direct evidence on how (if at all) the increase in root surface microorganisms influences the higher plants, but the dry weights, shown in Table 2, are relevant. In experiment 1,

Table 1 Percentage cover of bacteria, and fungal mycelium length per unit root surface area, on root surfaces of *Lolium perenne* and *Plantago lanceolata* grown either separately or together

	Bacteria Cover %		Fungi Mycelium length (mm mm ⁻²)			
	Experiment 1		Experiment 1		Experiment 2	
	<i>Lolium</i>	<i>Plantago</i>	<i>Lolium</i>	<i>Plantago</i>	<i>Lolium</i>	<i>Plantago</i>
Separate	4.3 a	5.6 ab	0.7 a	1.8 ab	8.4 a	8.8 a
Together	6.3 b	5.8 b	2.1 b	2.9 b	11.3 a	15.5 b
Significance†	<i>P</i> < 0.025		<i>P</i> < 0.01		<i>P</i> < 0.001	
Separate/together	<i>P</i> < 0.025		<i>P</i> < 0.01		<i>P</i> < 0.001	
Between species	ns		<i>P</i> < 0.025		ns	
Interaction	ns		ns		ns	

* Within each group of four, figures not followed by the same letter are significantly different (*P* < 0.05) by Duncan's multiple range test.

† Results of analysis of variance; ns, not significant.

Lolium showed an increased shoot weight in the mixture, while *Plantago* showed no change. The total yield by the mixture exceeded that of either pure stand (Table 2b). In experiment 2 there was a similar increase of weights in the mixture, though it was not quite statistically significant. These results differ from the commonest situation in direct competition, where increased growth by one species involves reduced growth by another. They could be caused by the increased microbial population having a beneficial effect on plant growth. Yield increases by mixtures, of the sort shown in Table 2b, have been reported previously, but do not always occur⁸⁻¹⁰. A recent survey of the literature on two-species and two-variety mixtures in pot and field experiments (E.I.N., unpublished), found that in 20% of cases the mixture yielded more than either pure stand. No satisfactory explanation has yet been offered as to why some mixtures show this increase and others do not, and it is possible that the nature of the microbial interactions is one of the factors determining this.

Table 2 Dry weight of shoots (g). Statistical significance expressed as in Table 1

a Mean weight per plant*.

	Experiment 1		Experiment 2	
	<i>Lolium</i>	<i>Plantago</i>	<i>Lolium</i>	<i>Plantago</i>
Separate	0.71 a	0.69 a	0.54 a	0.56 a
Together	1.01 b	0.69 a	0.71 a	0.62 a
Significance	<i>P</i> < 0.025		ns	
Separate/together	<i>P</i> < 0.025		ns	
Between species	<i>P</i> < 0.01		ns	
Interaction	<i>P</i> < 0.025		ns	

b Mean weight per pot (four plants)*.

	<i>Lolium</i> alone	<i>Plantago</i> alone	Mixture	Significance
Experiment 1	2.84 a	2.75 a	3.39 b	<i>P</i> < 0.025
Experiment 2	2.17 a	2.26 a	2.65 a	ns

* Letters (a and b) are for comparisons within each row.

Clearly the significance of our findings for higher plant growth is at present speculative. What we have definitely shown is that two plant species which commonly grow together can influence each other's root surface microbial population.

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¹ Muller, C. H., *Vegetatio*, **18**, 348–57 (1969).

- ² U.S. National Committee for the International Biological Program, *Biochemical Interactions among Plants* (National Academy of Sciences, Washington, D.C., 1971).
- ³ Whittaker, R. H., and Feeny, P. P., *Science*, **171**, 757-70 (1971).
- ⁴ Barber, D. A., *A. Rev. Pl. Phys.*, **19**, 71-88 (1968).
- ⁵ Bowen, G. D., and Rovira, A. D., in *Root Growth* (edit. by Whittington, W. J.), 170-99 (Butterworths, London, 1969).
- ⁶ Newman, E. I., *J. appl. Ecol.*, **3**, 139-45 (1966).
- ⁷ Rovira, A. D., Newman, E. I., Bowen, H. J., and Campbell, R., *Soil Biol. Biochem.* (in the press).
- ⁸ Syme, J. R., and Bremner, P. M., *J. appl. Ecol.*, **5**, 659-74 (1968).
- ⁹ Marshall, D. R., and Jain, S. K., *J. Ecol.*, **57**, 251-70 (1969).
- ¹⁰ Harris, W., and Thomas, V. J., *N.Z.J. agric. Res.*, **15**, 19-32 (1972).

Catalysomes of adipose tissue are artefacts of enzyme localisation

WHEN adipose tissue (insect fat body and gut, mouse mesentery) is stained to localise esterases (using 5-bromoindoxyl acetate as indigogenic substrate³) or dehydrogenases (using various substrates and nitro-blue tetrazolium⁷) the reaction product forms a single cap on each lipid droplet^{4,9,11-13}. The cap has been thought to be an organelle¹², perhaps a specialised mitochondrion concerned in lipid metabolism², for which Wigglesworth proposes the term catalysome. Although the localisation of the reaction products in caps is very precise, the nature of the organelle has remained a mystery. In *Calpodes* fat body, 5-bromoindoxyl acetate always gives one perfect blue cap of the indigo reaction product for each lipid droplet (Fig. 1a) and similar localisation is obtained with the dehydrogenases (Fig. 1b), but further studies have shown that the catalysomes are artefacts caused by the preferential precipitation of reaction products in lipid or at lipid-water interfaces.

Our initial intention was to locate the caps by electron microscopy, and thus discover the nature of the organelle. In *Calpodes* larvae, the fourth instar lipid droplets disappear and are replaced by a new generation within 24 h of ecdysis to the fifth instar (ref. 5). Esterase caps can be demonstrated from the moment that the new generation of lipid droplets becomes visible by light microscopy, that is when they are about the size of mitochondria. Electron microscopy (EM), however, showed no organelle preferentially associated with lipid droplets at this time when such an association should have been unmistakable. Esterase localisation for EM by the 8-acetoxyquinoline/bismuth oxynitrate method of Deimling¹ also failed to identify any esterolytic organelle associated with lipid droplets. Bismuth was deposited in mitochondria spread throughout the cytoplasm, and within some droplets (Fig. 2) but no cap-like organelle could be discerned. The bismuth precipitate associated with lipid which in EM sections often appeared to spread from one or occasionally two foci on the periphery of a droplet (Fig. 2) was seen in whole mounts as a large number of spots on the surface of each droplet (Fig. 1c).

Our failure to locate the catalysome as well as the rather different intracellular localisation of esterase reaction product demonstrated by Deimling's method suggested that the validity of the methods that produced the original cap localisations should be checked. An alternative substrate for the indigogenic demonstration of esterases, 5-bromo-4-chloroindoxyl acetate, has greater affinity for protein which causes it to be retained more strongly at the site of esterase activity⁴. The cytoplasm of fat body cells reacted intensely when this substrate was applied (Fig. 1d), but there was no localisation in lipid or caps. The localisation of esterase reaction products in caps may therefore be an artefact resulting from lipid solubility of the indoxyl intermediate in the indigogenic reaction⁴. An attempt was made to reproduce the artefact on free lipid droplets by mixing them with esterase *in vitro*. Fat bodies were homogenised

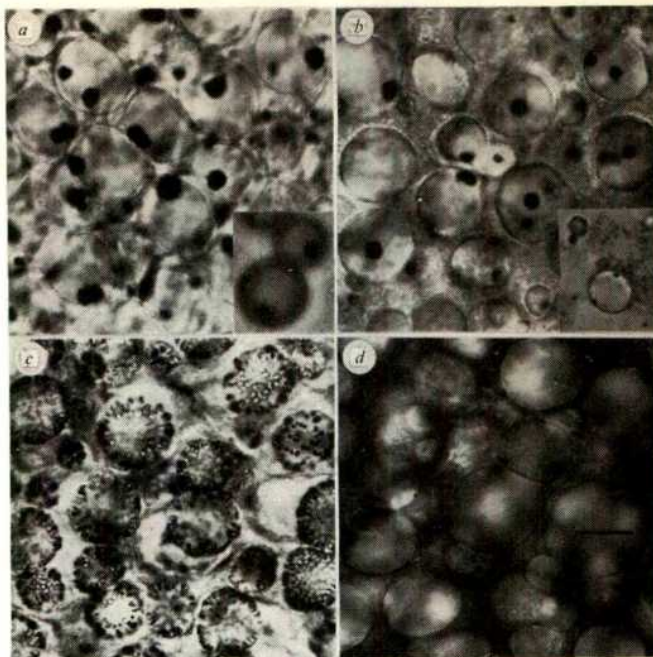


Fig. 1 Distribution of enzyme reaction products in whole mounts of *Calpodes* fat body: Scale 10 μ m. *a*, Caps of indigo on lipid droplets from esterase demonstration with 5-bromoindoxyl acetate. Insert, indigo caps on olive oil droplets. *b*, Caps of diformazan on lipid droplets from NADH diaphorase demonstration using Nitro B.T. Insert, caps of diformazan on olive oil droplets. *c*, Surface spots of reaction product on lipid droplets from esterase demonstration by Deimling's method. *d*, Cytoplasmic indigo precipitate with no lipid association from esterase demonstration with 5-bromo-4-chloroindoxyl acetate.

in buffer and the homogenate centrifuged at 70,000g for 1 h to produce a discrete fat layer above a supernatant and pellet. The supernatant and pellet were taken from below the fat layer and added to the indigogenic reaction mixture (substrate 5-bromoindoxyl acetate) together with a small amount of olive oil which was dispersed by stirring. After 1 h the numerous lipid droplets less than 14 μ m in diameter all carried one indigo cap similar in appearance to those produced in intact fat body (Fig. 1a, insert). No indigo precipitates were obtained in a control mixture lacking esterase. Repetition of the experiment adding only the supernatant to the indigogenic reaction mixture also produced single caps on droplets.

Since esterase localisation in caps seems to be an artefact, the similar localisation of various dehydrogenases must also be questioned. Eight dehydrogenases have been reported to be localised in the caps on lipid droplets¹². But since the final reaction in all of these histochemical tests involves a common enzyme 'Nitro B.T. reductase' the existence of an artefact common to all reaction mixtures can be decided using a mixture for a single dehydrogenase. We chose to investigate the most active, NADH diaphorase. A homogenate of *Calpodes* fat body was prepared as described previously and two fractions taken from it, the supernatant and a mixed supernatant and pellet. These were added to the NADH diaphorase reaction mixture. After 1 h caps of formazan had formed on many lipid droplets in the mixture containing pellet and supernatant (Fig. 1b insert). In contrast to the indigogenic result two or more caps per droplet was the most common situation which suggests a difference from the localisation reported in tissue. In our experiments, however, incubations of live fat body to demonstrate NADH diaphorase generally resulted in a small number of caps per lipid droplet (Fig. 1b). No caps were produced on lipid droplets in the supernatant fraction, a predictable result since NADH diaphorase is considered to be mitochondrial. These experi-



Fig. 2 Reaction product (arrowed) localised in mitochondria and lipid droplets in *Calpodes* fat body from esterase demonstration by Deimling's method. Scale 1 μ m.

ments provide strong evidence that the cap localisation of dehydrogenases is an artefact of Nitro B.T. formazan's attraction for lipid-water interfaces. This attraction has been observed in histochemical practice⁶ and has been demonstrated recently *in vitro*¹⁰.

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¹ Deimling, O. v., and Madreither, H., *Histochemie*, **29**, 83-96 (1972).

² Deimling, O. v., and Madreither, H., *Histochemie*, **29**, 83-96 (1972).

³ Holt, S. J., *Proc. R. Soc.*, **B142**, 160-169 (1954).

⁴ Holt, S. J., and Withers, R. F. J., *Proc. R. Soc.*, **B148**, 520-532 (1958).

⁵ Locke, M., *Tissue Cell*, **2**, 197-223 (1970).

⁶ Novikoff, A. B., Shin, W.-Y., and Drucker, J., *J. biophys. biochem. Cytol.*, **9**, 47-61 (1961).

⁷ Pearce, A. G. E., *Histochemistry, Theoretical and Applied*, **2** (Churchill Livingstone, London, 1972).

⁸ Subramoniam, T., *Acta histochem.*, **47**, 250-253 (1973).

⁹ Thomsen, E., *Z. Zellforsch.*, **75**, 281-300 (1966).

¹⁰ Warchol, von J. B., and Marzotko, D., *Acta histochem.*, **42**, 378-380 (1972).

¹¹ Wigglesworth, V. B., *Q. Jl microsc. Sci.*, **99**, 441-450 (1958).

¹² Wigglesworth, V. B., *Nature*, **210**, 759 (1966).

¹³ Wigglesworth, V. B., *The Principles of Insect Physiology*, seventh ed. (Chapman and Hall, London, 1972).

Genetic response to environmental heterogeneity

ONE of the major problems of contemporary population genetics is how to account for the large amount of genetic variation occurring in natural populations. Considerable controversy

exists between those people proposing that the variation is adaptively neutral, and those arguing that most of the variation is maintained by balancing selection. One of the processes of balancing selection postulated, is based on the idea that different genetic variants are favoured in different environmental niches. We report results from an experiment designed to test this hypothesis in which populations of *Drosophila* were exposed to experimental environments of various degrees of heterogeneity. The rationale is simple—if genetic variation is adaptively neutral, all populations should maintain about the same amount of genetic variation; if the 'environmental heterogeneity' hypothesis is correct, the degree of genetic variation in a population should be correlated with the degree of heterogeneity of its environment.

Eighteen population cages were started on August 20, 1972, each consisting of 423 *Drosophila pseudoobscura* that were F2 and F3 progenies of gravid females collected at McDonald Ranch, Napa County, California during May and June 1972. Two females and one male were introduced into each cage from each of 141 single female lines. The cages were similar to those described by Ayala¹ but each contained ten food cups. Four environmental factors were varied in the experiments: food, yeast, temperature and illumination (see Table 2 for the environmental treatments used). In four populations all four environmental factors were fixed for any one population. In the other 14 populations, one or more environmental factors were heterogeneous; that is, these populations were exposed to two kinds of food, and/or two kinds of yeast, and so on. The number of environmental factors varied were: one in four populations, two in five populations, three in two populations, and four in three populations. The cages were maintained for 1 yr (12-15 generations) at which time adult females were collected and assayed for genetic variability using the techniques of starch-gel electrophoresis². Twenty polymorphic enzyme loci were studied in all populations except for three cages which we had sampled for only 15 loci before they were destroyed accidentally. Forty-four individuals or 88 genomes from each population were assayed for each enzyme. All cages were assayed within 3 weeks.

Table 1 summarises the results. For each group of populations we calculated the average (over populations) of heterozygous individuals observed at each locus. There is a tendency for higher levels of heterozygosity in populations exposed to greater environmental heterogeneity. The heterozygosity among the groups of populations is significantly heterogeneous at only two loci, *Ald* and *Est-5*.

Table 1 also shows the average heterozygosity over the 20 loci studied for each group of populations. The average heterozygosity gradually increases as the number of heterogeneous environmental factors increases from zero to three, but it decreases from three to four. Given the large standard errors, heterozygosities would not be expected to reflect precisely the environmental effects. Yet, the overall trend towards increased heterozygosity with increased environmental heterogeneity seems well established; the average heterozygosities are significantly heterogeneous. An analysis of variance based on the locus heterozygosities for all cages shows that treatment (number of environmental factors heterogeneous) has a significant effect ($P \approx 0.01$). The heterozygosity values for each population have also been calculated using only the 15 loci assayed in all populations. The results do not differ in any significant way from those reported in Table 1.

The average proportion of heterozygotes per locus in the natural population from which the experimental flies were descended is 0.212 ± 0.041 for the 20 polymorphic loci studied. This statistic averaged over all 18 experimental populations is 0.184 ± 0.006 . In the experimental populations the amount of genetic variation has decreased somewhat on the average, although for some groups of populations the average heterozygosity is ostensibly very similar to that of the natural populations.

Table 2 gives for each environmental factor the heterozy-

Table 1 Proportion of heterozygous loci per individual in 18 experimental populations of *Drosophila pseudoobscura*

Loci	No. of heterogeneous environmental factors				
	0	1	2	3	4
<i>Acph-1</i>	0.080 ± 0.072	0.062 ± 0.011	0.158 ± 0.034	0.112 ± 0.021	0.092 ± 0.023
<i>Acph-2</i>	0.153 ± 0.022	0.170 ± 0.054	0.134 ± 0.020	0.136 ± 0.023	0.151 ± 0.059
<i>Ald</i> †	0.165 ± 0.022	0.098 ± 0.008	0.170 ± 0.019	0.295†	0.242 ± 0.033
<i>Aph-1</i>	0.193 ± 0.034	0.227 ± 0.031	0.273 ± 0.040	0.273 ± 0.000	0.235 ± 0.072
<i>Aph-2</i>	0.107 ± 0.019	0.119 ± 0.023	0.145 ± 0.026	0.193 ± 0.011	0.091 ± 0.023
<i>Est-4</i>	0.040 ± 0.019	0.074 ± 0.014	0.050 ± 0.018	0.125 ± 0.057	0.023 ± 0.013
<i>Est-5</i> §	0.341 ± 0.089	0.528 ± 0.035	0.591 ± 0.047	0.528 ± 0.063	0.568 ± 0.035
<i>G3pdh</i>	0.080 ± 0.015	0.091 ± 0.052	0.148 ± 0.022	0.136†	0.114 ± 0.023
<i>Hk-1</i>	0.074 ± 0.044	0.102 ± 0.035	0.052 ± 0.021	0.068 ± 0.000	0.013 ± 0.038
<i>Hk-4</i>	0.040 ± 0.017	0.098 ± 0.008	0.091 ± 0.023	0.023 ± 0.023	0.045 ± 0.026
<i>Idh</i>	0.000 ± 0.000	0.000 ± 0.000	0.014 ± 0.009	0.011 ± 0.011	0.000 ± 0.000
<i>Lap</i>	0.146 ± 0.058	0.250 ± 0.093	0.250 ± 0.025	0.223 ± 0.073	0.280 ± 0.059
<i>Me-1</i>	0.222 ± 0.049	0.290 ± 0.043	0.336 ± 0.032	0.384 ± 0.021	0.364 ± 0.013
<i>Me-2</i>	0.165 ± 0.023	0.176 ± 0.025	0.186 ± 0.023	0.159 ± 0.000	0.136 ± 0.013
<i>Mdh</i>	0.091 ± 0.076	0.061 ± 0.061	0.195 ± 0.077	0.000 ± 0.000	0.030 ± 0.030
<i>Odh</i>	0.136 ± 0.044	0.091 ± 0.023	0.068 ± 0.054	0.227†	0.068 ± 0.039
<i>Pgm</i>	0.318 ± 0.132	0.398 ± 0.031	0.472 ± 0.091	0.750 ± 0.114	0.470 ± 0.027
<i>Tpi-1</i>	0.017 ± 0.017	0.011 ± 0.011	0.018 ± 0.018	0.000 ± 0.000	0.000 ± 0.000
<i>Tpi-2</i>	0.023 ± 0.009	0.228 ± 0.134	0.045 ± 0.034	0.023 ± 0.023	0.038 ± 0.027
<i>Xdh</i>	0.523 ± 0.091	0.652 ± 0.053	0.631 ± 0.057	0.500†	0.689 ± 0.087
Average*					
20 loci	0.146 ± 0.029	0.186 ± 0.038	0.201 ± 0.040	0.208 ± 0.045	0.186 ± 0.044
17 loci	0.159 ± 0.032	0.193 ± 0.044	0.217 ± 0.046	0.224 ± 0.052	0.204 ± 0.051

* Averages have been calculated for all 20 loci sampled, as well as separately for 17 loci not located on the third chromosome. Analyses of variance using the interaction term as error show significant heterogeneity among the cages with different numbers of heterogeneous factors, $P \leq 0.01$.

† Only one cage sampled.

‡ Analysis of variance shows significant heterogeneity among the cages with different numbers of heterogeneous factors (classes 3 and 4 have been combined), $P < 0.01$.

§ $P < 0.005$.

gosities in populations exposed to each of two environments or to both. The values in Table 2 were obtained by averaging the average heterozygosities of the appropriate experimental populations. For every factor, populations exposed to heterogeneous environments have, on the average, greater amounts of genetic variation than populations exposed to uniform environments. The differences are highly significant for temperature and yeast, less so for food, and are statistically insignificant for light.

The idea that genetic polymorphism can be maintained by selection favouring two or more phenotypes in the same population is not new^{3,4}. Indeed, mathematical treatments of the subject have existed for 20 yr (refs 5 and 8). In spite of this early theoretical interest, field and laboratory data in support of multiple-niche polymorphism are far from extensive. The early work of Cain and Sheppard⁹ gave some indication that shell colour in *Cepaea* was related to the variety of habitats in which it was found. Likewise Da Cunha *et al.*¹⁰ presented evidence for a positive correlation between the frequency of gene arrangements in *D. willistoni* and the degree of habitat diversity. Laboratory experiments to demonstrate that polymorphism can be maintained by diversifying selection were carried out by Thoday and Boam¹¹ and Clarke and Sheppard¹² on the level of quantitative characters in *D. melanogaster* and *P. dardanus* respectively, and by Beardmore and his colleagues¹³ on the *esterase-6* locus in *D. melanogaster*. More recently Powell¹⁴ has reported the results of an experiment carried out in population cages with *D. willistoni*. Having an experimental design similar

to our own, Powell surveyed 13 populations for 22 enzyme loci and found that the average heterozygosity per individual was higher in populations maintained in heterogeneous environments than in populations in more constant environments. Since *D. willistoni* flies are polymorphic for multiple chromosomal inversions, however, it has been argued that the inversion polymorphisms and not the single loci may have been the direct targets of balancing selection in Powell's experiment¹⁵. This ambiguity does not arise in our experiment. The strains of *D. pseudoobscura* used to initiate our populations carry inversions only in the third chromosomes. Seventeen of the loci assayed in our study are located in chromosomes other than the third. The heterozygosities calculated using only these 17 loci are given in Table 1. Clearly, populations exposed to heterogeneous environments maintained more genetic variation than populations living in uniform environments. The parameters given in Table 2 have also been calculated for the 17 loci known to be in chromosomes other than the third. The results do not differ in any significant way from those obtained when all 20 loci are used.

Since our experiment was designed to test a model of selection we have taken care to minimise the role of random drift. Drift could have been a significant factor on the population heterozygosities if the population size were small at the start or at any time during the experiment. Each cage was started with 846 genomes (2×423 flies) descended from 282 wild flies (141 females and their mates); the number of flies in a cage was never smaller than 500.

In conclusion, our results indicate an overall positive correlation between environmental diversity and genetic polymorphism, and therefore support the notion that genetic variation is maintained in natural populations by selection.

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¹ Ayala, F. J., *Drosophila Information Serv.*, **43**, 185 (1968).

Table 2 Average heterozygosities for populations exposed to a given treatment

Factor	1	2	1/2	<i>t</i> *
Food	0.181 ± 0.021	0.173 ± 0.014	0.192 ± 0.004	1.90†
Yeast	0.176 ± 0.010	0.161 ± 0.012	0.194 ± 0.004	2.23‡
Temperature	0.168 ± 0.012	0.176 ± 0.012	0.207 ± 0.006	4.73§
Light	0.168 ± 0.012	0.192 ± 0.011	0.193 ± 0.007	1.38

1, Spassky's food, baker's yeast, 16° C, dark; 2, cornmeal medium, brewer's yeast, 25° C, light; 1/2, both 1 and 2 present.

* One-sided Student's *t* test calculated on the assumption of same variance for all three groups of populations within a given treatment.

† $P < 0.050$.

‡ $P < 0.025$.

§ $P < 0.005$.

- ² Ayala, F. J., Powell, J. R., Tracey, M. L., Mourão, C. A., and Pérez-Salas, S., *Genetics* 70, 113-139 (1972).
³ Dobzhansky, Th., *Genetics and the origin of species* (Columbia University Press, New York, 1951).
⁴ Mather, K., *Evolution* 9, 52-61 (1955).
⁵ Ludwig, W., *Zool. Anzeiger* (Neue Ergebnisse und Probleme der Zoologie) 145, 516-537 (1950).
⁶ Levene, H., *Am. Naturalist*, 87, 331-333 (1953).
⁷ Li, C. C., *Am. Naturalist*, 89, 281-296 (1955).
⁸ Dempster, E. R., *Symp. quant. Biol.*, 20, 25-32 (1955).
⁹ Cain, A. J., and Sheppard, P. M., *Am. Naturalist*, 88, 321-326 (1954).
¹⁰ Da Cunha, A. B., Burla, B. H., and Dobzhansky, Th., *Evolution*, 4, 212-235 (1950).
¹¹ Thoday, J. M., and Boam, T. B., *Heredity*, 13, 205-218 (1959).
¹² Clarke, C. A., and Sheppard, P. M., *Heredity*, 14, 163-173 (1960).
¹³ Beardmore, J., in *Essays in honor of Theodosius Dobzhansky*, 299-313 (Appleton-Century-Crofts, New York, 1970).
¹⁴ Powell, J. R., *Science*, 174, 1035-1036 (1971).
¹⁵ King, J. L., *Science*, 176, 545 (1972).

Inferred slow inward current in snail neurones

A NUMBER of excitable membranes exhibit a long-lasting depression of the delayed outward current during a depolarising pulse. One explanation is that there is a true inactivation of potassium gates¹⁻³, and another is that it is due to an accumulation of potassium ions in a limited volume extracellular space, reducing the electric driving force for potassium ions^{3,4}. We have examined the relationship between the depression of net outward current and concomitant changes in potassium ion activity recorded with a potassium-sensitive microelectrode positioned close to the surface of monopolar ganglion cells of the snail *Helix pomatia*.

Experiments were performed on two bursting pacemaker neurones in the right parietal ganglion⁵. One of these produces no fast outward current⁶⁻⁹ and is therefore useful as a control for possible artefacts due to activation or inactivation of an additional outward current. Electrodes of 2-20 M Ω , filled with 3 M KCl, were inserted into exposed cell bodies. The preparation was bathed in Ringer's solution¹⁰ with 5 mM HEPES (N-2-hydroxyethyl-piperazine-2-ethane sulphonic acid; Servo, Heidelberg) buffer in place of Tris at a temperature of 13°C-15°C. The electrochemical activity of K⁺ was monitored at distances below 50 μ m from the visible cell surface with a double-barrelled potassium sensing electrode. One barrel contained the K⁺ selective resin while the other barrel, containing 0.15 M NaCl, acted as a reference for the pair¹¹. The resolution time constant for transients in K⁺ activity was below 20 ms. Membrane current during voltage clamping of the cell was measured with glass pipettes from a limited area of the soma surface (50-80 μ m in diameter) by the patch clamp technique¹². This avoided current transients from unclamped regions of the neurone. All signals were stored on tape and averaged by a Didac computer. Net charge transfer across the membrane (integral of the current signal) was compared with net K⁺ flux (time integral of the K⁺-activity signal).

The major features characterising depression of delayed outward current can be seen in Fig. 1a. Long depolarising pulses produced a delayed outward current which gradually decayed to about 60% or less of its maximum intensity with a time constant of 300 to 700 ms (refs 1, 7). The presentation of paired short pulses of equal amplitude and duration demonstrated that long term depression of the slow outward current persisted even though the voltage was returned to the holding level between a conditioning pulse (pulse 1) and a test pulse (pulse 2). For a time interval of up to 1 s, the delayed current produced by the second pulse sometimes even failed to reach the level of the current produced at the same point in time by the prolonged first pulse. Recovery from depression of the pulse 2 outward current occurred with a time constant of up to 10 s. The length of pulse 1 necessary to depress the test pulse current corresponded with the rise of the pulse 1 outward current. Prolongation of pulse 1 beyond its peak did not produce much further depression

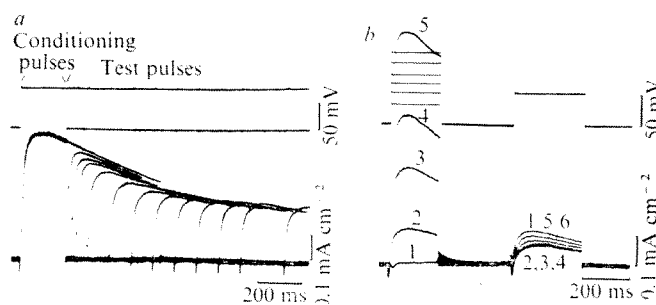


Fig. 1 *a*, Superimposed series of a conditioning, test pulse experiment. Voltage programme, at the top (holding potential -58 mV), current traces below. Pulse 1 is held at a fixed position while pulse 2 is delivered with various delays to show the envelop of the outward current depression. A single pulse of equal voltage but longer duration (650 ms) is also superimposed. *b*, Depression of pulse 2 current by increasing amplitude of pulse 1. Same cell and holding potential as in *a*. Small numbers identify corresponding sequences. Pulse 2 current slopes numbered 5 and 6 rise from considerably higher instantaneous currents as an aftereffect of the large pulse 1 currents. The pulse 1 current during the 120 mV step⁹ is not displayed.

of the pulse 2 current¹. This, together with the failure of sub-threshold conditioning pulses to produce the phenomenon, suggests that the activation of the current gates during pulse 1 may be causally related to the time-dependent depression of the pulse 2 outward current. Increasing pulse 1 further reduced pulse 2 currents, but only slightly (Fig. 1b). The maximum effect was reached at positive membrane voltages of pulse 1 (+40 to +100 mV).

The kinetics of closing of the activated gates at the termination of pulse 1 can be determined from the instantaneous current steps which appeared at the onset of subsequent pulses 2 at short intervals (Fig. 1a). The depression of pulse 2 current was much longer-lasting and must therefore have arisen by a different mechanism. In bursting pacemaker cells, the rising phase of pulse 2 outward currents is always considerably slower than that of pulse 1 (Fig. 2a, b), which may contribute to the failure of pulse 2 currents to reach the current level during maintained voltage steps (Fig. 1a). Although the pulse 2 outward current increased up to 30% if hyperpolarisation up to -150 mV preceded pulse 2, the retardation of the current remained and became more pronounced during the early part of the outward current trajectory.

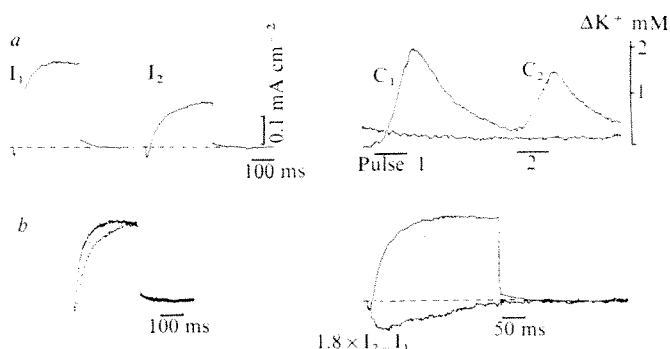


Fig. 2 *a*, Left, averaged pulse 1 and pulse 2 currents (I) ($n=8$) of a cell A during voltage steps of +45 mV from holding potential of -42 mV. The interpulse pause was 1 s. Right, simultaneously recorded extracellular K⁺ signals (C) in a continuous display. The tail K⁺ signal is superimposed. The prolonged slope from a sample of single pulses ($n=8$) at the same electrode position is marked by dots. Calibration of K⁺ signals is made with Ringer solutions with known K⁺ molarities^{1,11}. Note that current (left) and K⁺ signal (right) are displayed at different time scales. *b*, Left, currents normalised to same peak amplitude to show retardation in rising phase of pulse 2 current (digitalised display). Right, after normalisation, pulse 1 current subtracted from (enlarged) pulse 2 current; inward current downwards. Pulse 1 current is superimposed.

The long-lasting depression of outward current provided a convenient situation for comparing the depression of net outward current during pulse 2 with the concomitant reduction in K^+ accumulation at the outer neuronal surface using the K^+ -specific microelectrode (Figs 2-4). We standardised the pause between pulses 1 and 2 at 1,000 ms and delivered paired pulses at a repetition rate of once every 30 s to allow time for complete recovery from depression. The ratio $Q(2:1)$ was obtained by dividing the time integral of net outward current during pulse 2 by the equivalent integral during pulse 1 and was found to be 0.51 ± 0.12 (s.d.).

Since the outward current ceases almost immediately on termination of the pulse, accumulated extracellular K^+ (ΔK^+) should dissipate and its concentration fall progressively from the relatively early peak after the pulse is terminated. The role of K^+ accumulation was tested directly by measuring the K^+ activity with the electrode very near or just touching the visible cell surface during paired depolarising pulses. Single pulses were also applied to determine the baseline activity for the second pulse. After 1 s, when pulse 2 was initiated, the potassium signal evoked by pulse 1 was less than 15% of its peak value. Nevertheless, the outward current during pulse 2 was always depressed for periods longer than 1 s when the extracellular K^+ activity had approached even closer to pre-stimulus levels. Thus it seems unlikely that the depression of the pulse 2 net outward current is primarily due to extracellular accumulation of potassium ions.

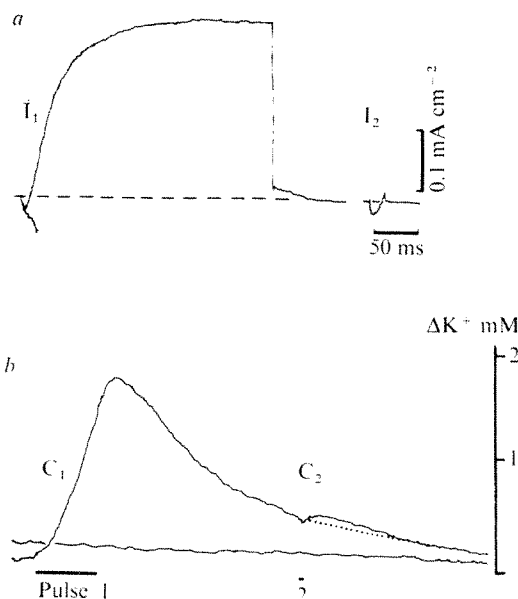


Fig. 3 Same experiment ($n=12$) and voltages as in Fig. 2, with pulse 2 of much shorter duration (20 ms). *a*, Current signal; *b*, K^+ activity signal. There is an obvious K^+ signal following pulse 2, concomitant with a predominantly inward going current during that pulse. Ratio of outward currents ($Q(2:1)$) is not proportional to the ratio of K^+ signals ($FK(2:1)$). Current and K^+ activity traces displayed at different time scales.

If it is assumed that the net outward current recorded under voltage clamp conditions is a pure potassium current, it follows that potassium activity changes recorded near the cell surface should parallel quantitatively the net charge transfer produced by the outward current. That is, the ratio $Q(2:1)$ should equal the ratio $FK(2:1)$ (K flux during pulse 2/ K flux during pulse 1) for any pair of pulses. From Figs 2 and 4 it is evident that $FK(2:1) < 1$; that is, K^+ efflux is reduced, as would be expected if the K channels are partially inactivated during pulse 2. Quantitative comparison, however, indicates that there is a 'deficit' in the net outward current of pulse 2 relative to the K^+ signal. In Fig. 2*a* this is obvious from the peak of the K^+ signal of pulse 2 which is more than half of the K^+ peak of pulse 1, whereas the ratio of the amplitudes of currents 2 and 1 is less than 0.5. This deficit ($FK(2:1) - Q(2:1)$) ranged from 0.10 to

0.25 in various units studied. The deficit in net current is most readily explained as the result of an inward current which makes its appearance during pulse 2 and shortcircuits a portion of the K^+ efflux. Such an inward current would result in a net charge transfer during pulse 2 lower than that transferred by K^+ efflux, and thus explain the deficit of net outward current recorded during pulse 2 relative to the K^+ signal.

The presence of a previously unrecognised slow inward current receives further support from a comparison of the kinetics of the outward currents of pulses 1 and 2 (Fig. 2*a, b*). The trajectory of the outward current characteristically has a slower rising phase during pulse 2 than during pulse 1 when the currents were normalised. (Normalisation was carried out by three methods. In the first, the pulse 2 current was simply additionally amplified to bring the current peaks of the two pulses to the same amplitude. In the second, the exponential falling phases of the current pulses were extrapolated back to the beginnings of the pulses and the pulse 2 current was then additionally amplified, so that the extrapolated decays began from the same amplitude. In the third method of normalisation the averaged pulse 1 current trajectory was reduced in proportion to the ratio $FK(1:2)$ and then subtracted from the averaged trajectory of pulse 2. Deficit currents determined by those three methods differed only slightly.) Comparison of current traces in Fig. 2*a* and 2*b* shows that the 'slow inward current' obtained by subtracting normalised pulses 1 from pulses 2 has a far slower time course than the fast inward current.

The difference in the trajectory of the outward current of pulses 1 and 2 was displayed by electronically subtracting, after normalisation, the averaged trajectories of pulse 1 currents from those of the enlarged pulse 2 currents from the same neurone (Fig. 2*b*). The result is a trajectory with a peak 20 to 50 ms after the onset of the current pulse, that is, during the rising phase of the outward current of pulse 1. The time integral of this deficit in pulse 2 current in the different cells was -0.08 to -0.21 times that of the corresponding pulse 1 currents and was always found comparable with the deficit in net outward current obtained by determining $FK(2:1) - Q(2:1)$.

We conclude that the long-term suppression of net outward current by a conditioning pulse consists of two components. The chief component is a true time and voltage-dependent inactivation of the delayed potassium system, similar in some respects to the inactivation of the sodium system described by Hodgkin and Huxley¹³. It is also manifested as a reduction in pulse 2 extracellular potassium accumulation relative to accumulation during pulse 1. Since it develops during interpulse periods with pulse durations approximating the duration of the action potential (Fig. 4), the inactivation contributing to the depression of outward current described here seems more likely to have functional importance under physiological conditions than any reduction in outward current which may be produced by the extracellular accumulation of K^+ with prolonged depolarising pulses⁴. The second component contributing to depression of delayed outward current during pulse 2 seems to be a previously unrecognised inward current which partially shortcircuits the outward potassium current. Its existence is supported by several independent lines of evidence: first, the reduction in K^+ efflux during pulse 2, resulting from a true K^+ inactivation, is smaller than the reduction in net outward current (Fig. 2*a*); second, at any given time after pulse 1 (up to 1 or 2 s) the peak outward current produced by pulse 2 does not reach the level of outward current produced at that time by the prolongation of pulse 1 (Fig. 1*a*). In addition, the rising phase of pulse 2 exhibits slower kinetics than that of pulse 1, consistent with a superimposed delayed inward current (Fig. 2*b*).

Certain molluscan neurones have a fast outward potassium current which is activated by conditioning hyperpolarisation and inactivated by depolarisation⁶⁻⁹. Inactivation of a fast current might produce the observed slowing of the pulse 2 outward current. This source of artefact can, however, be ruled out for several reasons, the most important of which is the use of bursting pacemaker cells (Figs 1-3) which fail to show a fast out-

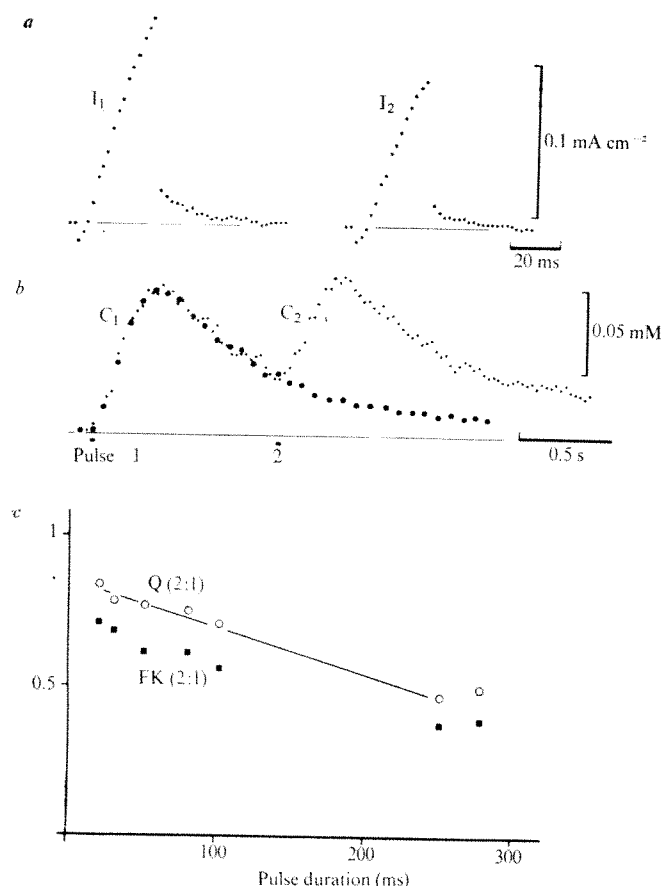


Fig. 4 *a*, Currents 1 and 2 evoked by +65 mV steps each of 30 ms duration from a holding potential of -45 mV. Simultaneous sampling of K^+ signals is shown in *b* ($n=25$). The superimposed averaged trajectory of single pulses is given by heavier dots. *c*, Ratios of pulse 2 to pulse 1 outward current integrals ($Q(2:1)$) and corresponding ratios of potassium fluxes ($FK(2:1)$) of this cell plotted as a function of pulse duration. Duration of pulse 1 and 2 was kept equal and was varied in unison. At shorter pulse durations the difference between K^+ flux and charge transfer ($FK(2:1) - Q(2:1)$) becomes smaller, but does not disappear.

ward current even if given a strong hyperpolarising conditioning pulse. Also, the deficit of pulse 2 outward current relative to measured K^+ accumulation cannot be explained by the inactivation of a fast outward current. In cells with a fast outward current this deficit was seen whether or not the fast outward current was activated.

The delayed inward current is difficult to identify and measure during pulse 1; its appearance or augmentation during pulse 2 has been demonstrated by using the pulse 1 current as a control for the pulse 2 current. It is nevertheless interesting that the activation of this inward current, unlike the other membrane current components, is facilitated by a preceding depolarisation. The two components of delayed outward current depression, true inactivation of the K^+ system and activation of a slow inward current, presumably both contribute to the progressive increase in spike duration, overshoot and positive shift in post-spike undershoot, which occur during the initial half dozen or so spikes of the train comprising a 'burst'. The ionic identity of the slow inward current and its possible role in neuronal bursting remain to be ascertained.

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- ¹ Lux, H. D., and Neher, E., *Proc. fourth Int. Congr. Biophys.*, **3**, 222-223 (1972).
- ² Gola, M., *Pflügers Arch., ges. Physiol.*, **346**, 121-140 (1974).
- ³ Alving, B. O., *J. gen. Physiol.*, **54**, 512-531 (1969).
- ⁴ Eaton, D. C., *J. Physiol., Lond.*, **224**, 421-440 (1972).
- ⁵ Kerkut, G. A., and Meech, R. W., *Comp. Biochem. Physiol.*, **19**, 819-832 (1966).
- ⁶ Hagiwara, S., and Saito, N., *J. Physiol., Lond.*, **148**, 161-170 (1959).
- ⁷ Neher, E., and Lux, H. D., *Pflügers Arch., ges. Physiol.*, **322**, 35-38 (1971).
- ⁸ Neher, E., *J. gen. Physiol.*, **58**, 36-53 (1971).
- ⁹ Connor, J. A., and Stevens, C. F., *J. Physiol., Lond.*, **213**, 31-53 (1971).
- ¹⁰ Gainet, H., *Brain Res.*, **39**, 369-385 (1972).
- ¹¹ Lux, H. D., and Neher, E., *Expl Brain Res.*, **17**, 190-205 (1973).
- ¹² Neher, E., and Lux, H. D., *Pflügers Arch., ges. Physiol.*, **311**, 272-277 (1969).
- ¹³ Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **117**, 500-544 (1972).

Neuromuscular blocking action of an alkylating local anaesthetic: site of action and effects of temperature and calcium ions

THE mechanism by which local anaesthetics block neuromuscular transmission has been under investigation for many years. Most of the evidence suggests that these drugs act primarily on nerve terminals either to inhibit acetylcholine (ACh) release¹⁻³ or exert some other effects⁴. However, other workers have shown inhibition of ACh receptor^{5,6} or direct depression of muscle excitability⁷⁻¹⁰. Inhibition of nerve conduction apparently has been ruled out as contributing to the blockade in rat phrenic nerve-diaphragm³ or cat soleus nerve-muscle preparations⁴.

In an attempt to determine the primary site of action of these drugs we have used the recently synthesised β -haloethyl amine compound, 2-[(2-chloroethyl) methylamino] ethyl 4-ethoxybenzoate, which irreversibly blocks nerve conduction¹¹ as well as neuromuscular transmission¹². We report here that when phrenic nerve-diaphragm transmission is irreversibly blocked, the compound has essentially no effect on the excitability of nerve or muscle. From this we conclude that the action of the irreversible local anaesthetic in this preparation is confined to the junctional region.

The rat phrenic nerve-diaphragm preparation was set up by conventional means¹³ for stimulation of the muscle either directly or through the nerve (indirect). Figure 1 shows the effect of the compound on contractions elicited by both types of stimulation: the compound blocked indirect contractions with no effect on directly elicited contractions. This occurred whether the drug was in the bath or removed by washout. When the phrenic nerve was removed at the time of irreversible block of indirect contractions, it still maintained excitation and conduction properties. Irreversible block of these electrical responses *in vitro* required a concentration of drug about five times greater than that needed for irreversible block of transmission¹¹.

These findings show that blockade of transmission by the compound is confined to the junction. The physico-chemical properties of the compound probably account for this selective action, unlike procaine which depresses both direct and indirect contractions concomitantly. At the pH of the bath we used (7.4) the compound exists almost exclusively in the aziridinium or positively charged form¹² and thus cannot penetrate well to the active membranes of nerve or muscle. On the other hand, the compound evidently can readily reach the unmyelinated nerve terminal membrane or post-junctional region, where it combines irreversibly with some receptor.

• If the drug was washed out after a fairly short time of contact with the tissue, for example, 10 min, the observed

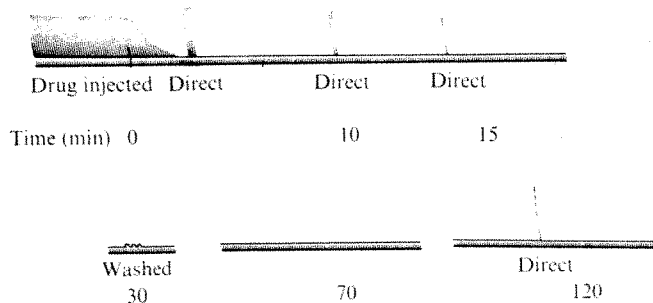


Fig. 1 Neuromuscular blocking action of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate, 0.2 mg ml^{-1} , applied to rat phrenic nerve-diaphragm preparation. Even in the presence of the drug, the directly elicited contraction was unaffected. On the other hand, the indirect twitch was abolished. The irreversible nature of the latter effect is indicated by the complete block even 2 h after washing.

block of contraction was reversed completely, indicating a two step inhibitory mechanism similar to that observed with other receptor alkylating agents¹⁴.

If the tissue was pretreated with decamethonium or *d*-tubocurarine at concentrations sufficient to block transmission completely, application of the compound still resulted in irreversible blockade after washout. This procedure has been shown¹⁵⁻¹⁷ to protect the active site on the ACh receptor against irreversible reaction with α -bungarotoxin. Based on this finding, we think it most likely that a receptor for local anaesthetics exists on the nerve terminal and that it can undergo the same type of alkylation reaction exhibited by receptors for neurohormones.

To obtain thermodynamic data for reaction with the receptor, the temperature dependence of rate of irreversible block of transmission was determined as follows. Before the drug was added, the nerve was stimulated with gradually increasing voltage, from threshold to the maximum for eliciting contraction of the muscle. This gave control voltage-response curves. This procedure was repeated at various times after the drug had been added to the bath and washed out, giving data for the irreversible change in

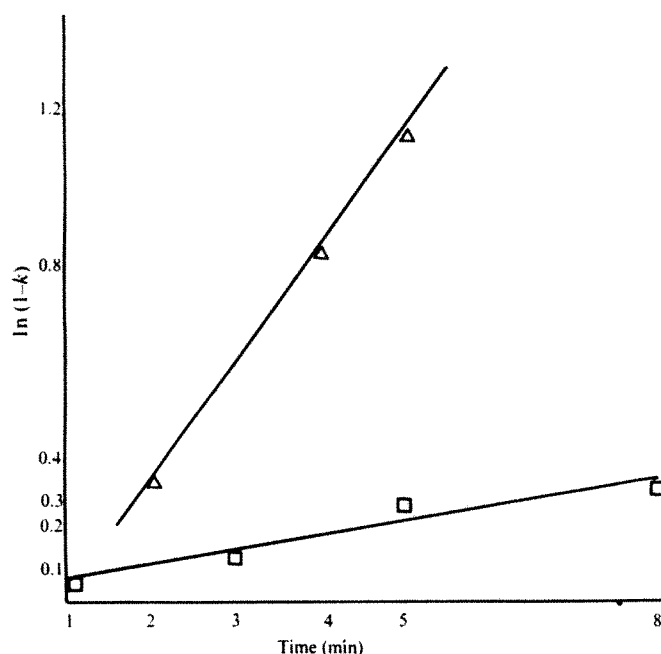


Fig. 2 Semi-log plot of rate of irreversible block of indirectly elicited twitch of the phrenic-nerve diaphragm by 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate. Rate of block was determined from midpoints of the voltage-response curve after washing out the drug (see text for details). □, k at $30^\circ \text{C} = 0.037 \text{ min}^{-1}$; △, k at $15^\circ \text{C} = 0.30 \text{ min}^{-1}$.

sensitivity. The initial effect of the drug was to shift the voltage-response curve to the right without reducing the maximum; with increasing time, the maximum declined and ultimately contractions could not be elicited at any voltage. The midpoints of the curves were plotted as log percentage change in voltage against time; straight lines were obtained (Fig. 2).

It is interesting that the rate constant at 15°C was much greater than at 30°C ; this was not anticipated since alkylation reactions would be expected to show a positive temperature dependence. Other findings indicated that the actual rate of reaction of the drug with the receptor is much lower at the low than the high temperature. If the tissue was incubated with the drug at 15°C to give complete irreversible block and the temperature raised to 30°C , transmission was completely restored (Fig. 3). This indicates that during the time of reaction at 15°C only a small fraction of the receptors had reacted, but this was sufficient to produce the complete block at this temperature. Another possibility is that reversal of block was due to cleavage of the drug-receptor bond at the higher temperature. To test this, the degree of reversal was determined when the temperature was progressively raised from 15° to 30°C and then lowered back to 15° (Fig. 4). The two curves are almost identical and thus the reversal of the block produced by raising the temperature was not due to a change in receptor occupancy. In addition, if the drug was applied at 30°C for a time during which no significant irreversible shift in the voltage-response curve was observed; lowering the temperature to 15°C resulted in inhibition of contractions.

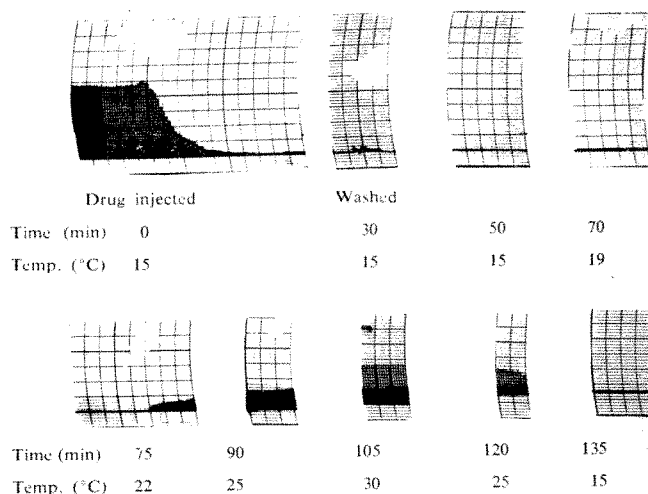


Fig. 3 Effect of increasing temperature of bath after application and washout of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate, 0.2 mg ml^{-1} , for 30 min. The indirect twitch was irreversibly blocked at 15°C and progressive increase in temperature resulted in relief of block. At 30°C the response was almost that of the 30°C control, which usually was somewhat smaller than at 15°C . Reduction of the temperature back to 15°C resulted in return of the block.

These results indicate that the number of receptor acting irreversibly is directly related to temperature as the rate of block is inversely temperature dependent. Thus the rate of receptor alkylation cannot be determined pharmacologically since there appears to be no relationship between absolute number of receptors and the degree of neuromuscular block. This was supported by the fact that although the same number of receptors was occupied by the drug at 15° and 30°C , the block differed. One possible explanation for the block at the lower temperature in spite of the same fraction of receptors occupied is that fewer nerve terminals are involved in transmission at the lower

Hence, occupation of a smaller fraction of nerve terminal receptors would be sufficient to block contractions. In support of this concept, Taylor reported that the output of acetylcholine is about 80% lower at 20°C as compared with 37°C in this preparation¹⁸.

It is well documented that curare-like drugs also show an inverse temperature dependence for potency of neuromuscular blockade^{18,19}. This has been interpreted in terms of a temperature-dependent dissociation of the drugs from the ACh receptor¹⁹ or to temperature dependent solubility

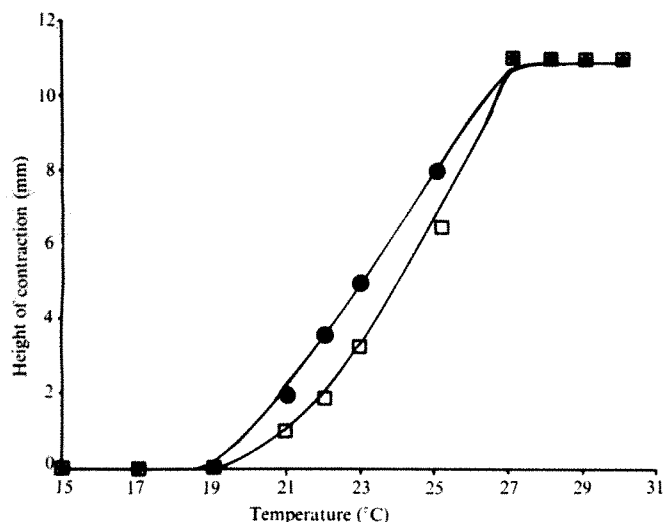


Fig. 4 Effect of progressive increases of temperature from 15°C to 30°C (□) and the subsequent return to 15°C (●) on a preparation blocked irreversibly at 15°C. The degree of irreversible block was not significantly affected by the increase in temperature, and thus the appearance of contractions did not result from dealkylation of receptors.

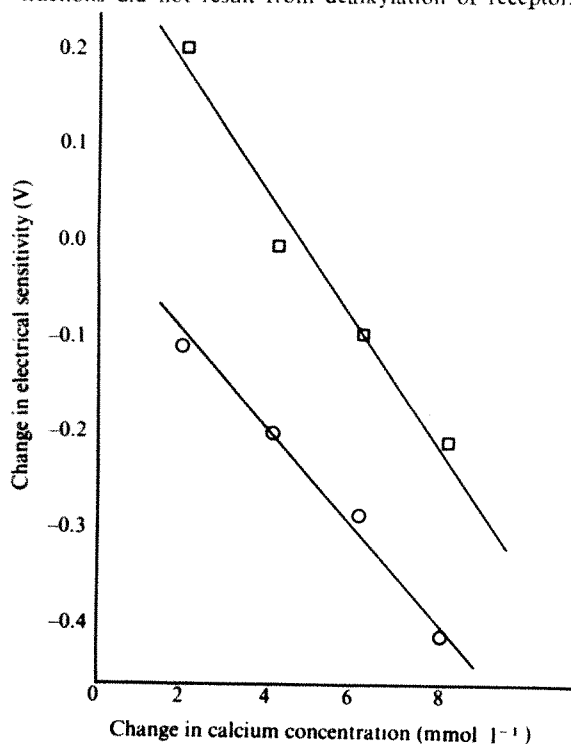


Fig. 5 Effect of increasing calcium ion concentration on change in sensitivity of indirectly elicited contraction by the alkylating local anaesthetic (□) and by procaine (○). Sensitivity (V) is the voltage required to produce half-maximum contraction as determined after applying the drugs for 10 min. An increase in ΔV indicates that the calcium increased the potency of the drug, that is a greater voltage was required to produce half-maximum contraction. A decrease in ΔV signifies antagonism of the drug by calcium.

of the drugs in the post-synaptic membrane²⁰. We have found that blockade of transmission by procaine shows a similar inverse temperature dependence. In view of the results reported here for the irreversibly acting compound, the explanation for temperature dependence of blockade by reversibly acting drugs may have to be revised.

The effects of calcium on the voltage-response curve of the alkylating compound and on procaine are shown in Fig. 5. In this concentration range, calcium had no effect on excitability of the preparation. Calcium ions had a biphasic effect on the irreversible compound, promoting its potency at low concentrations, inhibiting its blocking action at higher concentrations. On the other hand, calcium only inhibited procaine blockade of transmission. The potency of the alkylating compound was also enhanced when the preparation was stimulated tetanically or if the potassium ion concentration was increased. These conditions produce nerve terminal depolarisation. Since the compound was present primarily in the quaternary ammonium form, it would not penetrate well through the nerve terminal membrane. Penetration seems to have been promoted by depolarising conditions as manifested by the enhanced potency observed. These results are in harmony with Narahashi's evidence that the site of action of local anaesthetics is the inner surface of nerve axon membranes²¹. Higher concentrations of calcium ions probably reduce the rate of irreversible reaction of the aziridinium compound with its receptor by a competitive mechanism as observed on various nerve axons for reversible local anaesthetics^{22,23}.

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- ¹ Straughan, D. W., *J. Pharmacol.*, **13**, 49 (1961).
- ² Matthews, E. K., and Quilliam, J. P., *Br. J. Pharmacol.*, **22**, 415 (1964).
- ³ Galindo, A., *J. Pharmac. exp. Ther.*, **177**, 360 (1971).
- ⁴ Usubiaga, J. E., and Standaert, F. G., *J. Pharmac. exp. Ther.*, **159**, 353 (1968).
- ⁵ Del Castillo, J., and Katz, B., *Proc. R. Soc.*, **B146**, 339 (1957).
- ⁶ Maeno, T., *J. Physiol., Lond.*, **183**, 592 (1966).
- ⁷ Hirst, G. D. S., and Wood, D. R., *Br. J. Pharmacol.*, **41**, 105 (1971).
- ⁸ Inoue, F., and Frank, G. B., *J. Pharmac. exp. Ther.*, **136**, 190 (1962).
- ⁹ Lorkovic, H., *Arch. int. Pharmacodyn.*, **146**, 266 (1963).
- ¹⁰ Harris, E. J., and Ochs, S., *J. Physiol., Lond.*, **187**, 5 (1966).
- ¹¹ Rosen, G. M., Ehrenpreis, S., Mittag, T. W., and Stubbins, J. F., *J. med. Chem.*, **14**, 514 (1970).
- ¹² Rosen, G. M., Ehrenpreis, S., and Karoutsou, A., *Arch. int. Pharmacodyn.*, **204**, 242 (1973).
- ¹³ Bulbring, E., *Br. J. Pharmacol.*, **1**, 38 (1946).
- ¹⁴ Nickerson, M., and Gump, W. S., *J. Pharmac. exp. Ther.*, **97**, 25 (1949).
- ¹⁵ Chang, C. C., and Lee, C. Y., *Arch. int. Pharmacodyn.*, **144**, 241 (1963).
- ¹⁶ Changeaux, J.-P., Kasai, M., and Lee, C. Y., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1241 (1970).
- ¹⁷ Miledi, R., and Potter, L. T., *Nature*, **233**, 599 (1971).
- ¹⁸ Taylor, D. B., *Anesthesiology*, **20**, 439 (1959).
- ¹⁹ Lonsdale, K., and Milledge, H. J., *Nature*, **206**, 407 (1965).
- ²⁰ Canepa, F. G., *Nature*, **207**, 1149 (1965).
- ²¹ Narahashi, T., and Frazier, D. T., in *Neurosciences Res.* (edit. by Ehrenpreis, S., and Solnitzy, O. C.), **4**, 66 (Academic Press, New York, 1971).
- ²² Blaustein, M. P., and Goldman, D. E., *J. gen. Physiol.*, **49**, 1043 (1966).
- ²³ Sasa, M., Avner, B. P., and Albuquerque, E. X., *Eur. J. Pharmacol.*, **23**, 97 (1973).

Temperature-sensitive mutation affecting myofilament assembly in *Caenorhabditis elegans*

THE assembly of contractile and calcium-related regulatory proteins into functioning myofilament arrays within muscle cells and the genetic control of muscle differentiation are still largely unknown processes¹⁻³. Here we present evidence that a particular gene may regulate such lattice assembly in the body wall muscle cells of the nematode *Caenorhabditis elegans*. A temperature-sensitive mutation in this gene can prevent the appearance of normal myofilament lattices in this muscle without affecting the amounts of major contractile proteins present. The organised or defective adult structures once formed in mutant muscle are stable to changes in temperature.

The recessive mutation *e286* is induced by ethyl methane-sulphonate on chromosome III of *Caenorhabditis elegans* (strain collection of Dr Sydney Brenner)^{4,5}. This mutagen has proved of great use in producing temperature-sensitive mutations in *Drosophila*⁶. *N₂* (wild type) nematodes are highly motile between 15° and 25° C. The locomotive and reproductive rates of these animals increase over this temperature range. In contrast, when grown above 20° C, *E286* animals are paralysed with only very slow body movements apparent and they reproduce at a diminished rate. At 15° C, the movement and growth of *E286* mutants is indistinguishable from *N₂* populations. At both temperatures, the pharyngeal muscle in mutants behaves normally. *+ / e286* heterozygotes are not affected behaviourally or structurally by growth at either temperature. Thus, one genic dose of normal function is sufficient to overcome the presence of a mutant gene or its product.

If mutant eggs (chitin-encased embryos) are permitted to develop and hatch at 15° C, switching the temperature to 25° C will lead to paralysed larvae and adults. Conversely, mutant embryos hatching at 25° C will develop into motile adults by growth at 15° C. Such reversal of phenotype can be performed throughout the four larval stages of these nematodes. Behavioural reversibility at the *L₁* stage is demonstrated in Table 1. But when the sexually mature adult stage is reached, the phenotype is stable to temperature change within the above range. Maintenance of paralysed adults at 15° C or of motile adults at 25° C for periods up to several days (compare to 3 d generation time) does not alter either body movement, or the characteristic body wall muscle structure of either form (Fig. 1). Motile *E286* adults have body wall muscle cells exhibiting periodic sarcomeric structures such as A bands, I bands, dense bodies, and H zones that are very similar to *N₂* muscle elements as observed with the polarised light microscope. Paralysed *E286* adults do not exhibit these regular structures in their body wall muscle. When the body wall muscle cells of *E286* animals grown at either temperature are examined by electron microscopy, ultrastructural differences between the two populations are observed. The 15° C specimen demonstrates thick filaments encircled by parallel arrays of thin filaments (Fig. 2). The paralysed 25° C animal also possesses recognisable thick filaments. In this case, there does not seem to be any specific orientation of thin filaments. Fibrous structures of dimensions appropriate to thin filaments appear at various angles to the thick filament array.

This mutation seems to be affecting the formation of myofilament arrays during muscle growth periods as evidenced by temperature reversibility of phenotype during larval development. Adult muscle having constructed its myofilament arrays or not is then unaffected by this temperature-sensitive mutant function. Thus, the instability of a permanent component of muscle is probably not responsible for the loss of structure and function at high temperature.

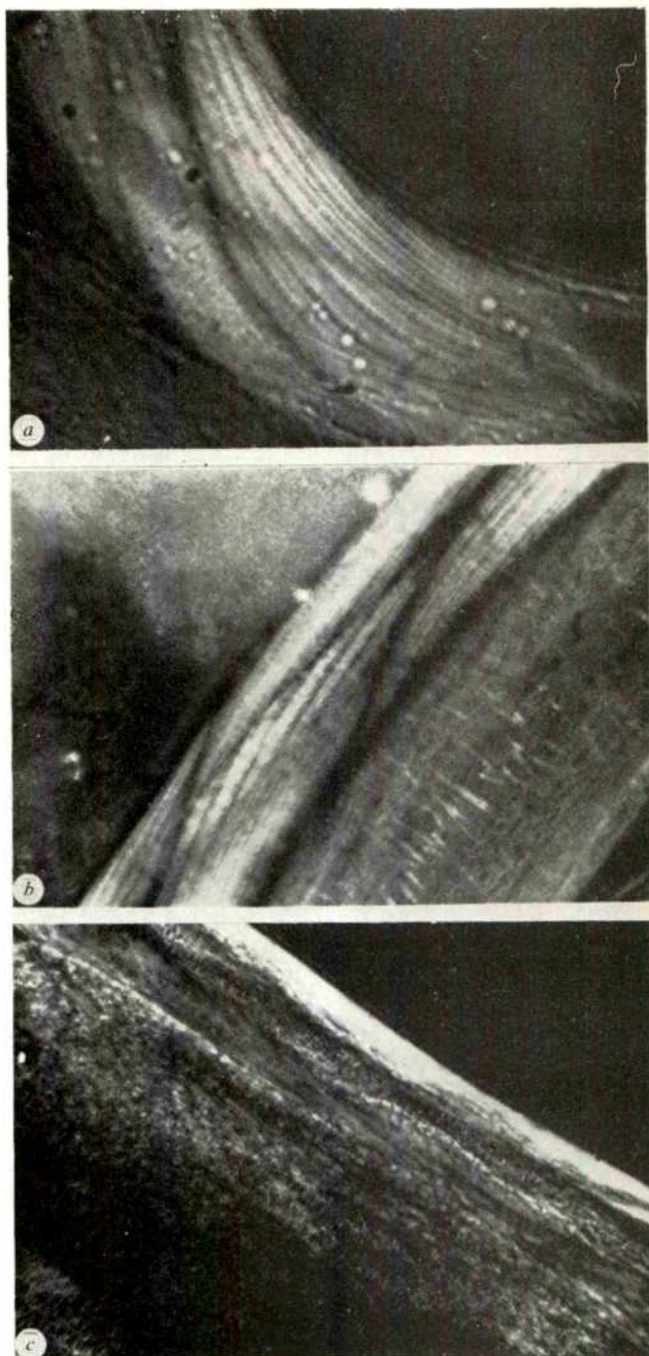


Fig. 1 Living animals were picked off lawns of *Escherichia coli*, OP50, and suspended in a 10 μ l drop of 0.8 Ascaris Ringer's solution (24.5 mM KCl, 11.8 mM CaCl_2 , 9.8 mM MgCl_2 , 3.9 mM NaCl, and 125.1 mM sodium acetate). A No. 1 or No. 1.5 glass coverslip was then placed over the drop. All observations were performed on a Reichert Zetopan research microscope fitted with strain-free objective and condenser. The uncalibrated magnification determined by instrumental and photographic settings was $\times 746$. *a*, *N₂* grown at 25° C; *b*, *E286* grown at 15° C; *c*, *E286* grown at 25° C. All observations were made at an ambient temperature of about 23° C.

The relative migration and amounts of several myofilament proteins of *E286* grown at either temperature and of *N₂* are similar to one another when separated according to molecular weight on sodium dodecyl sulphate-polyacrylamide gel slabs⁷. But a difference in a catalytic or minor structural protein present in low relative concentration or an alteration not affecting molecular weight would not be observed with this method.

Table 1 Reversibility of E286 locomotive behaviour

Temperature, condition* (° C)	No. of animals	Time (s) for 50 beats†
15	20	20.9 ± 1.8
25→15	20	31.2 ± 8.4
25	16	157 ± 74
15→25	16	189 ± 106

*Animals were either grown through adulthood at one temperature or switched at L₄ stage. All observations were of adults.

†One beat is defined as the formation of a semicircle by flexion at the mid-body when the animal is suspended in 0.8 Ascaris Ringer's solution. Note that about two thirds of the 25° C animals did not make any such movements and were not scored. The movements of 25° C and 15°→25° C animals that were motile at all were so slow that less than 50 beats were usually measured and then normalised to 50. Such biases may lead to underestimation of the time and greater variability in the paralysed cases.

What kind of function is altered by *e286*? The most attractive hypothesis is a defect in a catalytic function necessary for proper myofilament arrays to form, consistent with the fully recessive behavioural and morphological phenotype of E286 at 25° C. Mutation in a *cis* acting control element regulating synthesis of a myo-

fibrillar component would also explain the recessive character but would be unlikely to produce temperature sensitivity, whereas alteration of a structural protein which at 25° C prevented assembly might be expected to have a semi-dominant phenotype. Further biochemical and structural studies of E286 and the search for additional mutations within this gene are being pursued for better understanding of this function.

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- Etlinger, J. D., and Fischman, D. A., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 511-522 (1972).
- Yaffe, D., and Dym, H., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 543-547 (1972).
- Holtzer, H., Sanger, J. W., Ishikawa, H., and Strahs, K., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 549-566 (1972).
- Brenner, S., *Br. Med. Bull.*, **29**, 269-271 (1973).
- Brenner, S., *Genetics*, **77**, 71-94 (1974).
- Suzuki, D. T., *Science*, **170**, 695-705 (1970).
- Laemmli, U. K., and Favre, M., *J. molec. Biol.*, **80**, 575-599 (1973).
- Hirumi, H., Ruski, D. J., and Jones, N. O., *J. ultrastruct. Res.*, **34**, 517-543 (1971).
- Epstein, H. F., Waterston, R. H., and Brenner, S., *J. molec. Biol.* (in the press).
- Rosenbluth, J., *J. Cell Biol.*, **25**, 495-515 (1965).

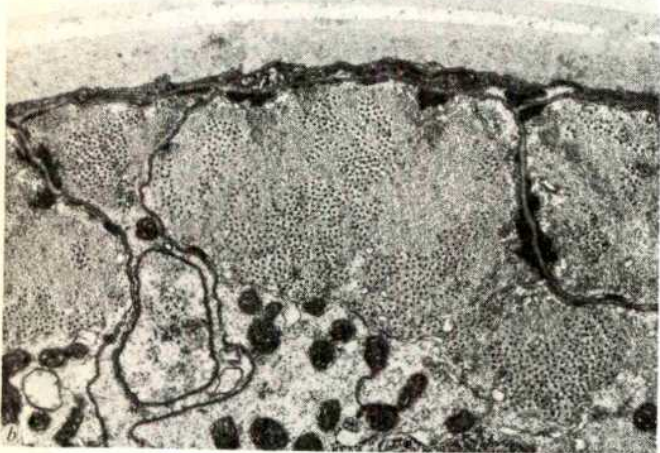
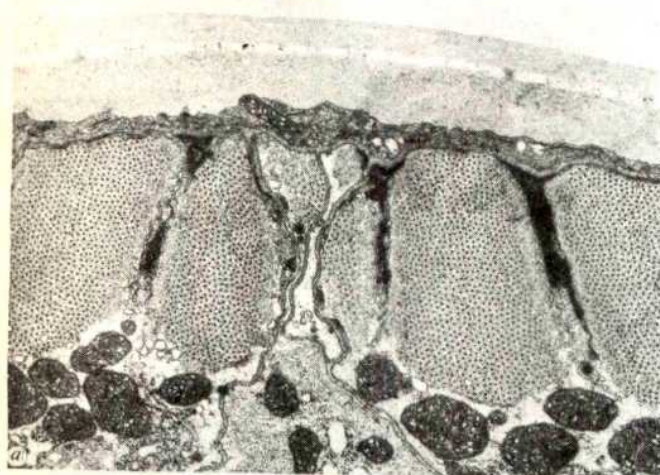


Fig. 2 Nematodes were grown on *E. coli*, OP50, lawns at either temperature. Living adults were cut in half, fixed in cold phosphate-buffered glutaraldehyde, and post-fixed in cold Veronal-buffered osmium tetroxide^{8,9}. Thin sections of body wall muscle cells were photographed on an AEI EM63 electron microscope. The uncalibrated magnification was $\times 14,400$. The dimensions of thick and thin myofilaments are about 2.4 and 0.6 nm, respectively^{8,10}. *a*, E286 at 15° C; *b*, E286 at 25° C.

Genetic complementation after fusion of Tay-Sachs and Sandhoff cells

THE N-acetyl- β -D-glucosaminidase (hexosaminidase) activity in cultured human fibroblasts consists of at least three components (A, B and C)¹⁻³. At least two of these (designated Hex A and Hex B) seem to be closely related, for (1) antiserum against Hex A reacts against Hex B, and *vice versa*^{1,3} (2) in Tay-Sachs disease only Hex A activity is deficient, accompanied by an increase in Hex B activity in certain tissues^{1,6}, and (3) both Hex A and Hex B activities are missing in Sandhoff disease, another autosomal recessive disorder⁷. But in spite of several theories⁸⁻¹¹, the precise relationship between these components remains unknown, as does the nature of the genetic and biochemical relationships between the two diseases. To investigate these questions we have fused Tay-Sachs with Sandhoff fibroblasts and obtained cultures containing heterokaryons which produce a hexosaminidase which is absent from the parent lines. It has the electrophoretic and heat lability characteristics of the Hex A found in normal fibroblasts.

Skin fibroblasts from a patient with Tay-Sachs disease (G.M. 221) and a patient with Sandhoff disease (G.M. 203) were obtained from the Genetic Mutant Repository, Camden, New Jersey, and experiments were carried out with cells at the eighth to eleventh passage. Electrophoretic analysis of extracts of these cells confirmed that the Tay-Sachs fibroblasts lacked Hex A, while the Sandhoff fibroblasts under our experimental conditions has neither Hex A nor Hex B. On rare occasions faint traces of Hex A and

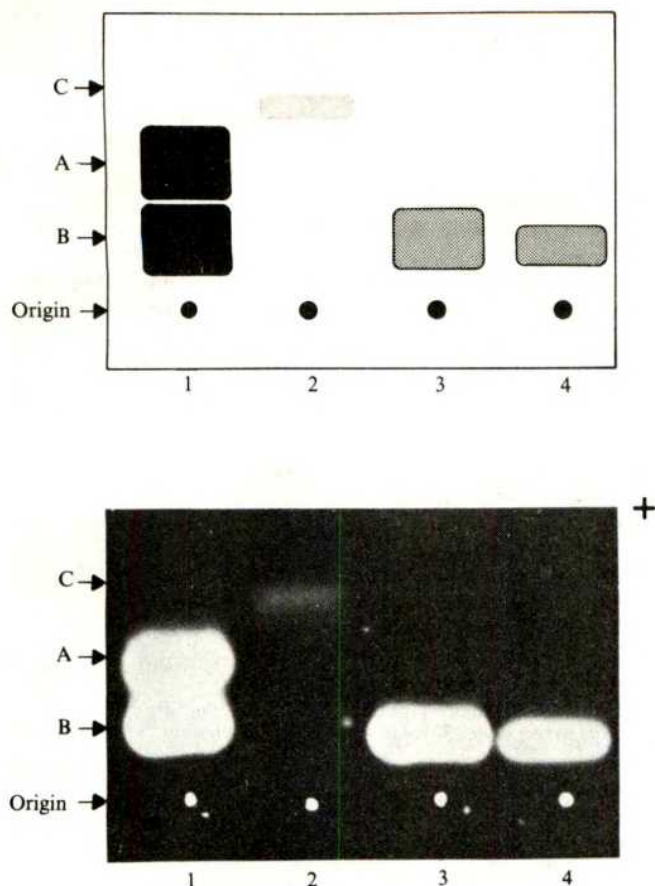


Fig. 1 N-acetyl- β -D-glucosaminidase electrophoresis. (1) Normal control fibroblasts; (2) virus-treated Sandhoff fibroblasts; (3) virus-treated Tay-Sachs cells; and (4) mixture of Sandhoff and Tay-Sachs fibroblasts cultivated together without virus fusion. The electrophoretic analysis was carried out on cell extracts using a slight modification¹³ of the CelloGel method of Rattazzi and Davidson¹⁴.

B could be detected in the Sandhoff fibroblasts when they had been maintained without subculturing for more than 15 d. In addition, both cell lines had, at times, Hex C^{2,3,11}, which in our experience appears only in cultures maintained for 5 or more days without transfer, the actual time apparently depending on the initial cell concentration.

In a series of fusion experiments (Table 1), fibroblasts from the two parental lines, after trypsinisation, were resuspended in Hanks salt solution without glucose. Cells were mixed in a ratio of 2:1, that is 4.8×10^6 Sandhoff cells to 2.4×10^6 Tay-Sachs cells. These cell mixtures, as well as separate aliquots of Tay-Sachs and Sandhoff fibroblasts, were exposed to β -propiolactone-inactivated Sendai virus¹² in the cold for 15 min. Cells were then resuspended in medium (Eagle's minimal essential medium with non-essential amino acids, penicillin, streptomycin and 15% foetal calf serum) and plated in a series of 60 mm plastic Petri dishes (10^6 – 10^8 cells per dish). In addition, mixtures of the parental lines (2 Sandhoff:1 Tay Sachs) not exposed to virus were plated into dishes as another control.

Sixteen to 24 h after fusion, cover slips in dishes containing the virus-treated Sandhoff–Tay-Sachs cell mixtures were fixed and analysed for the presence of multinucleated cells (Table 1). Cells from experimental and control dishes were collected at various times after fusion for the electrophoretic analysis of hexosaminidase activity (Table 1). The cells were washed twice with phosphate-buffered saline (PBS), trypsinised, and, after centrifugation, washed again with PBS. Each pellet was suspended in 0.05–0.2 ml (de-

pending on cell concentration) of 0.01 M citrate-phosphate buffer, pH 7.0, and disrupted by ultrasonication (three 10-s treatments). The ruptured cells were centrifuged for 10 min at 5,000g at 3–5° C and the supernatant was used for biochemical studies.

Without virus treatment, the cocultivation of the parental lines in a ratio of 2:1 (Sandhoff to Tay-Sachs) for as long as 16 d yielded Hex B, and in time Hex C, but no Hex A (Fig. 1). Virus-treated Tay-Sachs fibroblasts also showed Hex B, and on prolonged culture the C band, but never Hex A. The virus-treated Sandhoff cells showed neither Hex A nor Hex B, but on prolonged culture they also had Hex C.

In contrast, the virus-treated mixtures of parental lines had, in addition to the expected Hex B, a band comigrating with Hex A (Fig. 2). The latter appeared in one experiment as early as 24 h and in all cases seemed to increase to a maximum between 3 and 6 d after fusion (Table 1 and Fig. 2). The activity of Hex A seemed to correlate with the number of multinucleated cells present in the culture and declined when the nondividing heterokaryons had been overgrown by mononuclear parental cells.

As this new A-like band might represent a modified form of Hex B or C, its thermal lability was examined. Cell extracts were incubated under conditions in which the Hex A activity is destroyed while most of the Hex B activity remains. We found that the novel component formed by the virus-treated Sandhoff–Tay-Sachs mixture had the heat lability of the Hex A found in normal fibroblasts (Fig. 3).

We suggest that the novel band produced by virus-induced heterokaryons is the Hex A enzyme lacking in both parental lines. A more positive identification awaits the development of sensitive and specific assays involving antibodies or labelled substrates.

It is conceivable that the substance responsible for the appearance of this novel band is passed through the medium from heterokaryon to deficient parental cells. But, even when Sandhoff or Tay-Sachs cells were exposed for 2–11 d to medium in which virus-treated cell mixtures had been proliferating for at least 3 d, no evidence of Hex A was found. Furthermore, neither incubation of mixtures of cell extracts from the two parental lines at 37° C for up to 144 h, nor prolonged exposure of lysates from one parent line to proliferating cells of the other, resulted in the production of Hex A.

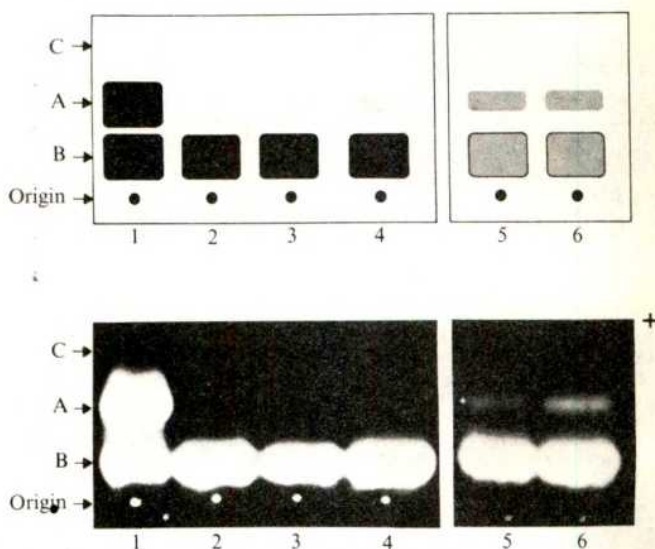


Fig. 2 N-acetyl- β -D-glucosaminidase electrophoresis. (1) Extract from normal fibroblasts and (2–6) extracts from virus-treated mixtures of Tay-Sachs and Sandhoff fibroblasts 16, 24, 48, 72, and 144 h after fusion. The novel band shown in this figure comigrates with Hex A when mixed with extracts of control fibroblasts.

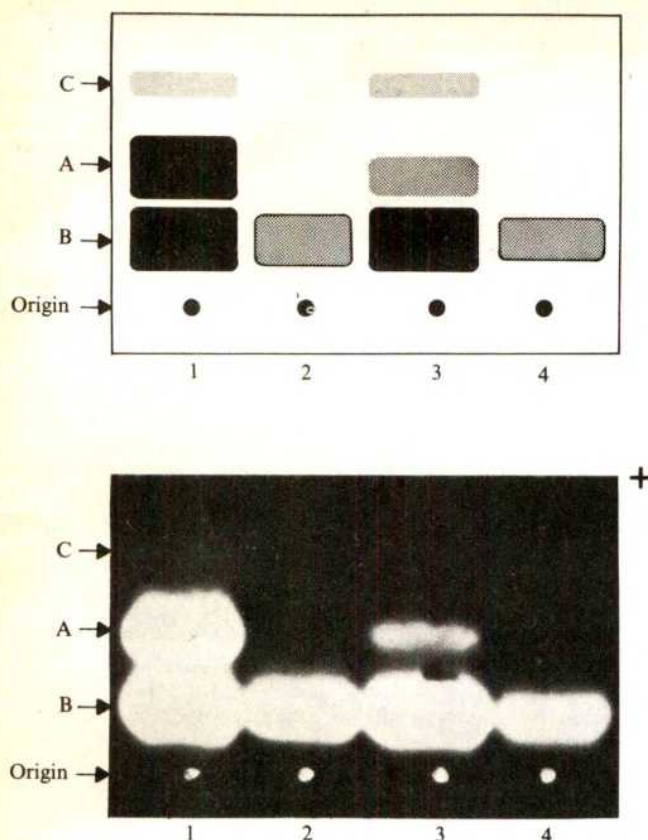


Fig. 3 Cellogel electrophoresis of cell extracts before and after incubation in 0.01 M phosphate-citrate buffer; pH 7.0, for 45 min at 52° C. Extract of normal fibroblasts before (1) and after (2) incubation; extracts of virus-treated Tay-Sachs-Sandhoff mixture before (3) and after (4) incubation.

Our results (Table 1) suggest that this enzyme persists in these heterokaryons for as long as 16 d, at a time when the cell population contains relatively few heterokaryons. Conceivably the product responsible for the appearance of the activity passes from heterokaryon by cell-mediated contact to surrounding parental cells or alternatively is stored for prolonged periods, as reported for β -glucuronidase¹⁵.

The mechanism underlying the appearance of the novel enzyme, believed to be Hex A, is uncertain. Perhaps the Sandhoff cells provide either an enzyme to convert the Hex B of Tay-Sachs origin into Hex A, or a subunit essential to the formation of Hex A. On the other hand, the Tay-Sachs cells might stabilise in some way a labile Hex B and Hex A of Sandhoff origin.

We have not attempted to isolate mononuclear hybrid cells because of the lack of an effective means to select against either parental cell.

Nevertheless, our studies of the heterokaryons derived

from Tay-Sachs and Sandhoff cells show that even in the absence of selection this approach can reveal intergenic complementation. Complementation tests have already provided evidence of genetical heterogeneity contributing to the phenotypes of galactosemia, xeroderma pigmentosum, and maple syrup urine disease¹⁶⁻¹⁸. The study of the interaction between different genomes within the heterokaryon, not only reveals genetical heterogeneity, but also indicates directions to follow in pursuit of the underlying disease mechanism.

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- Okada, S., Veath, M. L., Leroy, J., and O'Brien, J. S., *Am. J. Human Genet.*, **23**, 55 (1971).
- Gilbert, F., Kucherlapati, R., and Ruddle, F. H., *Abstract, The American Society of Human Genetics*, October 24-27, 1973, 29A.
- Poenaru, L., and Dreyfus, J. C., *Clin. chim. Acta*, **43**, 439 (1973).
- Carroll, M., and Robinson, D., *Biochem. J.*, **126**, 17 (1972).
- Srivastava, S. K., and Beutler, E., *Biochem. biophys. Res. Commun.*, **47**, 753 (1972).
- Okada, S., and O'Brien, J. S., *Science*, **165**, 698 (1969).
- Sandhoff, K., Andreae, U., and Jatzkewitz, H., *Life Sci.*, **7**, 283 (1968).
- Goldstone, A., Konecny, P., and Koenig, H., *FEBS Lett.*, **13**, 68 (1971).
- Snyder, jun., P. D., Krivit, W., and Sweeley, C. C., *J. Lipid Res.*, **13**, 128 (1972).
- Robinson, D., and Carroll, M., *Lancet*, **i**, 322 (1972).
- Ropers, H. H., and Schwantes, U., *Humangenetik*, **20**, 167 (1973).
- Neff, J. N., and Enders, J. F., *Proc. Soc. exp. Biol. Med.*, **127**, 260 (1968).
- Thomas, G. H., Taylor, H. A., Reynolds, L. W., and Miller, C. S., *Pediat. Res.*, **7**, 751 (1973).
- Rattazzi, M. C., and Davidson, R. G., in *Antenatal diagnosis*, (edit. by Dorfman, A.), 207 (The University of Chicago Press, Chicago, 1972).
- Langunoff, D., Nicol, D. M., and Pritzl, P., *Lab. Invest.*, **29**, 449 (1973).
- Nadler, H. L., Chacko, C. M., and Rachmeler, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 976 (1970).
- De Weerd-Kastelein, E. A., Keijzer, W., and Bootsma, D., *Nature new Biol.*, **238**, 80 (1972).
- Lyons, L. B., Cox, R. P., and Dancis, J., *Nature*, **243**, 533 (1973).

Table 1 Activity of novel-N-acetyl- β -D-glucosaminidase in cultures containing virus-induced heterokaryons of Tay-Sachs and Sandhoff fibroblasts

Experiment	% Multi-nucleated cells*	Days after fusion								
		1	2	3	4	5	6	7	8	16
1	20	+	+	+			+	+		•
2	9	-	-	+			+	+	+	+
3	10	-	+	+	+	+	+	-	-	

* 16-24 h after fusion.

Solvent exposure of specific nuclei of angiotensin II determined by NMR solvent saturation method

CONFORMATIONAL analysis of peptides in solution by nuclear magnetic resonance (NMR) spectroscopy generally involves determination of the relative exposure to solvent of specific NH hydrogens¹⁻⁴. The four methods used so far are based on: (1) rates of NH proton exchange with labile hydrogens of the solvent⁵⁻⁶; (2) temperature dependence of chemical shifts of NH resonances⁷⁻⁸; (3) dependence of NH chemical shifts on the composition of a suitable solvent mixture⁹⁻¹¹, and (4) degree of resonance broadening when a paramagnetic substance is added¹². Each method has limitations. Proton exchange rates reflect not only exposure to solvent,

but also proximity to functional groups of the peptide which catalyse exchange. Factors which determine the temperature dependence of NH chemical shifts are as yet poorly understood. Changes in solvent composition can alter the conformation of the peptide¹¹. Paramagnetic ions may associate preferentially with the solvent or with specific sites on the peptide.

We suggest here a method which offers the advantage of measuring the solvation of both NH and CH hydrogens while not physically altering molecular structure. This involves monitoring intensity changes of solute resonances resulting from saturation of the solvent resonance. Intensities of solute resonances originating from exposed labile hydrogens exchanging rapidly with the solvent are diminished by transfer of saturation¹³⁻¹⁶, whereas resonances of exposed nonexchangeable hydrogens are enhanced by a positive nuclear Overhauser effect (NOE)¹⁷. Although the method is generally applicable to any solute-solvent pair having suitable nuclei, it is particularly appropriate to investigations of peptides and other biomolecules in aqueous solution. Here we describe its application to the interaction of water with the pressor hormone (Asn¹, Val⁵) angiotensin II (AII'), whose primary structure appears in Fig. 1. Materials and the correlation spectroscopy technique used have been described before^{16,18,19}.

Transfer of saturation is governed by equation (1)

$$(M_\alpha^a - M_\alpha^b)/M_\alpha^a = [T_{1\alpha}/(T_{1\alpha} + \tau_a)] [(M_\beta^b - M_\beta^a)/M_\beta^b] \quad (1)$$

which was derived from modified Bloch equations using a procedure analogous to that used by Gupta and Redfield²⁰. The α and β states refer to solute and solvent nuclei, respectively, which are chemically exchanging. $T_{1\alpha}$, τ_a , M_α^a and M_α^b are the spin-lattice relaxation time, life time, observed magnetisation and equilibrium magnetisation of the α nucleus, respectively. Analogous notation is used for the β nucleus. $(M_\alpha^a - M_\alpha^b)/M_\alpha^a$ is the fractional decrease in resonance intensity of the α resonance resulting from double irradiation of the β resonance, whose intensity is diminished by a factor of $(M_\beta^b - M_\beta^a)/M_\beta^b$. Complete saturation of the β state causes a fractional decrease of the α resonance equal to $T_{1\alpha}/(T_{1\alpha} + \tau_a)$, which is significant only if the pseudo-first order rate constant for exchange of the α nucleus, $1/\tau_a$, is comparable with or greater than its relaxation rate, $1/T_{1\alpha}$. Measurement of the extent of saturation transfer and $T_{1\alpha}$ facilitates estimation of the exchange rate.

Saturation of the solvent resonance also results in positive NOEs for resonances originating from solute hydrogens exposed to the solvent or in close proximity to solute hydrogens experiencing saturation. Saturation of solute resonances can result either from exchange-mediated transfer of saturation from the solvent or from coincidence of solute and solvent resonances (which is usually also caused by rapid exchange with the solvent). Three mechanisms can contribute to the observed NOE: (1) direct dipole-dipole interaction between solute and solvent hydrogens (such intermolecular dipole-dipole effects have been observed, and a theoretical treatment of this phenomenon has been derived by Krishna and Gordon)²¹, (2) dipole-dipole interaction between the monitored hydrogen and a nearby partially or completely saturated solute hydrogen, and (3) scalar coupling between the monitored hydrogen and a solute hydrogen which is exchanging with the solvent at a rate comparable to the chemical shift difference between the two coupled solute hydrogens¹⁷. This exchange-modulated scalar coupling would yield a negative NOE (decrease in resonance intensity), whereas the dipolar mechanisms yield positive NOEs except when long correlation times are encountered (for example, macromolecules)²².

Figure 1a shows the region of the spectrum of AII' to low field of the water resonance when saturation power is applied 1,200 Hz to high field of the solvent peak. Resonance assignments are those obtained in this

laboratory^{16,23,24} and by Bleich, *et al.*^{25,26}. Saturation of the water resonance yields the spectrum shown in Fig. 1b. Figure 1c shows the difference (amplified) between the solvent saturated and the off resonance irradiated spectra (Fig. 1b-Fig. 1a). Negative peaks originate from solvent exposed labile hydrogens experiencing transfer of saturation—Arg² peptide NH , Asn¹ *trans*-amide NH , and Arg⁷ guanidino NH s. The Arg² peptide NH , Asn¹ amide NH and Arg⁷ guanidino NH resonances decreased in intensity by factors of 0.50 ± 0.05 , 0.09 ± 0.03 and 0.07 ± 0.02 , respectively. Positive peaks originate from solute hydrogens experiencing a positive NOE—His⁶ C_αH , His⁶ C_βH , Tyr⁴ *o*-CH and perhaps also the Phe⁸ $\text{C}_\alpha\text{H}_2$ (overlap with the His⁶ C_βH peak obscures this resonance). The His⁶ C_αH and Tyr⁴ *o*-CH peaks experienced fractional enhancements of resonance intensities of 0.17 ± 0.05 and 0.12 ± 0.03 , respectively. Larger NOEs might be obtained by minimising relaxation pathways not associated with solute-solvent interactions, for example, by degassing the sample or using partially deuterated samples. Perturbation of His⁶ and Tyr⁴ peptide NH peaks results from partial decoupling of their corresponding α -CH resonances, which are close to the solvent peak.

Transfer of saturation indicated that the NH protons of Arg² exchange rapidly with the solvent and are therefore

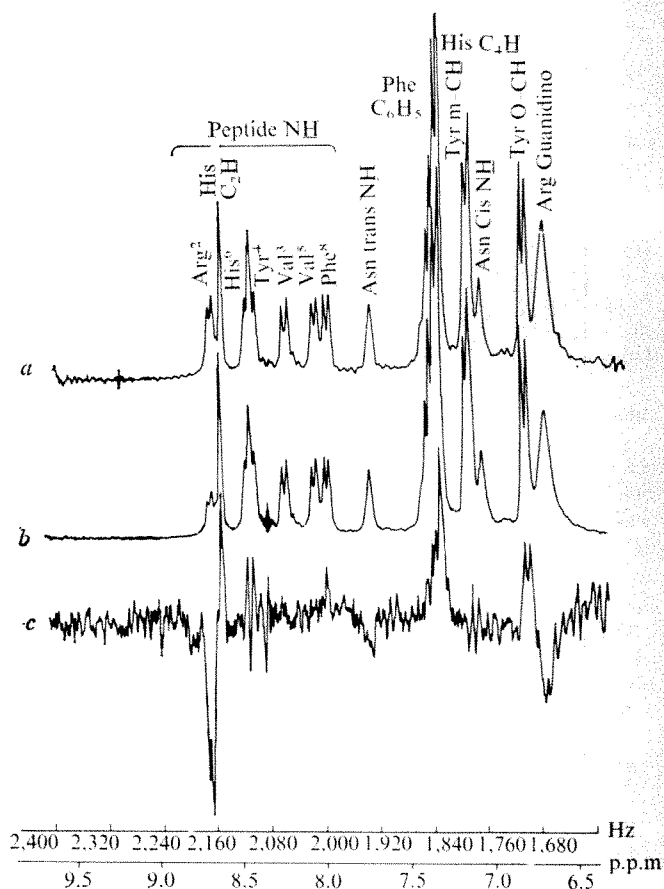
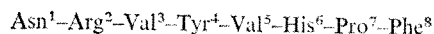


Fig. 1 a, Low field region of the 250 MHz PMR spectrum of AII' (6.9% (w/v)) in H_2O , pH 3.0 ± 0.1 , at 31.5°C . The spectrum was obtained by correlation spectroscopy (twenty scans) with saturating rf power applied 1,200 Hz to high field of the solvent resonance. Chemical shifts are referred to the methyl resonance of sodium 2,2-dimethyl-2-silapentane 5-sulphonate. b, Same as (a) but with the solvent peak saturated. c, Spectrum (b) minus spectrum (a) amplified thirteenfold.

probably well solvated. Rapid exchange of these hydrogens has been noted previously^{16,23,28}. Partial saturation of the Asn¹ *trans*-amide NH suggests that this hydrogen is also exposed to the solvent. Taken together the data for Asn¹ and Arg² suggest that the two N-terminal residues of AII are relatively well solvated. This conclusion is consistent with our report that the *pK_a* of the Asn¹ amino group is comparable with that of similar solvated functional groups^{23,24}.

Observations of positive NOEs indicates that the dipolar mechanisms dominate over any exchange-modulated scalar coupling which might be contributing to the relaxation of the His² imidazole CH and Tyr⁴ *o*-CH protons. These protons must be in intimate contact with the solvent and (or) the nearby exchangeable protons on the side chains of these residues must be substantially saturated as a result of rapid exchange with the solvent. In either case a solvated environment is indicated for these CH protons since dipolar relaxation depends on the inverse sixth power of the internuclear distance. Solvation of the imidazole and phenol of AII¹ is consistent with the normal *pK_a*s of these groups^{23,24,27,28}. Failure to observe an NOE from the Tyr⁴ *m*-CH hydrogens may indicate that this portion of the phenol group is not in contact with water. These studies show how solvent saturation effects can delineate the extent of solvation of both labile and nonexchanging hydrogens.

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- ¹ Urry, D. W., and Ohnishi, M., in *Spectroscopic approaches to biomolecular conformation* (edit. by Urry, D. W.), cha. 7 (American Medical Association Press, Chicago, Illinois, 1970).
- ² Bovey, F. A., Brewster, A. I., Patel, D. J., Tonelli, A. E. and Torchia, D. A., *Accounts of Chem. Res.*, **5**(6), 193-200 (1972).
- ³ Kopple, K. D., and Schamper, T. J., in *Chemistry and biology of peptides* (edit. by Meienhofer, J.), 75-80 (Ann Arbor Publishers, Ann Arbor, 1972).
- ⁴ Stern, A., Gibbons, W. A., and Craig, L. C., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 734-741 (1968).
- ⁵ Hvidt, A., and Nielson, S. O., *Adv. Protein Chemistry*, **21**, 287-386 (1966).
- ⁶ Molday, R. S., Englander, S. W., and Kallen, R. G., *Biochemistry*, **11**, 150-158 (1972).
- ⁷ Kopple, K. D., Ohnishi, M., and Go, A., *J. Am. chem. Soc.*, **91**, 4264-4272 (1969).
- ⁸ Ohnishi, M., and Urry, D. W., *Biochem. biophys. Res. Commun.*, **36**, 194-202 (1969).
- ⁹ Pitner, T. P., and Urry, D. W., *J. Am. chem. Soc.*, **94**, 1399-1400 (1972).
- ¹⁰ Pitner, T. P., and Urry, D. W., *Biochemistry*, **11**, 4132-4137 (1972).
- ¹¹ Walter, R., and Glickson, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1199-1203 (1973).
- ¹² Kopple, K. D. and Schamper, T. J., *J. Am. chem. Soc.*, **94**, 3644-3646 (1972).
- ¹³ Forsen, S., and Hoffman, R. A., *J. chem. Phys.*, **39**, 2892-2901 (1963).

- ¹⁴ Hoffman, R. A., and Forsen, S., in *Progress in nuclear magnetic resonance spectroscopy* (edit. by Emsley, J. W., Feeney, J., and Sutcliffe, L. H.), Volume 1, 15-204 (Pergamon Press Ltd, Oxford, 1966).
- ¹⁵ Von Dreele, P. H., Brewster, A. I., Dadok, J., Scheraga, H. A., Bovey, F. A., Ferger, M. F., and Du Vignaud, V., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2169-2173 (1972).
- ¹⁶ Glickson, J. D., Dadok, J., and Marshall, G. R., *Biochemistry*, **13**, 11-14 (1974).
- ¹⁷ Noggle, J. H., and Schirmer, R. E., *The nuclear overhauser effect*, (Academic Press, New York, 1971).
- ¹⁸ Dadok, J., and Sprecher, R. F., *J. Magn. Res.*, **13**, 243-248 (1974).
- ¹⁹ Gupta, R. K., Ferretti, J. A., and Becker, E. D., *J. Magn. Res.*, **13**, 275-290 (1974).
- ²⁰ Gupta, R. K., and Redfield, A. G., *Biochem. biophys. Res. Commun.*, **41**, 273-281 (1970).
- ²¹ Krishna, N. R., and Gordon, S. L., *J. chem. Phys.*, **58**, 5687-5696 (1973).
- ²² Balaram, P., Bothner-By, A. A., and Dadok, J., *J. Am. chem. Soc.*, **94**, 4015-4017 (1972); Balaram, P., Bothner-By, A. A., and Breslow, E., *ibid.*, 4017-4018 (1972).
- ²³ Glickson, J. D., Cunningham, W. D., and Marshall, G. R., in *Chemistry and biology of peptides* (edit. by Meienhofer, J.), 563-570 (Ann Arbor Publishers, Ann Arbor, 1972).
- ²⁴ Glickson, J. D., Cunningham, W. D., and Marshall, G. R., *Biochemistry*, **12**, 3684-3692 (1973).
- ²⁵ Bleich, H. E., Galaray, R. E., and Printz, M. P., *J. Am. chem. Soc.*, **95**, 2041-2042 (1973).
- ²⁶ Bleich, H. E., Galaray, R. E., Printz, M. P., and Craig, L. C., *Biochemistry*, **12**, 4950-4957 (1973).
- ²⁷ Paiva, T. B., Paiva, A. C. M., and Scheraga, H. A., *Biochemistry*, **2**, 1327-1334 (1963).
- ²⁸ Zimmer, S., Haar, W., Maurer, W., Ruterjans, H., Fermandjian, S., and Fromageot, P., *Eur. J. Biochem.*, **29**, 80-87 (1972).

Ferritin synthesis in normal and leukaemic leukocytes

SERUM ferritin concentration is normally directly related to body iron stores^{1,2} but abnormally high values are also found in patients with leukaemia³. The greatest amounts are present in patients with acute myeloblastic leukaemia and in this condition the concentration within circulating leukocytes is about six times higher than in normal leukocytes⁴. We have demonstrated an abnormally high rate of overall ferritin synthesis in leukaemic cells. Previous studies in other tissues have shown that ferritin synthesis is closely related to iron supply, but this does not seem to be the case in either normal or leukaemic leukocytes where production of the protein is independent of iron concentration and the protein formed contains little, if any, iron.

Peripheral blood leukocytes were obtained from healthy adults and patients with acute myeloblastic leukaemia by sedimentation and differential centrifugation⁴. In synthesis experiments the white cell pellet was suspended in Eagle's minimum essential medium (MEM) at a concentration of 2×10^6 cells ml⁻¹, to which 20% foetal calf serum and 1 μ Ci ¹⁴C-leucine were added. Cultures were incubated in triplicate at 37° C under 5% CO₂ in air for 22 h. Controls consisted of omitting the cells from the incubation medium or the addition of cycloheximide at a concentration of 10⁻⁴ M. At the end of the incubation, the cells were sonicated and dialysed against five changes of 0.3% saline for 3 h. One hundred micrograms of human spleen ferritin were added and the total volume brought to 9 ml. A 2 ml aliquot was removed and the protein precipitated with 10% trichloroacetic acid followed by heating at 90° C for 15 min. The precipitate was washed with trichloroacetic acid and finally dissolved in 0.1 M NaOH before scintillation counting. Ferritin was isolated by heating the cell extract containing carrier ferritin at 70° C for 10 min, followed by centrifugation at 1,300g for 10 min. Anti-human spleen ferritin was then added to the supernatant, incubated at 37° C for 1 h and left at 4° C overnight. The precipitate was

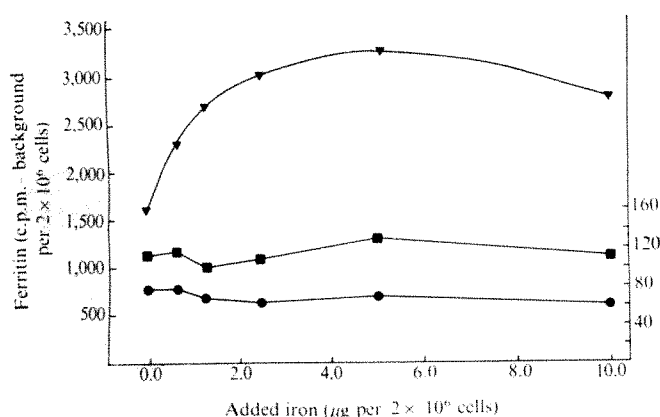


Fig. 1 ^{14}C -leucine incorporation into ferritin at different iron concentrations. The iron content of the basal medium is $0.67 \mu\text{g}$ per 2×10^6 cells. ▼, Chang cells; ●, leukaemic leukocytes (left hand scale). ■, Normal leukocytes (right hand scale).

washed three times with 0.9% saline and dissolved in 0.5 M HCl before scintillation counting. Chang liver cells³ (Flow Laboratories) were grown in MEM with 10% foetal bovine serum. Ferritin synthesis was measured under the same conditions and with the same cell concentration used with leukocytes. Sucrose density gradient fractionation of ferritin was carried out by the method of Drysdale and Munroe⁶. Twenty fractions of 0.5 ml were collected and ferritin was determined either by immunoradiometric assay or by the absorbance at 280 nm.

^{14}C -leucine incorporation into ferritin and total protein is expressed as c.p.m. per 10^6 cells. In seven normal subjects, the mean ferritin uptake was 54.2 ± 5.5 c.p.m. and the mean protein uptake $1,876 \pm 190$ c.p.m. The protein-ferritin ratio was 35.7 ± 4.1 . In seven patients with acute myeloblastic leukaemia, the mean ferritin uptake was 230.8 ± 36.6 c.p.m. and protein uptake $7,526 \pm 1,140$ c.p.m. with a protein-ferritin ratio of 33.0 ± 2.3 . Increasing the iron concentration in the medium from 0 to $10.0 \mu\text{g ml}^{-1}$ had no effect on ferritin synthesis in either normal or leukaemic cells. In experiments using Chang liver cells, a similar increase in iron concentration in the medium resulted in stimulation of ferritin synthesis (Fig. 1).

The largest amounts of purified human spleen ferritin appeared between fractions 8 and 11, in nine separate experiments. In three experiments using normal leukocyte extract, and six experiments using leukocyte extracts from patients with acute myeloblastic leukaemia, the maximum concentration of ferritin appeared in fraction 2 to 4 (Fig. 2) corresponding to apoferritin.

These results demonstrate increased ferritin synthesis by leukaemic cells and supports the suggestion that this is the cause of the raised serum and leukocyte ferritin concentration⁴ in leukaemia patients. Experimental studies on the control of ferritin synthesis have been carried out largely on liver cells and in this tissue, ferritin synthesis is closely related to iron supply⁶. Ferritin synthesis *in vitro* by HeLa cells is also stimulated by iron⁷ and *in vivo* studies have shown a similar iron dependence of ferritin synthesis by rat hepatoma⁸. Ferritin synthesis in Chang cells shows marked stimulation by iron, but without any increase in overall protein synthesis. In normal leukocytes, ferritin synthesis does not seem to be stimulated by increasing concentrations of iron, and leukaemic cells show the same insensitivity. In leukaemic cells, ^{14}C -leucine incorporation into ferritin takes place at about four times the normal rate and this seems to be a reflection of increased protein synthesis by these cells.

The characteristics of leukocyte ferritin on density gradient centrifugation suggests that this is either apoferritin or protein with a very low iron content. It has

been known for some time that the ferritins in different tissues may vary in their physical characteristics and probably their subunit composition. Linder-Horowitz *et al.*⁹ showed that the stimulation of ferritin synthesis by iron varies in different organs, being more marked in liver and heart than in kidney. While the iron content of liver ferritin remains constant that of heart and kidney ferritin falls with increased synthesis of the apoprotein. In the female rat heart, where there are two separate ferritins, the electrophoretically slow variant shows less response than the fast variant to stimulation by iron. In a study of abnormal ferritins in rat hepatomas, Linder *et al.*⁸ showed that protein induction by iron was less than in normal regenerating liver. Increasing growth rate of the tumours was associated with decreased iron uptake, a decreased concentration of ferritin in the tumour and a lower iron content of the ferritin. It was suggested that ferritin of low iron content might be characteristic of tissues undergoing rapid cell division but the possibility also exists that in malignant tissue, expression of an alternative gene locus for ferritin

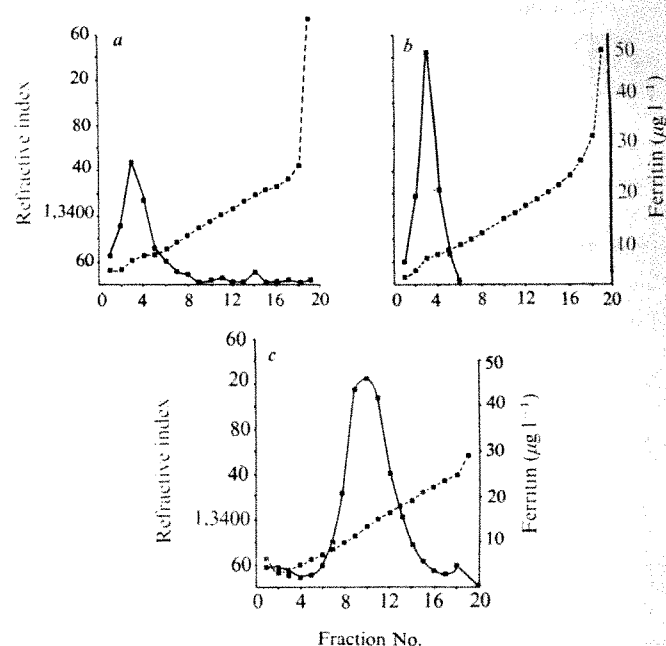


Fig. 2 Density gradient sedimentation of ferritin. ■—■ ferritin; ■—■ refractive index of medium. a, Normal leukocytes; b, leukaemic leukocytes; c, human spleen ferritin.

occurs and that this is associated with a low affinity of the protein for iron. The observation that synthesis in both normal and leukaemic leukocytes is not stimulated by iron and that the protein has the characteristics of apoferritin suggests the existence of a specific leukocyte ferritin. Its increased synthesis in acute myeloblastic leukaemia and its subsequent release into the plasma reflects the overall increase in protein synthesis by leukaemic cells and may provide a useful marker for the progress of the disease.

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* Walters, G. O., Miller, F., and Worwood, M., *J. clin. Path.*, **26**, 770-772 (1973).

- ³ Jones, P. A. E., Miller, F., Worwood, M., and Jacobs, A., *Br. J. Cancer*, **27**, 212-217 (1973).
- ⁴ Worwood, M., Summers, M., Miller, F., Jacobs, A., and Whittaker, J. A., *Br. J. Haemat.* (in the press).
- ⁵ Chang, R. S., *Proc. Soc. exp. Biol. Med.*, **87**, 440-443 (1954).
- ⁶ Drysdale, J. W., and Munro, H. N., *J. biol. Chem.*, **241**, 3630-3637 (1966).
- ⁷ Chu, L. L. H., and Fineberg, R. A., *J. biol. Chem.*, **244**, 3847-3854 (1969).
- ⁸ Linder, M., Munro, H. N., and Morris, H. P., *Cancer Res.*, **30**, 2231-2239 (1970).
- ⁹ Linder-Horowitz, M., Ruettinger, R. T., and Munro, H. N., *Biochim. biophys. Acta*, **200**, 442-448 (1970).

β , γ Unsaturated amino acids as irreversible enzyme inhibitors

IRREVERSIBLE enzyme inhibitors whose mechanisms of action are based on the k_{cat} term rather than on K_s are highly specific. Inhibitors of this type possess hidden reactive moieties which are unmasked enzymatically. On generation, the reactive product engages in a chemical reaction with an active site residue resulting in irreversible inactivation of the enzyme. Thus, the enzyme produces its own irreversible

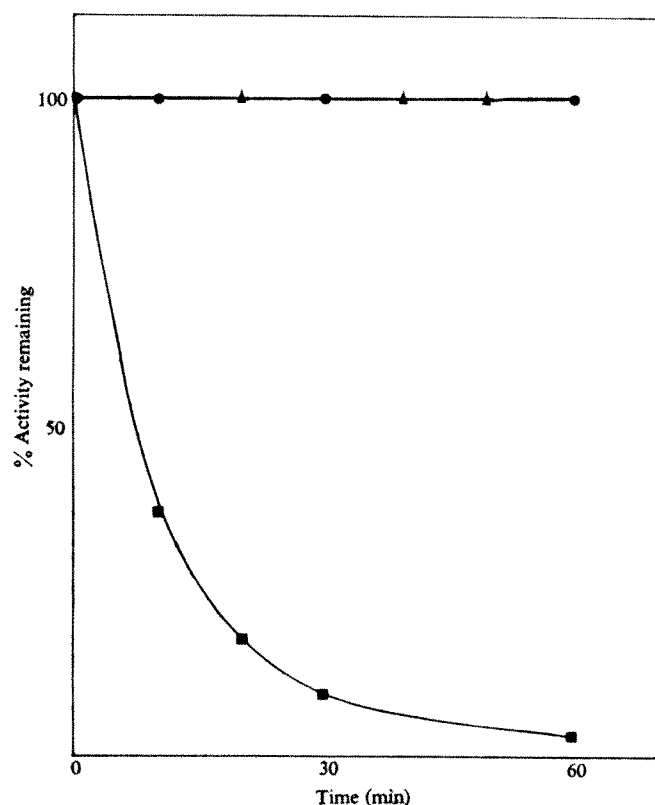


Fig. 1 Inactivation of aspartate amino transferase by AMB. Aspartate amino transferase from pig heart (Sigma Chemical Co.), specific activity 234 units mg^{-1} (where one unit of enzyme will convert 1 μmol of α -ketoglutarate to glutamic acid per min at 25°C) was used. In these experiments 0.008 units of enzyme (■) were incubated at 25°C in 0.1 M potassium phosphate buffer, pH=7.6 with 10 mM AMB. At the times indicated, 5 μl of the enzyme was removed and its activity determined by the standard assay system containing aspartic acid, α -ketoglutaric acid, NADH and malic dehydrogenase⁶. Identical experiments were performed in the presence of 0.2 M aspartic acid (▲) and in the absence of AMB (●) (control). The activity of the inactivated enzyme was not restored by dialysis against the phosphate buffer with several changes over a 36 h period attesting to the enzymes being irreversibly inactivated. Furthermore, the activity of the inactivated enzyme could also not be restored by incubation with 1 mM pyridoxal phosphate for several hours.

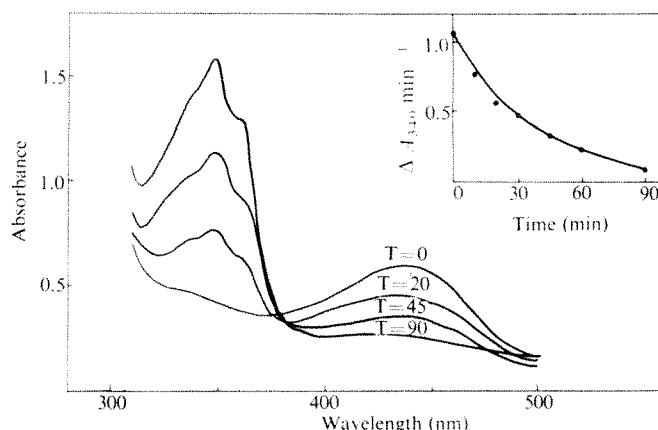


Fig. 2 The ultraviolet spectra and inactivation of aspartate amino transferase by AMB. In a 1 cm cuvette 936 units of enzyme in 1 ml (0.1 M potassium phosphate at pH=6.0) was charged with 10 mM AMB at 25°C. The ultraviolet spectra of the sample were determined at the indicated times on a Cary 118 double beam spectrophotometer. The activity of an aliquot of the enzyme was determined by the standard method⁶ and compared with an untreated control. Inset shows the inactivation profile at the indicated times.

inhibitor from a chemically unreactive substrate. Inhibitors of this type will be referred to as k_{cat} inhibitors, and several synthetic inhibitors of this type have been reported¹. Here I describe an example of a naturally occurring molecule which acts by this mechanism: specifically, the irreversible inhibition of soluble, pyridoxal linked, L-aspartate amino transferase by the bacterial toxin L-2-amino-4-methoxy-trans-3-butenoic acid (AMB) isolated from *Pseudomonas aeruginosa*².

Incubation of soluble L-aspartate amino transferase with AMB led to the inactivation of the enzyme as shown in Fig. 1. The substrate, in this case L-aspartic acid, protects against this inactivation when co-incubated with AMB (Fig. 1) and is consistent with the view that AMB is an active-site titrant. Also, activity of the inactivated enzyme cannot be restored by dialysis. This observation means that the mode of inhibition is irreversible.

The assertion that AMB requires chemical activation suggests that no inhibition should result in the absence of enzymatic conversion. This is confirmed in that neither holoenzyme in the pyridoxamine form nor apoenzyme, are in the least affected by AMB. The notion that the inactivation step requires enzymatic conversion can be directly demonstrated by following the ultraviolet absorption changes of the holoenzyme as a function of its inactivation (Fig. 2). As the inactivation proceeds, both the activity and the spectrum of the enzyme were recorded and as can be seen, the fall of the pyridoxal phosphate imine peak (approximately 440 nm) and the rise of the new peaks at approximately 350 nm are simultaneous with the inactivation course. The appearance of the peaks at 350 nm are unusual and cannot be pyridoxamine phosphate ($\lambda_{max}=325$ nm) (ref. 3). These new peaks represent a reaction product, or complex, between the converted AMB and cofactor. This may mean that the inhibitor functions by reacting with the cofactor. This was tested by trying to resolve the inactivated holoenzyme into apoenzyme and cofactor by the usual method⁴. The 'resolved' enzyme still retained an absorption peak centred at 350 nm, representing about 30% of the total. This peak cannot be diminished even under forced resolution conditions. Of primary importance is that the resolved enzyme cannot be reactivated by incubation with fresh pyridoxal phosphate, therefore the primary action of AMB must involve a chemical reaction with an active-site amino acid residue. Of secondary importance is the formation of a further reaction pro-

duct(s) with the cofactor. Had a chemical reaction simply ensued between the cofactor and AMB, 70% of the activity should have been regained after fresh cofactor was added to the resolved enzyme.

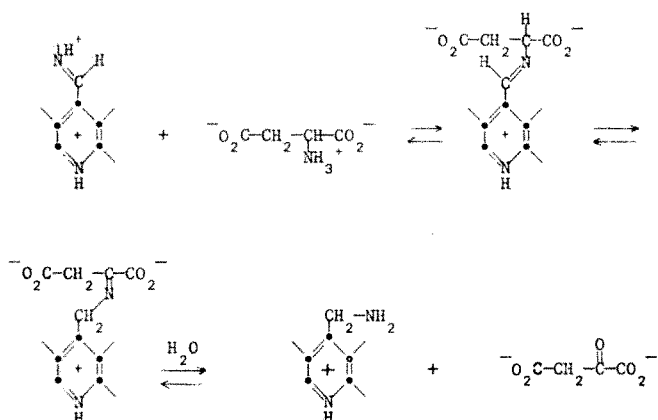


Fig. 3 Initial steps in the catalysis with aspartate and α -ketoglutarate as substrate.

The initial steps in the catalytic process effected by aspartate amino transferase with aspartate and α -ketoglutarate as substrates are shown in Fig. 3 (ref. 5). With AMB as a substrate the conversions shown below can intervene once the α C—H bond is cleaved (Fig. 4). That both (B+B') and C are exceedingly reactive chemically is important because they can act as Lewis acids in the Michael reaction. The carbon atoms indicated with stars * designate where reaction would occur. The conversion shown in equation 1 is preferred on the basis of its being a normally occurring one for the enzyme, and is more consistent with the spectral changes which occur during the course of the inactivation process. Also, it is apparent that these proposed reactive intermediates are chemically bi-functional; for example, B', being a conjugated enol ether, can undergo two successive reactions with nucleophiles. This is precisely what is required here, because a minimum of two reactions must occur—one with an active-site

residue and one with the cofactor—to account for the spectral changes and the inactivation process.

In conclusion, AMB has been shown to be a potent irreversible inhibitor of the k_{cat} type, the first naturally-occurring toxin to be reported as such. It is likely that this is in no sense unusual but that other β,γ unsaturated acids will be found to be irreversible inhibitors of many pyridoxal-linked enzymes involved in amino acid metabolism, and that other naturally occurring toxins will also be found to be k_{cat} inhibitors.

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- ¹ Rando, R. R., *Science*, (in the press).
- ² Scannell, J. P., Preuss, D. L., Demmy, T. L., Sello, L. H., Williams, T., and Stempel, A., *J. Antibiotics*, **25**, 122–127 (1972).
- ³ Jenkins, W. T., and D'Ari, L., *Biochem. biophys. Res. Comm.*, **22**, 376–382 (1966).
- ⁴ Bertland, L. H., and Kaplan, N. U., *Biochemistry*, **7**, 134–141 (1968).
- ⁵ Hammes, G. G., and Fasella, P., in *Chemical and Biological Aspects of Pyridoxal Catalysis*, (edit. by Snell, E. E., et al.) 185 (Macmillan & Co., New York, 1963).
- ⁶ Amador, E., and Wacker, W. E. C., *Clin Chem.*, **8**, 343–350 (1962).

Chelating agents for the binding of metal ions to macromolecules

POLYAMINOCARBOXYLATE chelating agents such as ethylenediaminetetraacetic acid (EDTA) form stable chelate complexes with the ions of many heavy metals. These metal ions exhibit a wide range of useful spectral and radioactive properties, such as electronic absorption, scattering of electrons and X rays, electron paramagnetic resonance spectra, the production of line-broadening and chemical shifts in nuclear magnetic resonance spectra, the emission of correlated gamma-ray cascades and various radioactive lifetimes and nuclear radiations. The preparation of chelating agents whose complexes can interact, in some selected manner, with biological macromolecules could make possible several new applications of metal ions as probes of biological systems.

The synthesis of 1-(*p*-aminophenyl)-EDTA (Fig. 1, R = NH₂) is a step toward this goal. In principle, its aromatic amino group can be acylated, alkylated or otherwise modified to form biologically active molecules or covalent labelling reagents. The specific interaction between a precursor of this compound, 1-(*p*-nitrophenyl)-EDTA (Fig. 1, R = NO₂), and an antibody molecule has been described previously¹.

We describe here the use of a derivative of the amino compound, 1-(*p*-benzenediazonium)-EDTA (Fig. 1, R = N₂⁺), as a reagent for labelling proteins. We also present data bearing on the usefulness of proteins labelled covalently with ¹¹¹In chelates as radiopharmaceuticals for the localisation of tumours.

The synthesis of 1-(*p*-aminophenyl)-EDTA (Fig. 1, R = NH₂) was accomplished in six steps, starting from benzaldehyde (details will be furnished on request). The amino compound then was diazotised² to form the chloride salt of 1-(*p*-benzenediazonium)-EDTA, or ⁺N₂Ph-EDTA (Fig. 1, R = N₂⁺).

The ⁺N₂Ph-EDTA was coupled to human serum albumin or to bovine fibrinogen (Blombäck fraction I-4 (ref. 3)) by reaction overnight at 4° C with 2% protein solutions in aqueous 0.01 M EDTA/0.12 M NaHCO₃ buffer, pH 8.1. The buffer ions and any

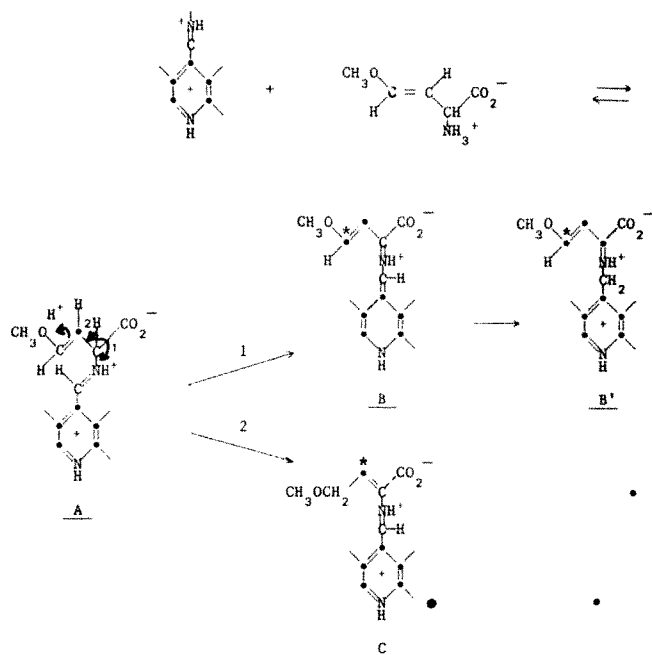


Fig. 4 Possible conversions of the catalytic product with AMB as substrate.

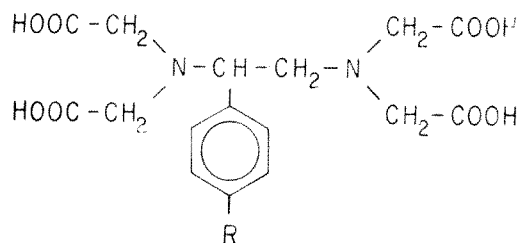


Fig. 1 The chelating agents discussed in the text.
R = NO₂, NH₂, or N₂⁺.

unbound reagent then were removed by extensive dialysis against heavy-metal-free 0.1 M citrate, pH 6. The presence of azotyrosine groups in the products was indicated by increased absorbance at 330 nm. Citrate buffer was chosen to facilitate the chelation of carrier-free ¹¹¹In ions by protein-bound -N₂Ph-EDTA groups. In this buffer, indium ions do not undergo hydrolysis or bind to native serum albumin or fibrinogen, but do bind readily to the conjugated proteins.

Indium-binding was studied experimentally by the technique of γ -ray perturbed angular correlations (PAC), using the ¹¹¹In ion as a radioactive probe of molecular motion¹. Based on the coincidence detection of γ rays emitted in cascade from a single nucleus, PAC depends on the rotational correlation time for fluctuating electric field gradients at the radioactive nucleus. The binding of ¹¹¹In ions to macromolecules is revealed by a change in the measured value of the integral perturbation factor, $\overline{G_{22}(\infty)}$. For carrier-free ¹¹¹InCl₃ in 0.1 M citrate at pH 6, $\overline{G_{22}(\infty)} = 0.60 \pm 0.01$, a value consistent with an indium complex of low molecular weight (in the same buffer, the NO₂-Ph-EDTA chelate of ¹¹¹In yields $\overline{G_{22}(\infty)} = 0.58 \pm 0.02$). For a similar solution containing 1% bovine fibrinogen, $\overline{G_{22}(\infty)} = 0.57 \pm 0.02$, indicating little interaction between indium ions and the macromolecule. For 1% human serum albumin, $\overline{G_{22}(\infty)} = 0.59 \pm 0.02$. When 0.3 ml of a 2% solution of human serum albumin, which had been reacted with an equimolar amount of +N₂Ph-EDTA, was added to 0.3 ml of citrate buffer containing ¹¹¹InCl₃, binding was complete in less than 1 min. This was indicated by the perturbation factor; $\overline{G_{22}(\infty)} = 0.40 \pm 0.01$, a value which was not changed by further addition of the conjugated albumin. The addition of 1% bovine fibrinogen, which had been reacted with a two- to three-fold excess of +N₂Ph-EDTA, to ¹¹¹InCl₃ in citrate led to complete binding within several minutes; the equilibrium value of $\overline{G_{22}(\infty)}$ was 0.37 ± 0.02 .

Albumin and fibrinogen differ greatly in molecular size and shape; yet, the perturbation factors for ¹¹¹In bound to the two labelled proteins are quite similar. This suggests that in each case the link between chelate and macromolecule is flexible⁴.

We have determined the organ distribution and tumour uptake of radioactivity after injection of the ¹¹¹In-labelled proteins into specially prepared BALB/c mice. A tumour line, KHJJ, derived from a primary mammary carcinoma arising spontaneously in a mouse and maintained for over 100 transplant generations was used for the assay⁵. Three mice were injected with each compound, and the per cent dose per gram of organ was measured 24 h after injection. Results are given in Table 1, together with data obtained using commercial ¹³¹I-labelled human serum albumin in the same model system. These data suggest that proteins conjugated with -N₂Ph-EDTA chelates will be useful radiopharmaceuticals. The observed tumour:organ radioactivity ratios would be favourable for scanning studies on human patients, and the radioactive properties of ¹¹¹In are ideal for providing scans up to 1 week after administration with a minimum of radiation exposure to the patient^{6,7}. In addition, PAC studies can provide direct information on the stability *in vivo* of ¹¹¹In-labelled compounds⁸.

The mild reaction conditions and rapid metal-binding achieved here suggest that +N₂Ph-EDTA will be useful for labelling

Table 1 Distribution and uptake of labelled macromolecules in BALB/c mice with KHJJ Tumour

Organ	¹¹¹ In-albumin	¹¹¹ In-fibrinogen	¹³¹ I-albumin
Blood	4.6 ± 0.6	6.7 ± 1.1	6.8 ± 1.6
Lungs	3.4 ± 0.6	3.1 ± 0.3	3.4 ± 1.2
Liver	8.6 ± 0.3	9.2 ± 0.5	1.1 ± 0.3
Spleen	3.3 ± 0.1	7.0 ± 0.7	0.7 ± 0.2
Kidneys	11.4 ± 0.1	18.1 ± 1.9	2.4 ± 0.6
Tumour	9.7 ± 0.3	8.1 ± 0.8	3.3 ± 1.0
Muscle	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2
Bone	2.0 ± 0.4	1.9 ± 0.4	1.0 ± 0.5
Brain	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Skin	2.4 ± 0.4	2.0 ± 0.3	—

Each of the compounds listed was injected into three BALB/c mice which carried a KHJJ tumour (implanted in the flank 14 d before). The tumours were about 1 cm³ in volume. Twenty-four hours after injection, the mice were killed and samples of blood and major organs were taken, weighed and counted. Results are reported in terms of per cent total dose per gram organ, a measure of the concentration of radioisotope in the various tissues.

biologically active molecules with short-lived radioisotopes. Related chelating agents have potential applications to a broad range of problems. A full account of this work will be published elsewhere⁹.

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¹ Meares, C. F., Sundberg, M. W., and Baldeschwieler, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3718-3722 (1972).

² Vogel, A. I., *Practical organic chemistry*, 567-568 (Longmans, Green, New York, 1948).

³ Blombäck, B., and Blombäck, M., *Ark. Kemi.*, **10**, 415-443 (1956).

⁴ Marshall, A. G., Werbelow, L. G., and Meares, C. F., *J. chem. Phys.*, **57**, 364-370 (1972). Erratum: page 4508.

⁵ Rockwell, S. C., Kallman, R. F., and Fajardo, L. F., *J. natn. Cancer Inst.*, **49**, 735-749 (1972).

⁶ Goodwin, D. A., Finston, R. A., Colombetti, L. G., Beaver, J. E., and Hupf, H., *J. nucl. Med.*, **10**, 337 (1969).

⁷ Hunter, W. W., and de Kock, H. W., *J. nucl. Med.*, **10**, 343 (1969).

⁸ Goodwin, D. A., Meares, C. F., and Song, C. H., *Radiology*, **105**, 699-702 (1972).

⁹ Meares, C. F., et al., *J. med. Chem.* (in the press).

Light-dependent phosphorylation of rhodopsin in living frogs

PHOSPHORYLATION of rhodopsin has been studied so far only *in vitro*. When suspensions of isolated and purified rod outer segments (ROS) from cattle and frogs have been mixed with γ -³²P-ATP and Mg²⁺, upon illumination ³²P-phosphate has been found to be bound covalently to rhodopsin in a slow dark reaction after bleaching. The activity of the water-

extractable kinase which mediates the phosphate transfer was found to be independent of light, but rhodopsin was not acceptable as a substrate until it had been bleached by light⁴. The slow rate at which phosphorylation occurs (half time 3–5 min (refs 1–4)) suggests that it may be involved in light adaptation of the rods which also seems to be a slow process⁵.

If the phosphorylation reaction plays a role in vision, it should be reversible; rhodopsin should undergo a cycle of phosphorylation and dephosphorylation. This important question, however, could not be clarified by the *in vitro* experiments: in some instances, dephosphorylation could be observed to occur to some extent^{2,3}, but mostly, the phosphorylation seemed to be irreversible^{1,4,8}. If there is capacity for dephosphorylation, it seems to be easily lost in the system of isolated ROS. Therefore the reaction had to be studied in living animals. That protein is phosphorylated in the ROS of living frogs was reported by Hall and Bacharach⁷ as early as 1970, but they were not interested in the light dependency of the effect. My experiments show that phosphorylation and dephosphorylation of rhodopsin are physiological reactions in the photoreceptors of the frog.

³²P-inorganic phosphate, in two portions of about 2 mCi each, was injected into the dorsal lymphatic sac of frogs (*Rana esculenta*, body weight 40–50 g) at an interval of 15 h. Each injection solution contained 20 μ mol of phosphate in 0.3 ml. The frogs were maintained individually in glass jars containing water, at 23°C in the dark. During the full incubation period of 41 h they excreted on the average $7.5 \pm 4\%$ (s. d.) of the total injected ³²P into the water, but

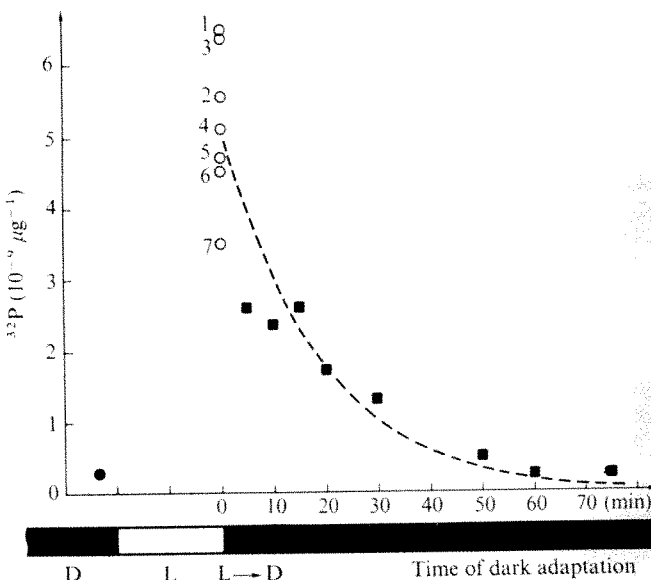


Fig. 2 ³²P bound to rhodopsin as a function of light/dark adaptation of 16 frogs. ●, Dark adapted (D), for 2 d; ○, light adapted (L) for 20 min; ■, light adapted for 20 min and then dark adapted for the times indicated in the abscissa. The seven light-adapted frogs are numbered as 1–7. ----, A theoretical first order decay curve based on a half time of 13 min, starting from the average phosphorylation level of the seven light-adapted frogs. The radioactivity was determined by scintillation counting of gel slices⁴. The amount of rhodopsin was determined from the area of the rhodopsin peak in stained and scanned gels; a calibration curve was obtained with 40 stained and scanned gels containing different amounts of purified cattle rhodopsin of known concentration. The staining and destaining procedure⁹ was carried out in strictly standardised conditions, and it was assumed that cattle and frog rhodopsin both have the same staining properties with Coomassie brilliant blue. The amount of rhodopsin isolated in the ROS was in general about 100 μ g per frog but varied from 150–40 μ g. The ³²P activity was related to the amount of rhodopsin, and to the total ³²P in each frog body at the moment of its death (total injected d.p.m. minus total excreted c.p.m. during the incubation). In a typical light-adapted frog, for example frog No. 2, there were 5,260 d.p.m. of ³²P bound to 146 μ g of rhodopsin, and 11,200 d.p.m. bound to phospholipid. 7×10^9 d.p.m. had been injected into the frog, 7% of which it had excreted during the incubation. Thus the value plotted on the ordinate is $5,260/146 \times (7 - 0.5) \times 10^9$ d.p.m./ μ g \times d.p.m. = 5.5×10^{-9} μ g⁻¹.

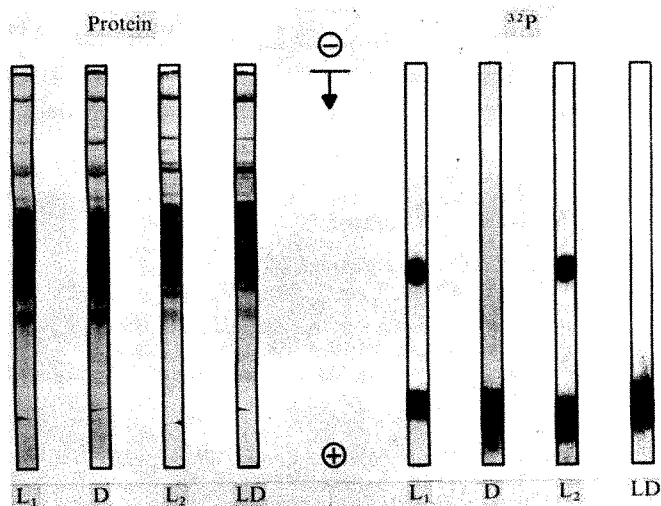


Fig. 1 Electrophoresis of ROS preparations obtained from four different frogs, on gels (length 11 cm) containing 5.8% polyacrylamide and 1% sodium dodecylsulphate (SDS) (ref. 9). To isolate the ROS, both retinas of each frog were shaken vigorously, using a Heidolph Reax I mixer, for 60 s in 2 ml of 45% sucrose solution containing 5 mM Tris-acetate (pH 7.3), 65 mM NaCl, and 1 mM MgCl₂. Differential centrifugation was carried out according to McConnell⁸. The final ROS pellet was solubilised with 100 μ l of 2% SDS containing 2% 2-mercaptoethanol, for 20 min at 45°C. Insoluble material derived from pigment epithelium was removed by centrifugation. L₁, Light-adapted frog, with retinas and ROS prepared in white light; L₂, light adapted with retinas and ROS prepared in dim red light; D, dark adapted for 2 d; LD, light adapted and then dark adapted for 75 min. The three light-adapted frogs were exposed for 20 min to diffuse white light of 450 lx. On the left side, photographs of gels stained with Coomassie brilliant blue are shown; the corresponding autoradiographs, exposed for 4 d on X-ray film, are shown on the right. The ink mark near the lower end of the stained gels indicates the position of the marker dye, pyronin Y, which ran to about the same position as the phospholipids. The autoradiographed gels contain 2–5 times more ROS material than the stained gels.

this value was as high as 13 and 14% in some frogs and as low as 0.5% in another. Forty to forty-one hours after the first injection, the frogs were subjected to different light/dark conditions: some were left in the dark, others were light adapted, and others first light and then dark adapted. Light adaptation was accomplished in all cases by exposing the frogs for 20 min to white diffuse light (450 lx) from a fluorescent lamp. At the end of the light/dark treatment (41 h after the first injection) the frogs were killed and the retinas were dissected in dim red light. The two retinas of a frog contained on the average 10^6 decompositions per minute (d.p.m.) of ³²P. The ROS were shaken off and purified by a modification of McConnell's procedure⁸ (Fig. 1). To minimise enzymatic reactions during the preparation, all operations were done at 0°C as fast as possible and, unless otherwise stated, in dim red light. The elapsed time between killing a frog and solubilising its ROS was 35–40 min.

The solubilised ROS were separated by SDS gel electrophoresis. Results obtained from four different frogs are shown in Fig. 1. The purity of the ROS preparation is indicated by the relatively large rhodopsin band in the stained gels shown at the left, demonstrating that rhodopsin is the predominant protein¹⁰. The two radioactive bands in the autoradiographed gels (Fig. 1, right)

correspond to ^{32}P -rhodopsin (middle of gells) and ^{32}P -phospholipids (lower end). The phospholipid nature of the lower band was demonstrated by extracting the ROS with chloroform/methanol¹¹ followed by thin layer chromatographic analysis of the extract. This phospholipid band was found in all frogs, independent of their light/dark treatment, and represents freshly synthesised phospholipids which were incorporated into the ROS during the 41 h incubation period. Inorganic phosphate was also found when the gels were electrophoresed for shorter times, but normally most of the phosphate was eluted into the anode buffer because of its faster mobility, compared to the phospholipids.

The rhodopsin obtained from the light-adapted frogs was phosphorylated (Fig. 1, L_1 and L_2), but that from the dark-adapted frogs was not. One of the dark-adapted frogs (Fig. 1, D) was dark-adapted for 2 d before being killed and the other (LD) was dark-adapted for only 75 min following an illumination period of 20 min; ^{32}P was not bound to the rhodopsin of either animal. This indicates that rhodopsin was phosphorylated when the frogs were exposed to light and dephosphorylated during dark adaptation.

Quantitative analyses indicate that phosphorylation of rhodopsin in the light adapted frogs was about 20 times that in the completely dark-adapted frogs (Fig. 2). The average ^{32}P bound to the rhodopsin of the seven light-adapted frogs was 5.1×10^{-8} per μg of rhodopsin (s. d.: $\pm 1.1 \times 10^{-9} \mu\text{g}^{-1}$), compared to $0.25 \times 10^{-9} \mu\text{g}^{-1}$ in completely dark-adapted frogs. No significant difference in the final yield of ^{32}P -rhodopsin could be observed between retinas and ROS from light-adapted frogs prepared in white light (Fig. 2, frogs 1, 4, 6, and 7) and those prepared in dim red light (Fig. 2, frogs 2, 3 and 5). This indicates that no significant, light-dependent changes in the phosphorylation level occurred during the 40 min preparation period at 0°C .

In the eight frogs which were dark adapted after a 20 min illumination period, the phosphorylation level of rhodopsin decreased slowly with increasing time in the dark, and after approximately 1 h the dephosphorylation was complete. If the dephosphorylation kinetics were first order, its half time would be about 13 min (Fig. 2, dotted line); but quantitative kinetic analysis is not possible because of animal to animal variations and the small number of animals used.

The slow rate of dephosphorylation shown in Fig. 2 is comparable to published rates of dark adaptation measured by psychophysical^{12,13} and electrophysiological^{14,15} methods. Kohlrausch¹² has shown that complete recovery of psychophysical sensitivity during dark adaptation in the human eye takes about 1 h. Similarly, electrophysiological studies on isolated retinas of frog¹⁴ and *Necturus*¹⁵ show that, following extensive bleaching, recovery of sensitivity occurs within about an hour.

Evidence has increased in the past few years that, besides 'neural' control mechanisms, substantial parts of dark adaptation take place in the photoreceptors themselves¹⁴⁻¹⁷. Rushton¹⁶ has demonstrated by combined psychophysical and spectrophotometric measurements that during slow dark adaptation, recovery of the logarithm of sensitivity occurs exactly in parallel with photopigment regeneration. On the other hand, electrophysiological experiments^{14,17} on isolated retinas in which regeneration of rhodopsin is believed to be negligible, demonstrated that slow recovery of sensitivity by 2-4 decades occurs at the receptor level even though regeneration is minimal. Thus there must be mechanism(s) of slow dark adaptation at the receptor level which are independent of photopigment regeneration. My experiments give evidence of a chemical reaction, namely dephosphorylation of phosphorylated rhodopsin, which takes place in the photoreceptors under the conditions and at about the rate of dark adaptation. This dephosphorylation seems to be independent of photopigment regeneration,

as recent experiments with isolated frog retinas have indicated (H.K., and Bader, unpublished). We have also shown⁴ that the regeneration of rhodopsin *in vitro* is not affected by the binding of phosphate to opsin, nor is the binding affected by regeneration; phosphorylated opsin regenerated with 11-*cis* retinal to form phosphorylated rhodopsin, and gave as good a result as in the nonphosphorylated control experiment, without any loss of phosphate during the regeneration.

The molecular mechanism by which this phosphorylation reaction regulates the sensitivity of the rods remains to be elucidated. As suggested earlier⁴, the phosphate group bound to rhodopsin and/or opsin in the light adapted state could alter the permeability of the membrane in which it is located and thus regulate ion fluxes through adjacent pores. This membrane could be the disk as well as the cell plasma membrane which has also been shown to contain rhodopsin¹⁸. Phosphorylation could also influence the binding of Ca^{2+} to the photoreceptor membranes. It is likely that during dark adaptation the phosphate is cleaved from rhodopsin by the action of a phosphatase.

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- ¹ Kühn, H., and Dreyer, W. J., *FEBS Lett.*, **20**, 1-6 (1972).
- ² Bownds, D., Dawes, J., Miller, J., and Stahlman, M., *Nature new Biol.*, **237**, 125-127 (1972).
- ³ Frank, R. N., Cavanagh, H. D., and Kenyon, K. R., *J. biol. Chem.*, **248**, 596-609 (1973).
- ⁴ Kühn, H., Cook, J. H., and Dreyer, W. J., *Biochemistry*, **12**, 2495-2502 (1973).
- ⁵ Dowling, J. E., and Ripps, H., *J. gen. Physiol.*, **60**, 698-719 (1972).
- ⁶ Bownds, D., Dawes, J., and Miller, J., in *Biochemistry and Physiology of Visual Pigments* (edit. by Langer, H.), 267-273 (Springer-Verlag, Berlin, Heidelberg, New York, 1973).
- ⁷ Hall, M. O., and Bacharach, A. D. E., *Nature*, **225**, 637-638 (1970).
- ⁸ McConnell, D. G., *J. Cell Biol.*, **27**, 459-473 (1965).
- ⁹ Fairbanks, G., Steck, T. L., and Wallach, D. F. H., *Biochemistry*, **10**, 2606-2617 (1971).
- ¹⁰ Bownds, D., Gordon-Walker, A., Gaide-Huguenin, A. C., and Robinson, W., *J. gen. Physiol.*, **58**, 225-237 (1971).
- ¹¹ Bligh, E. G. and Dyer, W. J., *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).
- ¹² Kohlrausch, A., *Handb. Norm. Pathol. Physiol.*, **12/2**, 1499-1594 (Springer-Verlag, Berlin, 1931).
- ¹³ Rushton, W. A. H., *J. Physiol., Lond.*, **156**, 193-205 (1961).
- ¹⁴ Hood, D. C., Hock, P. A., and Grover, B. G., *Vision Res.*, **13**, 1953-1963 (1973).
- ¹⁵ Norman, R. A., and Werblin, F. S., *J. gen. Physiol.*, **63**, 37-61 (1974).
- ¹⁶ Rushton, W. A. H., and Powell, D. S., *Vision Res.*, **12**, 1073-1081 (1972).
- ¹⁷ Grabowski, S. R., Pinto, L. H., and Pak, W. L., *Science*, **176**, 1240-1242 (1972).
- ¹⁸ Jan, L. Y., and Revel, J. P., *J. Cell Biol.* (in the press).

Regulation of arginine catabolism in *Aspergillus nidulans*

In *Aspergillus nidulans*, mutations at more than 20 loci result in increased levels of two arginine catabolic enzymes, arginase and ornithine δ -transaminase (OTase)¹. The large number of genes involved either directly or indirectly in regulation of arginine catabolism makes interpretation of the regulatory mechanism(s) complicated. Some of these genes at least are concerned with catabolite repression rather than with control of specific induction and repression processes². Here we

present evidence that the synthesis of arginase and OTase in *A. nidulans* is regulated in a positive fashion. Interrelations between the gene responsible for positive control and several other genes involved in the regulation of these enzymes were established. This enabled us to propose a model of the regulatory mechanism involving interaction between a specific positive and a nonspecific negative mode of regulation and providing a possible explanation of ammonium repression.

In *A. nidulans* arginine can serve as a source of proline both in the wild-type strain and in proline mutants blocked in the first two steps of the main pathway of proline synthesis. Use of arginine for proline synthesis depends on the presence and on the inducibility of arginine catabolic enzymes, arginase and OTase. Mutants with derepressed arginase and OTase were obtained in *A. nidulans* as suppressors of proline mutations^{3,4}. A mutant designated *arcA*^d 47 (former symbol *suG* 47 *pro*) (ref. 2) shows approximately 10-fold higher levels of both enzymes compared with those in the wild-type strain (Table 1). In heterokaryons and diploids *arcA*^d 47 is semidominant over the wild-type allele. By means of the haploidisation technique⁵ the *arcA* locus was assigned to linkage group VIII. The *arcA* locus is not linked to the structural genes for arginase and OTase.

We selected for mutants unable to use arginine as a source of proline and isolated mutants in the arginase and OTase structural genes, mutants defective in arginine transport (unpublished data), mutants hypersensitive to catabolite repression² and mutants designated by the *arcA*^r symbol. In *arcA*^r1 mutant the uninduced levels of arginase and OTase are the same as in the wild type, but neither enzyme is inducible by exogenous arginine. On the basis of recombination tests the *arcA*^r and *arcA*^d mutations seemed likely to be located within the same gene as no *arcA*⁺ recombinants were found among 600 progeny of the cross *arcA*^r × *arcA*^d. This assumption was strengthened by the results of studies on the ultraviolet-induced revertants of the *arcA*^r1 mutant. One of the revertants was found to exhibit all properties of the *arcA*^d47 mutant. By crossing this revertant to the wild type and *arcA*^d47 strains it was established that the reversion was due to mutation in or very close to the *arcA* locus. The *arcA*^r1 mutation is recessive to the *arcA*^d47 and *arcA*⁺ alleles.

The simplest interpretation of the properties of the *arcA*^r and *arcA*^d mutants is that the *arcA* gene specifies the product (inducer) which is necessary for expression of the arginase and OTase structural genes. In the wild type the inducer requires activation by the coinducer, arginine, whereas in the *arcA*^d47 mutant the inducer is active even in the absence of arginine. In the *arcA*^r1 mutant the inducer is inactive or it is not produced.

Table 1 Arginase and OTase activities in various mutants

No. Mutant*	Enzyme activity†			
	Arginase MM	OTase MM	MM + arg	MM + arg + NH ₄
(1) Wild type	0.20	7	110	30
(2) <i>arcA</i> ^r 1	0.20	5	5	5
(3) <i>arcA</i> ^d 47	4.0	50	105	95
(4) <i>suA25pro</i>	2.0	70	130	120
(5) <i>suD25pro</i>	2.0	75	140	130
(6) <i>arcA</i> ^r 1 <i>suA25pro</i>	0.20	10	22	—
(7) <i>arcA</i> ^r 1 <i>suD25pro</i>	0.15	6	7	—
(8) <i>arcA</i> ^d 47 <i>suA25pro</i>	4.0	180	190	—
(9) <i>arcA</i> ^d 47 <i>suD25pro</i>	4.0	200	200	—

Mycelia for enzyme assays were grown on standard minimal medium (MM) (10 g glucose l⁻¹ as carbon source, 10 mM sodium nitrate as nitrogen source) and transferred when necessary to media containing 5 mM arginine or 5 mM arginine and 5 mM ammonium tartrate for 6h.

* Genotypes of mutants: nos 1–4—*proA6 paba9 biA1*; nos 5, 7, 9—*proA6 adF9 y; phenA2*; nos 6, 8—*proA6 paba9 biA1; phenA2*.

† Units of arginase and OTase activity are that amount of enzyme which forms 1 μmol of urea and 1 μmol of glutamic γ-semialdehyde per min per mg protein respectively.

Arginase and OTase synthesis in *A. nidulans* is subject to both glucose and nitrogen catabolite repression. The mechanism of catabolite repression in fungi is not yet fully understood, but ammonium seems to be involved in nitrogen catabolite repression.⁶ One of the effects of ammonium is the prevention of induction of arginase and OTase by arginine⁶. Similar effects of ammonium on arginase and OTase induction were observed in yeasts^{7,8} and on induction of several other catabolic enzymes in *A. nidulans* and *Neurospora crassa*⁹.

The arginase and OTase levels in the *arcA*^d47 mutant are not fully derepressed and the synthesis of both enzymes can still be induced by exogenous arginine (data for OTase are given in Table 1). In contrast to the wild type, the presence of ammonium in the culture medium does not prevent induction of arginase and OTase in the *arcA*^d47 mutant. Assuming that the function of the *arcA* gene is interpreted correctly, it can be concluded that ammonium interferes with the process of activation of the *arcA* gene product (inducer) by arginine. As a result of the *arcA*^d47 mutation the inducer is able to function in the absence of arginine (although it retains some affinity for arginine) and at the same time becomes insensitive to the effects of ammonium.

Apart from the *arcA*^d47 mutation, mutations in six *supro* loci (designated respectively *A*, *D*, *E*, *H*, *J* and *L*) result in derepression of arginase and OTase to the level exceeding

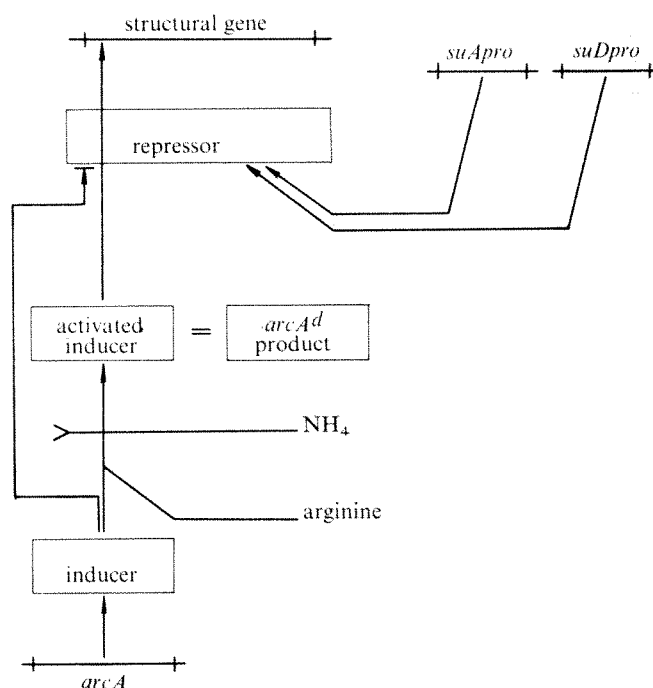


Fig. 1 Regulation of arginine catabolism.

approximately 10-fold the basal level of these enzymes observed in the wild-type strain¹. The levels of both enzymes are not repressed when *supro* mutants are grown in the presence of ammonium. All 25 mutations at these loci which we have studied are recessive and none of the loci is closely linked to any other or to the loci of the arginase and OTase structural genes. Two mutants, *suA25pro* and *suD25pro* were chosen and crossed to the *arcA*^d47 and *arcA*^r1 strains. The appropriate double mutants were selected from the progeny of these crosses and assayed for arginase and OTase activity. The results given in Table 1 can be summarised as follows: (1) The effects of *suA25pro* and *arcA*^d47 mutations are additive, as are the effects of *suD25pro* and *arcA*^d47. The level of both enzymes in double mutants corresponds to their maximal level observed in the wild type grown in the presence of arginine. (2) The *arcA*^r1 mutation cancels the effects of both *suA25pro* and *suD25pro* mutations and makes arginase and OTase

noninducible by arginine in *suA* and *suD* strains. The *arcA*¹ mutation is thus fully epistatic to the two suppressor mutations studied.

It can be concluded that the *suApro* and *suDpro* genes participate in the formation of a repressor which maintains the arginase and OTase synthesis at the low level observed in the wild-type strain grown in the absence of arginine. The function of the repressor is abolished by the activated inducer specified by the *arcA* gene. Activation of the inducer is necessary only to overcome the effects of repression exerted by the products of *suApro*, *suDpro* and possibly other *supro* genes: when the repressor is not active as in *suA25pro* and *suD25pro* mutants, the synthesis of arginase and OTase is derepressed despite the absence of arginine. On the other hand, inactivation of repressor alone is not sufficient to cause derepression of enzyme synthesis. Low levels of arginase and OTase in *arcA*¹ *suA25pro* and in *arcA*¹ *suD25pro* double mutants indicate that the presence of the normal product of the *arcA* gene is obligatory for derepression to occur. All above considerations are summarised in Fig. 1.

We have no information concerning the nature of the *suApro* and *suDpro* gene products. Thus we do not claim that these genes participate in formation of repressor equivalent to that of the *Escherichia coli* lactose system. This would be premature, especially knowing that in its formation not only *suApro* and *suDpro* but probably also four other genes are involved. Moreover, there is some information that mutations in these genes, in addition to controlling arginine catabolism, affect some other systems; for example they cause derepression of nitrate reductase synthesis (unpublished results). It was also found that the *suD19pro* mutation affects the synthesis of ornithine transcarbamylase¹⁰ and cancels certain effects of mutations in the *areA* gene which controls use of a variety of nitrogen sources⁹. It seems therefore that if *suDpro* and other genes of this group are responsible for formation of repressor of some kind, this repressor is highly nonspecific.

As pointed out by Gross¹¹, in fungi the positive rather than negative control of gene expression is the rule. The only exception so far was found by Wiame and his coworkers⁸ who proved the existence of operator genes in the arginine system in yeasts. The universality of positive regulation suggests that in fungi general regulatory mechanisms (those which control expression of genes concerned with many different enzymatic systems) can operate at the level of activation of inducers specific for structural genes of a single system. Such an interpretation was used in this paper to explain the effects of ammonium on the synthesis of arginine catabolic enzymes. We do not postulate that this is the only way in which ammonium affects the synthesis of catabolic enzymes. For example, ammonium also seems to participate in the mechanism responsible for maintaining the basal level of several enzymes^{2,6,12,13}. It is also probable that the inhibitory effect of ammonium upon induction of catabolic enzymes is mediated by the product(s) of particular gene(s), possibly including the *areA* gene which has recently been studied in several laboratories (see ref. 9).

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¹ Klimczuk, J., and Weglenski, P., *Acta microbiol. pol.*, **A6**, 93-100 (1974).

² Bartnik, E., Guzewska, J., and Weglenski, P., *Molec. gen. Genet.*, **126**, 85-92 (1973).

³ Weglenski, P., *Genet. Res.*, **8**, 311-321 (1966).

⁴ Weglenski, P., *J. gen. Microbiol.*, **47**, 77-85 (1967).

⁵ McCully, K. S., and Forbes, E., *Genet. Res.*, **6**, 352-359 (1965).

⁶ Bartnik, E., Piotrowska, M., and Weglenski, P., *Molec. gen. Genet.*, **126**, 75-84 (1973).

⁷ Middelhoven, W. J., *Antonie van Leeuwenhoek*, **36**, 1-19 (1970).

⁸ Wiame, J. M., *Curr. Top. Cell. Regulation*, **4**, 1-38 (1971).

⁹ Arst, H. N., jun., and Cove, D. J., *Molec. gen. Genet.*, **126**, 111-141 (1973).

¹⁰ Cybis, J., Piotrowska, M., and Weglenski, P., *Molec. gen. Genet.*, **118**, 272-277 (1972).

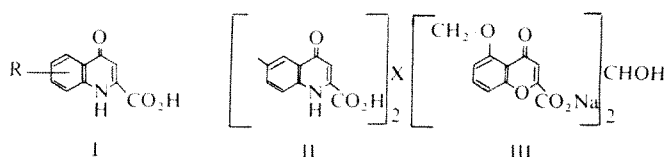
¹¹ Gross, S. R., *A. Rev. Genet.*, **3**, 395-424 (1969).

¹² Hynes, M. J., *J. Bact.*, **103**, 482-487 (1970).

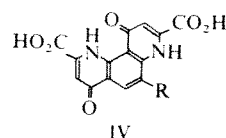
¹³ Pateman, J. A., Kinghorn, J. R., Dunn, E., and Forbes, E., *J. Bact.*, **114**, 943-950 (1973).

Inhibition of allergic reactions by a novel phenanthroline ICI 74,917

FOR 4 yr we have been investigating the biological properties of a series of kynurenic acids (I) and bridged analogues (II), related to the chromone carboxylic acids, of which disodium cromoglycate (III) is an example. Some of the quinolone acids I and II were found to be as effective as disodium



cromoglycate in inhibiting passive cutaneous anaphylaxis (PCA) in rats^{1,2}. Compounds of much greater activity in PCA were however discovered among a series of tricyclic quinolone acids, the 2,8-dicarboxy-4,10-dioxo-1,4,7,10-tetrahydrophenanthrolines (IV)³. Here we describe the properties of one of the more active of these compounds, ICI 74,917 (IV, R = *n*-butyl). It can be prepared by the reaction of 1,3-diamino-4-*n*-butyl benzene with oxaloacetic ester and thermal cyclisation of the bis-anil to the phenanthroline-2,8



dicarboxylate which is then hydrolysed to ICI 74,917. It melts at 300° C (decomposition) and is insoluble in most organic solvents although its alkali metal salts are soluble in water. It may exist in tautomeric forms other than that indicated.

Administered intravenously as the sodium salt, at the time of antigenic challenge, ICI 74,917 inhibited PCA provoked by reaginic (IgE-like) antibody prepared according to Mota⁴ against egg albumin or according to Ogilvie⁵ against the nematode parasite *Nippostrongylus brasiliensis*. A comparison of the ID₅₀ (the dose required to produce a 50% inhibition of PCA) of each compound revealed that ICI 74,917 was some 300 times more active than disodium cromoglycate (Table 1).

In vitro, ICI 74,917 was not an antagonist of histamine or 5-hydroxytryptamine. When administered intravenously, it did not inhibit blueing reactions provoked in the normal rat by intradermal injections of histamine, 5-hydroxytryptamine or Compound 48/80 but PCA reactions induced in this species by heat-stable (IgG) homocytotropic and heterologous (Guinea pig and rabbit) antibodies were significantly and consistently inhibited to a slight extent (30%).

It seemed possible, on the basis of these findings that ICI 74,917 might prevent the release of inflammatory medi-

ators from mast cells which follows interaction of antibody and antigen. This hypothesis was supported by the demonstration that ICI 74,917, when present at antigenic challenge at concentrations between 10^{-8} M and 10^{-6} M, reduced significantly the amount of histamine liberated from rat peritoneal mast cells sensitised *in vitro* with reaginic antibody.

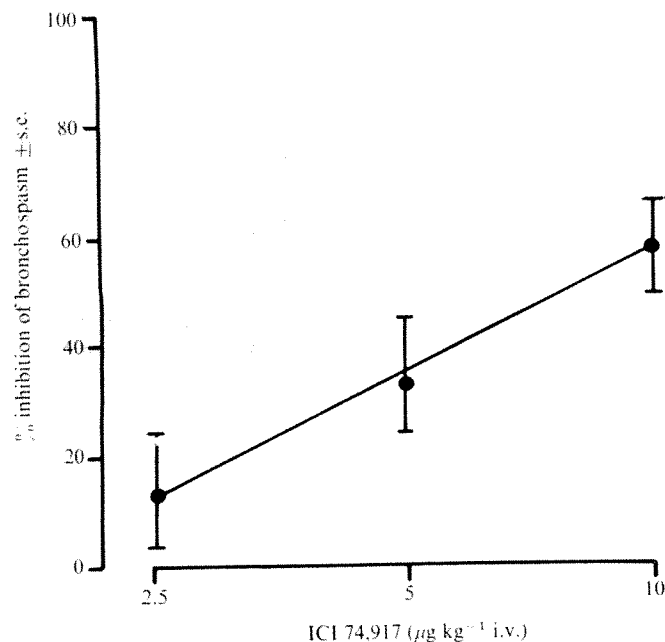


Fig. 1 Inhibition of allergic bronchospasm in the guinea pig by ICI 74,917. Groups of 10 animals were passively sensitised with guinea pig anti-egg albumin serum and challenged 18 h later with antigen (1 mg), and with saline or varying doses of ICI 74,917 in saline. Bronchospasm was assessed by the Konzett-Rössler technique as modified by Davies and Johnston⁷. Results are expressed as % inhibition of bronchospasm (\pm s.e.) 4 min after antigenic challenge compared with sensitised, control animals given antigen and saline alone.

ICI 74,917 also possesses anti-allergic activity in other species. In the mouse, PCA induced by a reaginic antibody prepared according to Mota⁶ was inhibited, but the amount (0.5–2.5 mg kg⁻¹ intravenously (i.v.)) of compound required to achieve a regular dose dependent inhibition (27 to 67%) was higher than that required in the rat. Although there was no evidence of bronchodilator activity in the guinea pig, the compound, administered intravenously, conferred partial protection against systemic anaphylaxis and at doses comparable to those necessary to inhibit PCA in the rat, reduced in a dose-dependent manner allergic bronchospasm (Fig. 1) in guinea pigs passively sensitised with an homologous nonreaginic antibody prepared according to Davies *et al.*⁷. On the other hand, ICI 74,917, even at relatively high doses, did not inhibit PCA induced with the same antibody, suggesting in this species, some tissue specificity for the compound. Disodium cromoglycate showed no activity in these models in the mouse or guinea pig at doses up to 50 mg kg⁻¹ i.v..

Table 1 A comparison of ICI 74,917 and disodium cromoglycate on reagin-mediated PCA

Compound	Antigen-antibody system	ID ₅₀ (mg kg ⁻¹ i.v. \pm 95% confidence limits)	Relative potency (\pm 95% confidence limits)
Disodium cromoglycate	Egg albumin	2.8 \pm 0.2	1.0
	<i>N. brasiliensis</i>	3.4 \pm 0.2	1.0
ICI 74,917	Egg albumin	0.009 \pm 0.0005	323 \pm 30
	<i>N. brasiliensis</i>	0.01 \pm 0.0006	308 \pm 24

These studies have shown that ICI 74,917 exhibits anti-allergic properties additional to those possessed by disodium cromoglycate in that it is effective in laboratory species other than the rat. ICI 74,917 may therefore be of value in the treatment of allergic conditions in man.

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- ¹ Evans, D. P., Gilman, D. J., O'Mant, D. M., and Thomson, D. S., UK Patent 1,334,705.
- ² Waring, W. S., UK Patent 1,305,820.
- ³ Waring, W. S., UK Patent 1,308,787.
- ⁴ Mota, I., *Immunology*, **7**, 681–699 (1964).
- ⁵ Ogilvie, B. M., *Immunology*, **12**, 113–131 (1967).
- ⁶ Mota, I., *Immunology*, **12**, 343–348 (1967).
- ⁷ Davies, G. E., and Johnston, T. P., *Int. Archs Allergy*, **41**, 648–654 (1971).

Regulation of clonal development of immune responding cells by antibody of maternal origin

PASSIVELY administered antibody can regulate homologous antibody production in adult animals but the mechanisms are not completely understood¹. Maternal antibody transmitted to the foetus also has such an effect². This effect is usually transient and reversible; animals regain the ability to respond to antigen after passively administered antibody is removed^{3,4}. Adoptively transferred spleen cells from donor animals previously exposed to antibody are also able to respond normally⁵. Mature B and T cells seem also not to be affected by exposure to passively administered antibody^{6,7}.

It is not known if passively administered antibody has any influence upon the clonal development of immune cells. So, we studied the effect of the administration of specific maternal antibody on the ability of progeny to mount antibodies against the appropriate antigen. This was undertaken because in certain species the progeny are exposed to maternal antibody *in utero* during the course of foetal clonal development of immune cells⁸. We found that clonal cells responding to at least some antigens may proliferate as a consequence of being exposed to homologous antibody during foetal or neonatal life.

Female C₃H/He mice were immunised several times with sheep red blood cells (SRBC) before mating. The progeny,

Table 1 Plaque-forming ability of normal and SRBC mice to SRBC in adoptive transfer experiments

Donor spleen	No. of mice	Average PFC in recipient spleens per 10 ⁶ donor spleen cells \pm s.e.
Normal mice	10	538 \pm 344
SRBC mice*	11	506 \pm 291

Spleen cells of 3-week-old normal or SRBC mice were transferred to syngeneic normal X-irradiated mice. Recipient mice were immunised intravenously with 4×10^8 SRBC 8 d before being assayed for their splenic PFC response. PFC to SRBC was assayed as described⁹. PFC in recipients that received either no antigen or no donor cells were negligible.

* Progeny mice whose mother had received SRBC immunisation several times before mating.

SRBC mice, were bled at 3 weeks of age and spleen cells were transferred to normal syngeneic X-irradiated mice. The numbers of plaque-forming cells (PFC) to SRBC amongst the recipient spleen cells were assayed 8 d after antigen injection. Spleen cells from progeny of nonimmune mothers were treated and tested in the same way as a control.

It was found that the number of PFC to SRBC amongst the spleen cells of recipient mice were similar whether recipients received donor spleen cells from normal or SRBC mice (Table 1). At the time of cell transfer, SRBC mice had a high titre of anti-SRBC antibody in their serum which should have been sufficient to suppress active production of antibody to SRBC. These results suggest that maternal antibody against SRBC does not affect the foetal development of specific immune responding cells.

When similar experiments were done using DNA as the test antigen different results were obtained. Female C₃H/He mice were immunised several times with denatured calf thymus DNA to attain high titres of anti-DNA antibody. The progeny, DNA mice, from immunised mothers were then treated in the same way as SRBC mice substituting DNA as the specific antigen. The numbers of PFC to calf thymus DNA amongst spleen cells from recipients who received spleen cells from DNA mice were three to four times those of the controls (Table 2). Although the number

Table 2 Plaque-forming ability of normal and DNA mice to DNA in adoptive transfer experiments

Donor spleen	No. of mice	Average PFC in recipient spleens per 10 ⁶ donor spleen cells \pm s.e.	
		Exp. 1	Exp. 2
Normal mice	3	40.1 \pm 2.7	41.2 \pm 12.9
DNA mice*	3	117.9 \pm 19.0	161.7 \pm 37.7

Experimental procedures are essentially the same as those in Table 1. Heat-denatured calf thymus DNA was conjugated with equal amounts of methylated bovine serum albumin and emulsified with complete Freund's adjuvant. Recipient mice were challenged to a single injection of antigen (100 μ g as DNA) subcutaneously. Anti-DNA PFC were assayed using SRBC coupled with DNA by chromium chloride¹¹. PFC in recipients that received either no antigen or no donor cells were negligible.

* Progeny mice whose mother had received DNA immunisation several times before mating.

of animals studied and the number of antibody-producing cells were small, the differences were significant.

Another experiment also showed the increased PFC response to DNA in DNA mice. Maternal anti-DNA antibody in DNA-mice became undetectable by 6 weeks of age. Normal and DNA mice were challenged with DNA at this age and the PFC response in their spleens was compared 4 d after DNA injection. As shown in Table 3, PFC in the spleen cells of DNA mice were much more than those of control mice.

The enhanced ability of DNA mice to respond to DNA is probably mediated by passively transferred maternal antibody, although the possible involvement of other factors such as passively transferred antigen, carrier protein, and adjuvant were not considered in these experiments. The observed effect of anti-DNA antibody (presumably maternal) in the progeny seems to be different from the suppressive effect of antibody on active antibody production in adult mice, as the enhanced ability to respond to DNA in DNA mice remained even after removal of antibody.

Increased PFC response to DNA in DNA mice may be interpreted as a result of maternal anti-DNA antibody influencing the clonal development of DNA-specific immune responding cells in the progeny. We do not know to what extent these observed effects of maternal antibody are

Table 3 PFC response in normal and DNA mice challenged to DNA

Group	No. of mice	Average PFC per spleen \pm s.e.
Normal mice	7	1,644 \pm 750
DNA mice	6	4,642 \pm 2,111

Six-week-old normal and DNA mice were challenged to a single injection of DNA and were assayed for their splenic PFC response 4 d after antigen injection.

general. Antigen selectivity clearly exists. DNA is a weak antigen, probably because of its self-antigen nature¹⁰. The clonal development of immune responding cells to DNA may be suppressed during ontogeny of the immune systems, and this may in some way be modified by maternal anti-DNA antibodies. Antigen selectivity and the mechanism of this phenomenon need further clarification. A detailed paper will be published separately.

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- Uhr, J. W., and Möller, G., *Adv. Immun.*, **8**, 81-127 (1968).
- Solomon, J. B., Rindell, G. S., and White, D. F., *Immunology*, **22**, 219-226 (1972).
- Graf, W. M., and Uhr, J. W., *J. exp. Med.*, **130**, 1175-1186 (1969).
- Möller, G., *J. exp. Med.*, **127**, 291-306 (1968).
- Möller, G., *Transplantation*, **2**, 405-415 (1964).
- Haughton, G., and Cooper, M. Y., *Cell. Immun.*, **8**, 384-394 (1973).
- Abrahams, S., Phillipsad, R. A., and Miller, R. G., *J. exp. Med.*, **137**, 870-892 (1973).
- Brambell, F. W. R., in *Transmission of passive immunity from mother to young*, **18**, 13 (North-Holland Research Monographs, Frontiers of Biology, 1971).
- Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell bound antibodies* (edit. by Amos, B., and Koprowski, H.) 109 (Wistar Institute Press, Philadelphia, 1963).
- Plescia, O. J., and Braun, W., *Adv. Immun.*, **6**, 231-252 (1967).
- Sweet, G. H., and Welborn, F. L., *J. Immun.*, **106**, 1407-1410 (1971).

Activation of suppressor T cells by tumour cells and specific antibody

IMMUNOSUPPRESSION by passively administered antibody has long been known (see ref. 1 for review) and as antibody can cause enhancement of tumour growth^{2,3}, particular attention has been paid to the role of antibody-mediated immunosuppression in tumour immunology. Hellstrom *et al.* have emphasised that blocking factors, most likely antigen-antibody complexes⁴, suppress cell-mediated immunity and thus play a major part in the progression of tumour growth⁵. Such blocking factors may be an important factor in the induction and/or maintenance of some forms of immunological tolerance⁶⁻⁸. But the mechanism remains obscure.

Some thymus-derived lymphocytes (T cells) may suppress

the immune response of other cells^{9,10}. These suppressor T cells¹¹, like antigen-antibody complexes, may play an important part in the induction of some forms of tolerance^{12,13}, and antigen-antibody complexes may exert their immunosuppressive effects indirectly by preferential activation of suppressor T cells. Since the effective cell-mediated immune response to tumours usually requires the presence of T cells, it is difficult to demonstrate an additional suppressor population of T cells by such manoeuvres as thymus deprivation, which may deplete effector as well as suppressor cells.

Previous studies have shown that hyperimmune isoantibody to leukaemia L-1210 can 'centrally' inhibit the development of spleen cell-mediated immunity in C57BL/6 mice¹⁴. Antibody was also shown to inhibit the development of macrophage-mediated immunity¹⁵. Mice treated with antibody lacked the enlarged vacuolated 'activated' macrophages which are usually observed in these mice 10 d after challenge with tumour. The peritoneal cells of mice pre-treated with antibody before inoculation with L-1210 were small, lacked stainable acid phosphatase granules, and showed no attached or engulfed tumour cells. Moreover, the addition of proved cytophilic antibody to L-1210 cells. The monocytes could, however, form rosettes with sheep erythrocytes¹⁵ or EL-4 leukaemia cells¹⁶ in the presence of specific cytophilic antibody. The suppression was long lived, lasting 14-18 d, and requiring specific antigen as well as antibody to be produced.

Since the assay for this macrophage function does not require the presence of T cells, we used it to investigate whether suppressor T cells are involved in passive enhancement of tumour growth. Our results suggest they do, since thymus deprivation completely eliminates the suppressive effects of passive antibody, as judged by the ability of macrophages to bind specific tumour cells in the presence of cytophilic antibody.

C57BL/6 male mice from Jackson Laboratories, Bar Harbor, Maine, were thymectomised when 7 weeks old or sham thymectomised. One week later all mice were given 850 rad of X irradiation from a Siemens stabilipan 250 kV source, at a dose rate of 85 rad min⁻¹. On the same day 5×10^6 bone marrow cells from 7-8-week-old syngeneic mice were inoculated into the tail vein.

Two experiments were performed; one 5 weeks after irradiation and the other after 13 weeks. The results of the two separate experiments were similar and the pooled results are given in Table 1. A single dose of 0.4 ml of hyperimmune antibody raised in C57BL/6 mice by multiple immunisations with 5×10^6 L-1210 cells was given intraperitoneally (i.p.) 1 d before tumour. Mice were then challenged i.p. with either 5×10^6 live L-1210 cells or 25×10^6 or 50×10^6 L-1210 cells, killed by previous treatment with mitomycin C (2 mg per 80×10^6 tumour cells at 37° C for 60 min).

Cytophilic antibody (0.3 ml) was injected with 2.5×10^6 L-1210 cells i.p. into mice 10 d later, 5 h before killing.

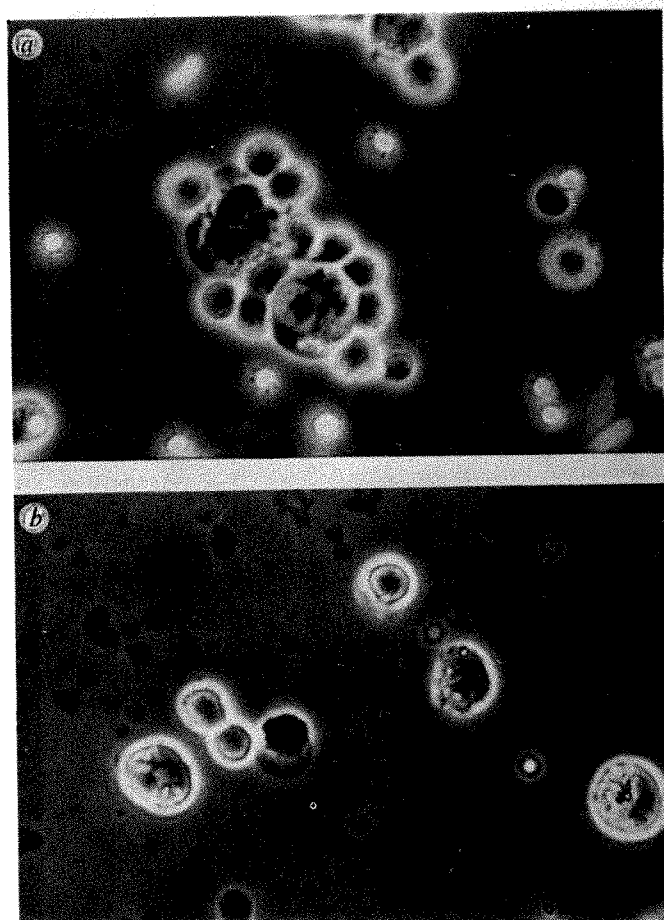


Fig. 1 *a*, Representative field from a 3+ positive slide. Thirteen macrophage cells surround three tumour cells in a clump. *b*, Representative field from a negative slide. Three macrophages without any attached tumour cells are present. Three tumour cells are also present. The dark cell in the middle is unidentifiable.

After killing the peritoneal cavity was lavaged with 6 ml of Fischer's medium. The lavage fluid was centrifuged at 1,000 r.p.m. for 6 min and the cell pellet was gently re-suspended. Covered wet-droplet preparations on a glass slide were coded and then examined 'blind' using a phase contrast microscope. Previous studies have shown that the assay can be done entirely *in vitro* and gives similar results. Results were given an arbitrary 'score' of 0 to 4+ after recording the percentage of macrophages with attached tumour cells and the number of tumour cells that attached to each macrophage. The appearance of the tumour cells and the macrophages (size, presence of refractile granules,

Table 1 Ability of macrophages of lethally-irradiated bone marrow restored C57BL/6 mice given various treatments to attach L-1210 tumour cells in the presence of cytophilic antibody

Treatment	No. of mice	Score assay for rosettes (\pm s.d.)†	% of macrophages with attached tumour cells (\pm 5%)
Thymectomy			
Sham	6	2.3 ± 0.5	50
Sham	10	0.4 ± 0.5	< 10
Sham	6	0.0 ± 0	< 10
+	7	2.6 ± 0.5	45
+	9	2.2 ± 0.4	45
+	4	2.3 ± 0.3	40
Antibody (day 11) Tumour (day 10)			
—	6	2.3 ± 0.5	50
+	10	0.4 ± 0.5	< 10
+	6	0.0 ± 0	< 10
+	7	2.6 ± 0.5	45
+	9	2.2 ± 0.4	45
+	4	2.3 ± 0.3	40

* Mitomycin C-treated tumour

† See text

degree of vacuolation and the presence or absence of clumping were also recorded. A score of 0=less than 10% of peritoneal macrophages with attached tumour cells; 1+ = 10–30%; 2+ = 30–50%; 3+ = 50–75%; and 4+ = 75–100% with attached tumour cells.

At least two slide preparations were read for each experimental animal. The mean and standard deviation of the arbitrary scores were calculated for ease of comparison.

Pretreating mice with isoantibody without also giving tumour has no effect on the attachment of macrophages to tumour cells¹⁵. Also, the peritoneal macrophages of normal mice not given isoantibody and inoculated with tumour 10 d previously, bind the tumour extensively whether or not additional cytophilic antibody is given¹⁵. These controls were not repeated in the present studies.

The results (Table 1) show that more than 30% of the macrophages from sham-thymectomised, lethally-irradiated, bone-marrow-reconstituted mice which were otherwise untreated attached L-1210 cells (about 3–5 cells per macrophage) (Fig. 1a) in the presence of cytophilic antibody. If these mice were treated with viable or mitomycin C-killed tumour cells plus isoantibody 10 d before assay, less than 10% of the macrophages attached tumour cells (Fig. 1b). Significantly, pretreatment with isoantibody and L-1210 did not alter the capacity to attach L-1210 cells in the thymus-deprived mice which attached tumour cells in a way indistinguishable from those of the sham-thymectomised untreated ('normal') animals.

These data clearly demonstrate the thymus dependence of the ability of antibody and tumour cells, perhaps acting as complexes, to suppress the binding of tumour cells to macrophages in the presence of cytophilic antibody.

The mechanism by which the T cells exerted their suppressive effect on macrophages is not clear. Others have shown that lymphocytes can make factors which specifically arm macrophages¹⁷. Perhaps they can also make specific factors which act to disarm macrophages by serving as receptors which transmit an inactivating signal when triggered by antigen. Alternatively, the arming factors in excess may act in an inhibitory fashion. Whatever the mechanism, the results clearly show that at least one aspect of antibody-induced immunosuppression is indirectly mediated through T cells. Another form of T-cell dependent antibody-mediated suppression is that produced by anti-allotype antibody¹⁸. Previous work has shown that T cells can suppress the immune functions of other T cells and B cells^{9–12}. Macrophages must now be added to the list of cells affected by suppressor T-cell activity. The precise significance of inhibition of macrophage-mediated immunity in the enhancement phenomenon remains to be determined.

The way in which antigen and antibody, perhaps acting as complexes, activate the suppressor T cells is also unclear. One possibility is that the antibody changes the molecular configuration of the antigen. It is well known that the immunogenicity of antigen is highly dependent on the physical state of antigen²⁰ and in so doing, perhaps, change the T cell response to it. The demonstration by Chan and Sinclair that removal of the Fc fragment from antibody reduces its immunosuppressive qualities²¹ suggests another possible mechanism. Some T cells may have receptors for the Fc portion of some antibodies and the attachment of antigen-antibody complexes at this site might cause them to exert a suppressor function.

In any case, our results offer a possible bridge between two recent observations: the thymus dependence of tolerance induction to some antigens^{12,13} and the presence of immunosuppressive antigen-antibody complexes in otherwise tolerant mice^{6–8}. The suppressive complexes may act through the agency of suppressor T cells.

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- ¹ Uhr, J. W., and Möller, G., *Adv. Immun.*, **8**, 81 (1968).
- ² Kaliss, N., and Molomut, N., *Cancer Res.*, **12**, 110 (1952).
- ³ Kaliss, N., and Kandutsch, A. A., *Proc. Soc. exp. Biol. Med.*, **91**, 118 (1956).
- ⁴ Sjögren, H. O., Hellstrom, I., Bansal, S. C., and Hellstrom, K. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1372 (1971).
- ⁵ Hellstrom, K. E., and Hellstrom, I., *A. Rev. Microbiol.*, **24**, 373 (1970).
- ⁶ Hellstrom, I., Hellstrom, K. E., and Allison, A. C., *Nature*, **230**, 49 (1971).
- ⁷ Phillips, M., Martin, W. J., Shore, A. R., and Wegmann, T. G., *Nature*, **234**, 146 (1971).
- ⁸ Voisin, G. A., *Progr. Allergy*, **15**, 328 (1971).
- ⁹ Katz, D. H., and Benacerraf, B., *Adv. Immun.*, **15**, 1 (1972).
- ¹⁰ Gershon, R. K., *Contemporary Topics in Immunobiology* (in the press).
- ¹¹ Gershon, R. K., Cohen, P., Hencin, R., and Liebhafner, S. A., *J. Immun.*, **108**, 586 (1972).
- ¹² Gershon, R. K., and Kondo, K., *Immunology*, **18**, 723 (1970).
- ¹³ Gershon, R. K., and Kondo, K., *Immunology*, **21**, 903 (1971).
- ¹⁴ Mitchell, M. S., *Cancer Res.*, **32**, 825 (1972).
- ¹⁵ Mitchell, M. S., and Moky, M. B., *Cancer Res.*, **32**, 832 (1972).
- ¹⁶ Mitchell, M. S., Moky, M. B., and Goldwater, D. J., *Proc. Am. Assoc. Cancer Res. (Abstr.)*, **13**, 62 (1972).
- ¹⁷ Evans, R., Grant, C. K., Cox, H., Steele, A., and Alexander, P., *J. exp. Med.*, **136**, 1318 (1972).
- ¹⁸ Herzenberg, L. A., and Herzenberg, L. A., *Contemporary Topics in Immunobiology* (in the press).
- ¹⁹ Dresser, D. W., *Immunology*, **6**, 378 (1962).
- ²⁰ Conway-Jacobs, A., Schechter, B., and Sela, M., *Biochemistry*, **9**, 4870 (1970).
- ²¹ Chan, P. L., Sinclair, N. R. StC., *Immunology*, **24**, 289 (1973).

Polyanions and lipopolysaccharide acts on different subpopulations of B cells

WITH respect to maturation stage, function, and cell surface constituents, lymphocytes bearing immunoglobulin on their (B cells) comprise a heterogeneous population. It has been suggested¹ that binding of mitogens to membrane surface constituents triggers the events leading to stimulation of cell mitosis. The various B cell mitogens can be divided into different chemical classes and different types of mitogens presumably react with different membrane surface constituents. According to these assumptions we tested whether various B cell mitogens act on the same or on different subpopulations of B cells.

Two kinds of experiments were performed. First, spleen cells of nude mice (BALB/c nu/nu lacking functioning T cells) were cultured with optimal doses of various B cell mitogens or with a combination of two different mitogens (both added to the cultures in optimal doses). Second, BALB/c mice 8–10 weeks old were thymectomised. One week later the mice were lethally irradiated and injected with syngeneic bone marrow cells (TXBM-mice) as described earlier². Spleen cells derived from TXBM-mice were cultured at various times after irradiation and after injection of bone marrow cells (XBM-procedure) with dextran sulphate (DS), with lipopolysaccharide from *Escherichia coli* O 55: B5 (LPS, Difco) or with phytohaemagglutinin-P (PHA, Difco). The results and the experimental details are given in Tables 1 and 2.

As already reported^{3,4} polyanions, for example DS (molecular weight 5×10^5 , Serva, Heidelberg) and double-stranded

Table 1 Effect of combinations of DS, poly (I), poly (C) and LPS on activation of DNA synthesis in nude spleen cells

Mitogen	Dose (μgml^{-1})	c.p.m. per culture	Stimulation index
—	0	852	1.0
DS	50	14,850	18.0
LPS	50	10,640	12.5
poly (I), poly (C)	200	7,071	8.3
DS + LPS	50 + 50	31,950	37.5
DS + poly (I), poly (C)	50 + 200	9,031	10.6
poly (I), poly (C) + LPS	200 + 50	17,380	20.4

Spleen cells suspensions ($2 \times 10^6 \text{ ml}^{-1}$) in a total volume of 3 ml were cultured in RPMI-1640 medium (Biocult) supplemented with 5% fresh heat inactivated (56°C for 30 min) human serum; 1-glutamine (2 mmol ml^{-1}), penicillin (100 U ml^{-1}) and streptomycin ($100 \mu\text{g ml}^{-1}$). The cultures were incubated in a humidified atmosphere of CO_2 : air, 5 : 95 at 37°C for 48 h. The mitogens were dissolved in sterile saline (LPS boiled for 1 h) and 0.1 ml of each solution added to the cultures. Controls received saline instead of mitogens. Two μCi of tritiated thymidine (specific activity 2 Ci mmol^{-1} , Radiochemical Centre, Amersham) were added for the last 4 h of incubation. The radioactivity in the trichloroacetic acid-insoluble fraction was determined as described earlier². The results are expressed as c.p.m. per culture and as stimulation indices (c.p.m. in mitogen treated culture/c.p.m. in control culture). Each value represents the mean of c.p.m. detected in five individual cultures. Standard deviation was less than 10%.

homopolyribonucleic acids are B cell mitogens. As shown in Table 1, spleen cells gave an additive response to DS and LPS or to poly (I) + poly (C) and LPS, when both types of mitogens were added simultaneously to the cultures. These results indicate that LPS and polyanions probably act on different subpopulations of B cells. Since dose response studies (data not given here) showed that higher doses of polyanions gave a smaller response than the optimal doses used in this study, the decreased response of the cells to two different polyanions when they were given simultaneously may indicate that polyanions acts on the same subpopulation of B cells.

One week after the XBM procedure, spleen cells derived from TXBM mice responded to DS and to PHA but not to LPS. LPS-positive cells are first seen in the spleen 2 weeks after XBM-procedure. According to the kinetic studies (Table 2) we postulate that the B cell population responding to DS represents a less matured B cell population than the LPS-reactive B cells. Both populations, however, seem to belong to cells bearing

Table 2 Kinetics of appearance of DS-, LPS- and PHA-reactive cells in spleen of TXBM mice

Time after week XBM-procedure	Stimulation index		
	DS	LPS	PHA
1	6.4	0.7	2.9
2	15.9	2.1	4.7
5	8.4	28.4	5.3
7	10.2	10.1	5.8

Spleen cells derived from TXBM-BALB/c mice (1–7 week after irradiation and bone marrow injection) were cultured for 48 h and the incorporation of ^3H -thymidine into the DNA was measured. Cultures stimulated by PHA received $2 \mu\text{g}$ PHA per ml medium. For details see Table 1. The results are expressed as stimulation indices. Each value represents the mean of c.p.m. detected in three individual cultures. Standard deviation was less than 10%.

immunoglobulin on their surface since pretreatment of a normal spleen cell population by polyvalent rabbit anti-mouse- γ -globulin serum and complement (data not given here) destroyed the capacity of the cells to respond to both DS and LPS but not to PHA. On the other hand it has been shown³ that DS but not LPS stimulates DNA synthesis in bone marrow cells of mice. Moreover, the stimulation rate of bone marrow cells derived from TXBM mice by DS was significantly enhanced compared with the reactivity of normal bone marrow cells³. These results and the observation that DS stimulates mice fibroblasts *in vitro* (U. Saar and T. D., in preparation) indicate that the mitotic activity of DS is not restricted to lymphoid cells and that DS

may also be useful for studies on cell differentiation in other systems.

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- 1 Melchers, F., and Andersson, J., *Transplant Rev.*, **14**, 76 (1973).
- 2 Diamantstein, T., Wagner, B., L'age-Stehr, J., Beyse, I., Odenwald, M. V., and Schulz, G., *Eur. J. Immun.*, **1**, 302 (1971).
- 3 Diamantstein, T., Vogt, W., Rühl, H., and Bocher, G., *Eur. J. Immun.*, **3**, 488 (1973).
- 4 Rühl, H., Vogt, W., Bocher, G., and Diamantstein, T., *Immun.*, (in the press).

Brain-associated tumour antigens demonstrated by immunofluorescence

It has been reported that lymphocytes from patients with cancer react with a basic protein of myelin, suggesting that brain shares antigen(s) with malignant tumours¹. The brain also possesses an antigen in common with the thymus^{2–5}. Using a heterologous anti-brain serum we have investigated the presence of brain-associated antigens in tumour cells and in lymphoid cells.

Anti-rat brain serum was produced in rabbits by three subcutaneous weekly injections of 1 ml of brain homogenate, containing 10 mg nitrogen, in an equal volume of complete Freund's adjuvant⁶; the rabbits were bled 7 d after the third immunisation.

The rat tumours studied were: gliomas and schwannomas induced in the offspring of pregnant DA Agouti rats injected intravenously with ethylnitrosourea ($10 \text{ mg per kg body weight}$)⁶, spontaneous mammary carcinomas in inbred Agouti rats, a squamous cell carcinoma⁷ in inbred Wistar rats, and Walker carcinoma in outbred Hooded Wistar rats. Unfixed frozen sections, impression films and smears of brain, thymus, bone marrow, tumours and normal non-lymphoid tissues were examined by standard immunofluorescence procedures⁸. Cell suspensions from tumours, thymus, spleen and lymph node were made by gentle mechanical teasing in tissue culture medium 199. Liver and kidney cell suspensions were made by perfusion of the organs with 10 ml of calcium- and magnesium-free Hanks balanced salt solution containing 0.05% collagenase and 0.1% hyaluronidase, followed by incubation of the diced tissue in the same enzyme solution at 37°C for 30 min. Peripheral blood leukocytes, largely ($>90\%$) lymphocytes, were separated from heparinised blood by centrifugation in a Hypaque-Ficoll gradient⁹.

Sandwich immunofluorescence staining of cell suspensions and tissue preparations was carried out by procedures described in detail elsewhere^{8,10,11}. The conjugate was fluorescein isothiocyanate-labelled goat anti-rabbit globulin with a protein concentration of 0.8 g per 100 ml and a fluorescein: protein molar ratio of 4.2. Before use, it was absorbed with human liver powder and rat liver homogenate, so that by itself it gave no staining of the various microscopical preparations.

The unabsorbed anti-rat brain serum stained the membrane and cytoplasm of all the rat tissues examined. After absorptions⁸ with rat erythrocyte membranes, liver and lung

Table 1 Immunofluorescent staining of rat brain, tumours and thymus by anti-rat brain serum

Antiserum absorbed with	Staining of unfixed frozen sections, smears and impression films of					Membrane staining of cell suspensions of			
	Brain	Tumours†	Thymus	Bone	Normal nonlymphoid tissues‡	Tumours†	Thymus	Bone marrow*	Liver or kidney
Erythrocytes, lung, liver and bone marrow*	+++	+	+++	—	—	100%	100%	<1%	—
+ Brain	—	—	—	—	—	—	—	—	—
+ Thymus	+++	+	—	—	—	—	—	—	—
+ Kidney	+++	+	+++	—	—	100%	100%	<1%	—
+ Mammary carcinoma	+++	—	+++	—	—	—	100%	<1%	—
+ Glioma	+++	—	+++	—	—	—	100%	<1%	—

* Bone marrow cells from thymectomised, irradiated and bone marrow-reconstituted rats.

† Rat gliomas, schwannomas, mammary carcinomas, squamous cell carcinoma and Walker carcinoma.

‡ Normal rat heart, muscle, liver, kidney, testis and skin.

homogenates, bone marrow cells from thymectomised irradiated and bone marrow-reconstituted rats, and dilution 1:4, this antiserum stained both the white matter and the cytoplasm of neurones and glia in unfixed frozen sections and impression films of the brain. Neuronal cytoplasm staining was best seen in sections of the cerebellum where there was strong reaction with the granular layer. The antiserum also stained the surface membranes of 100% of thymocytes, 61% of peripheral blood lymphocytes, 32% of spleen cells, 12% of lymph node cells, 12% of normal bone marrow cells and less than 1% of bone marrow cells from thymectomised, irradiated and bone marrow-reconstituted rats. It also stained the cytoplasm of thymocytes in unfixed frozen sections, smears and impression films, but did not stain smears of bone marrow cells. In contrast, the antiserum stained neither the membranes of cell suspensions of liver and kidney, nor unfixed frozen sections of the following normal rat tissues: heart, muscle, liver,

homogenates of mammary carcinoma, glioma or kidney. Tumour cell membrane staining was inhibited by absorption of the antiserum with brain homogenate and cell suspensions of thymus and tumours (glioma or mammary carcinoma) but not by absorption with kidney cell suspensions. Tumour cell cytoplasm staining was inhibited by absorption with homogenates of brain or tumours (glioma or mammary carcinoma) but not by absorption with homogenates of thymus or kidney (Table 1).

The prevention of brain staining only by brain homogenates points to the presence of an organ-specific brain antigen. The staining of the membranes of thymocytes and thymus-derived lymphoid cells and the inhibition of thymocyte membrane staining by brain or thymic absorptions support the findings of others of "brain-associated thymus antigen". Our observations suggest that there is also a cytoplasmic thymic antigen common to brain and thymus. In addition there seem to be at least two brain-associated tumour antigens: the first is shared by brain and the cell membranes of tumour cells and thymocytes, because tumour cell membrane staining by our antiserum is inhibited by absorption with brain homogenate or thymocyte suspension; the second is shared by brain and the cytoplasm of the tumour cells investigated since tumour cytoplasm staining was inhibited only by absorption with homogenate of brain or either of the two tumours used.

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Fig. 1 Immunofluorescent staining of tumour cells by rabbit anti-rat brain serum. *a*, Membrane staining of cell suspensions of rat mammary carcinoma ($\times 268$); *b*, cytoplasm staining of impression films of rat squamous cell carcinoma ($\times 171$).

kidney, testis and skin. Moderate to strong membrane and cytoplasm staining in 100% of cells was, however, observed in all the tumours tested: gliomas, schwannomas, mammary carcinomas, squamous cell carcinoma and Walker carcinoma (Fig. 1). In all cases, no staining was observed in the parallel control tests with preimmune serum, absorbed in the same way as the test serum.

The staining of brain sections was inhibited only by serum absorption with brain homogenates. However, thymocyte membrane staining was inhibited by serum absorption with rat brain homogenate or thymocyte cell suspensions but not by cell suspensions of mammary carcinoma, glioma or kidney, and thymocyte cytoplasmic staining by absorption with homogenates of brain and thymus but not by

- Caspari, E. A., and Field, E. J., *Br. med. J.*, **2**, 613-617 (1971).
- Reif, A. E., and Allen, J. M. V., *J. exp. Med.*, **120**, 413-433 (1964).
- Golub, E. S., *Cell Immun.*, **2**, 353-361 (1971).
- Peter, H. H., Clagett, J., Feldman, J. D., and Weigle, W. O., *J. Immun.*, **110**, 1077-1083 (1973).
- Gyongyossy, M. I. C., and Playfair, J. H. L., *Cell Immun.*, **7**, 118-123 (1973).
- Druckrey, H., Ivanovic, S., and Preussman, R., *Nature*, **210**, 1378-1379 (1966).
- Flannery, G. R., Chalmers, P. J., Rolland, J. M., and Nairn, R. C., *Br. J. Cancer*, **28**, 118 (1973).
- Nairn, R. C., *Fluorescent Protein Tracing* third ed. (Livingstone, Edinburgh, 1969).
- Boyum, A., *Scand. J. clin. Lab. Invest.*, **21**, Suppl. 97 (1968).
- Rolland, J. M., Nairn, R. C., and Davies, D. J., *J. Immun. Methods*, **1**, 83-90 (1971).
- Nairn, R. C., Cusdin, C. H., and McNaughtar, J., *Clin. exp. Immun.*, **8**, 835-838 (1971).

Susceptibility of xeroderma pigmentosum cells to chromosome breakage by adenovirus type 12

DNA repair-deficient microbial systems have attracted considerable attention because they offer an insight into the genetic control of sensitivity towards mutagens and carcinogens¹. These studies have become highly relevant to man with Cleaver's discovery² that cells of most patients with xeroderma pigmentosum (XP) have a reduced level of DNA repair synthesis following ultraviolet-irradiation or exposure to several chemical carcinogens³⁻⁶. The impaired repair capacity of XP cells seems to result in their greater sensitivity towards the lethal⁶⁻¹⁰ and chromosome-damaging action¹¹⁻¹² of those physical and chemical carcinogens which induce irreparable DNA alterations. On the other hand the behaviour of XP cells does not differ from that of controls when treated with several potent alkylating mutagens and carcinogens^{5,10,12,13}.

To further characterise the response of DNA repair-deficient cells towards carcinogens and mutagens which belong in different categories, we have examined the effect of infectious and impaired human adenovirus type 12 (AD12) on cultured fibroblasts of three unrelated XP patients and three control subjects. AD12 was used because it readily infects cultured human cells, it replicates within the cell nucleus, it has a capacity to induce chromosome breaks in mammalian cells, including humans cells¹⁴⁻¹⁷, and it forms intranuclear inclusion bodies (IB) which can be used to identify virus-producing cells^{15,16,18,19}. Since the repair capacity of the three XP cultures varied, we examined further whether different degrees of repair deficiency give rise to different sensitivities towards the chromosome-damaging effect of infectious and ultraviolet-impaired AD12.

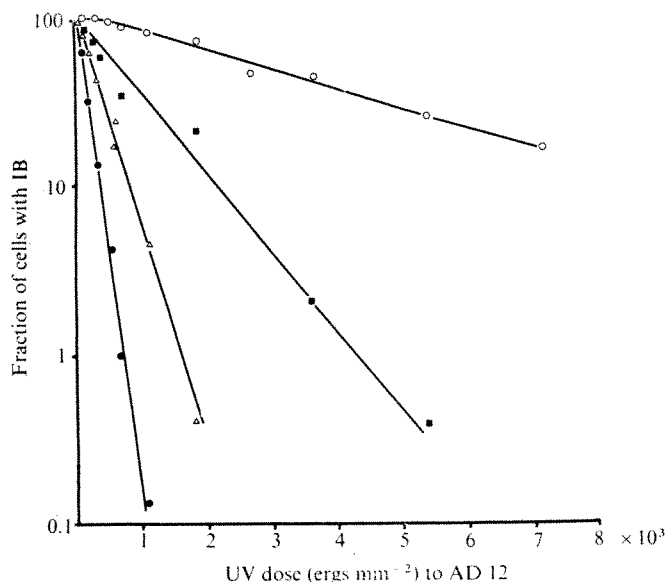


Fig. 1 Effect of ultraviolet irradiation on the capacity of AD12 to form IBs in three XP cell cultures (XP-E, XP-C, XP-K) and control. Samples were taken 48 h after infection. Virus suspensions were spread in a thin layer in a 60 mm Petri dish and irradiated under constant agitation with a Sylvania germicidal lamp (G15T8). At a distance of 7 inch this gave a dose of 30 ergs mm⁻² s⁻¹. Following irradiation the virus preparation was immediately added to the fibroblast cultures. The virus absorption time was 4 h at 37° C. The original nonirradiated AD12 preparation had an input multiplicity of 50 TCD₅₀ per cell. In each sample 2,000 to 2,100 nuclei were screened for the presence of IBs. Two coverslip cultures were used for each sampling point. ○, Controls (average of three nonaffected persons); ●, XP-E; △, XP-C; ■, XP-K.

Cultured fibroblasts were obtained from small skin punch biopsies of three unrelated XP patients and three control persons of comparable age. The stock cultures were kept in 10 cm diameter plates (Falcon plastic) placed in a CO₂ incubator and were fed Eagle's minimum essential medium (MEM) supplemented with 20% foetal calf serum (FCS) and antibiotics. For the experiments monolayers of cells were grown on glass cover slips (20 × 20 mm) which were kept in small plastic plates. The level of DNA repair synthesis, which was estimated by measuring the unscheduled uptake of ³HdR³, was about 21% for XP-E, 32% for XP-C and 56% for XP-K as compared to that found in three control subjects following ultraviolet irradiation. The stock preparations of AD12, prototype strain Huie, contained 10⁷ TCD₅₀ per 0.2 ml as determined by titration in human embryonic kidney cells. The AD12 preparations seemed to be free of PPLO and adeno-associated virus (AAV), as judged by electron-microscopic examination. Cells with replicating virus were identified by the presence of intranuclear inclusion bodies (IB) which are the replication site of adenovirus^{15,16,18,19}. The frequency of IB parallels results obtained on plaque assays^{16,20} and is dependent on the input multiplicity of the virus. IB and chromosome aberrations were analysed on preparations which were pre-treated with citrate solution, fixed with ethanol-acetic acid (3:1) and stained with a 2% solution of aceto-orcein.

The frequency of XP cells which support viral replication was compared with that in control cultures by counting the proportion of fibroblasts containing IB. In samples taken 30, 48, 72 and 96 h after infection with nonirradiated AD12 the frequency of cells containing IB in the three XP cultures examined depended on the ratio of viruses to cells and closely resembled that of controls. But there was a marked difference between XP cells and controls following infection with ultraviolet-irradiated virus. Relatively small doses of ultraviolet-irradiation which did not alter the frequency of IBs in control cultures reduced the capacity of AD12 to replicate and form IBs in the XP cells (Fig. 1). The capacity of ultraviolet-impaired AD12 to form IB was abolished faster in XP cells with a severe repair deficiency than in those with a less affected DNA repair capacity.

There was a slight increase in the frequency of IB-containing XP cells with time after infection with ultraviolet-irradiated (90 to 1,800 ergs mm⁻²) AD12, but even after 96 h their frequency did not reach the level found in cell populations of nonaffected persons. The extent of recovery of the replicative capacity of ultraviolet-impaired AD12 also depends on the degree of repair deficiency of the host cell. For example, ultraviolet doses of more than 1,800 ergs mm⁻² destroyed the capacity of the AD12 to recover its replicative potential in XP-E cells, whereas a slight recovery occurred in the less repair-deficient XP-C and XP-K cells and a restoration of about 60% took place in cells of nonaffected persons.

The extent of AD12-induced chromosome aberration was estimated by counting the number of metaphase plates with at least one chromatid or chromosome break or one chromatid exchange and by calculating the average number of chromosome aberrations per metaphase plate. There was no significant difference between the frequency of XP cells with chromosome aberrations and that of controls when infected with the same numbers of nonirradiated AD12 and sampled 30, 48, 72 or 96 h after infection. But the behaviour of the three XP cell cultures (XP-E, XP-C and XP-K) departed from that of the controls following infection with ultraviolet-irradiated AD12. Virus preparations irradiated with relatively small doses of ultraviolet were less capable of inducing chromatid and chromosome breaks in XP cells during the first two days after infection (Table 1). The frequency of chromosome aberrations was lower in those XP cells which had a severe DNA repair deficiency than in XP cells with a less impaired repair system. This pattern of survival of a viral function, which can be clearly observed in early samples after infection, closely resembles

that of IB formation in XP and normal cells following infection with ultraviolet-irradiated AD12 (Fig. 1).

In the XP cells examined the chromosome damaging action of ultraviolet-irradiated AD12 seemed to become restored at a later stage after infection (Table 1). The severity of chromosome breakage was also greater in samples taken 72 or 96 h after infection. In the 48 h samples chromatid breaks (Fig. 2a) were the major type of aberration, whereas fragmentation of the entire chromosome complement (Fig. 2b), which leads to the formation of micronuclei (Fig. 2c), predominated in the later samples. The IB-forming capacity of AD12 which received a dose of $3,600 \text{ ergs mm}^{-2}$ was either not restored (XP-E and XP-C cells) or showed only a slight recovery (XP-K cells) within the 96 h period.

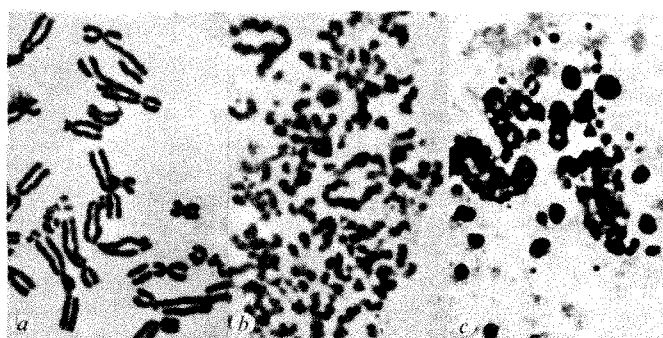


Fig. 2 Part of a metaphase plate of an XP-E culture infected with ultraviolet-irradiated AD12 (720 ergs mm^{-2}). *a*, Sampled 30 h after infection. Several chromatid and chromosome breaks. *b*, Sampled 96 h after infection. Fragmented chromosome complement. *c*, Part of a cell with multiple micronuclei of an XP-E culture infected with ultraviolet-irradiated AD12 (720 ergs mm^{-2}) and sampled 96 h after infection.

Controls and XP cells seemed to differ in the extent of chromosome damage rather than in the type of chromosome aberrations which followed infection with nonirradiated or ultraviolet-irradiated AD12. Chromosome fragmentation occurs in the examined XP cells as well as in controls (Table 1). Furthermore the nonrandom distribution of chromosome aberrations which was observed in cultured fibroblasts of normal persons¹⁵ and patients with Edward's syndrome¹⁷ also occurred in XP cells following infection with nonirradiated or ultraviolet-irradiated AD12. For example following infection with ultraviolet-irradiated (180 ergs mm^{-2}) AD12 59%, 67%, 58% and 65% of all chromosome aberrations accumulated in chromosome 1 and 17 of XP-E, XP-C, XP-K and controls respectively, although their combined length was just over 10% of the entire chromosome complement. Furthermore most

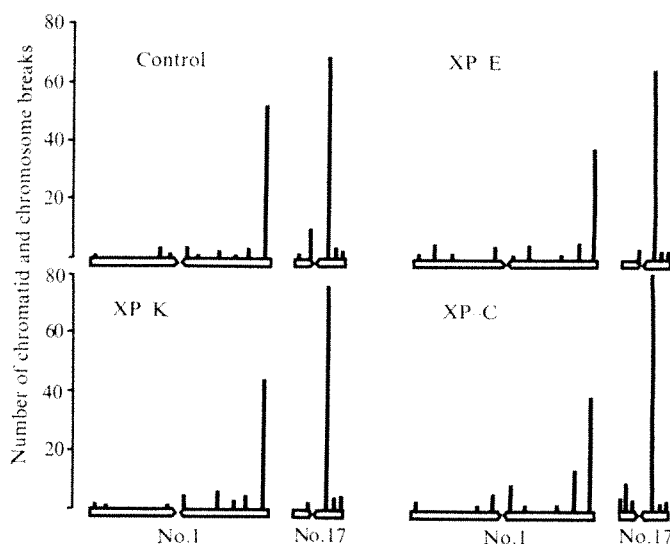


Fig. 3 Distribution of chromatid and chromosome breaks along chromosome 1 and 17 of controls and XP cells (XP-K, XP-E, XP-C) following infection with ultraviolet-irradiated (180 ergs mm^{-2}) AD12. Samples were taken 30 h after infection. For each sample, 60 cells with chromosome aberrations were analysed.

chromatid aberrations were located near the end of chromosome 1 and in the region of the secondary constriction of chromosome 17 (Fig. 3), which agrees with previous observations on cells with a normal¹⁵ and trisomic karyotype¹⁷.

Thus the repair of ultraviolet-irradiated viral genomes, and hence the restoration of replication and chromosome-damaging function, occur at lower levels in XP cells than in host cells with an unimpaired DNA repair system. But even in XP cells ultraviolet-irradiated AD12 induces a relatively high frequency of chromosome aberrations, which are manifested because the impaired virus cannot replicate and thus does not cause cell lysis. The data also show that the chromosome-damaging function is more readily restored than the replicative capacity of the virus. This may be because the first property requires only a part of a functional genome²¹. Furthermore, the degree of survival of these two viral functions depends on the level of DNA repair capacity of the host cells. These results can be compared with the reduced T antigen formation and cell transformation in XP cells infected with ultraviolet-impaired SV40²² and the diminished virus production following infection with ultraviolet-irradiated herpes simplex virus²³. The marked increase in chromosome damage at the third and fourth day after infection with ultraviolet-irradiated AD12 is probably caused by a delayed recovery of viral function, either due to a slow-working excision repair in XP cells or to an exchange

Table 1 Frequency and type of chromosome aberrations of xeroderma pigmentosum cells and controls following infection with ultraviolet-irradiated ($3,600 \text{ ergs mm}^{-2}$) AD12

	Sampling time after infection (h)															
	XP-E				XP-C				XP-K				Control			
	30	48	72	96	30	48	72	96	30	48	72	96	30	48	72	96
Metaphase plates with chromosome aberrations (%) [*]	0	4	12	45	0	6	30	59	0	16	60	79	63	44	31	16
Types of aberrations [†]																
Chromatid breaks (1—9) [‡]	0	100	40	0	0	98	28	0	0	50	4	1	96	25	28	26
Chromatid breaks (10—60)	0	0	9	34	0	2	34	8	0	33	11	4	4	52	44	36
Fragmentation (> 60)	0	0	51	66	0	0	66	92	0	17	85	95	0	23	28	38

^{*}Between 120 to 140 metaphase plates were analysed for each sample. The figures include only chromatid and chromosome breaks since chromatid exchanges were only occasionally seen.

[†]Figures show the percentage of the total number of chromosome aberrations.

[‡]Figures indicate the number of chromatid or chromosome breaks per chromosome complement.

type of repair between viral genomes in the host cell.

In conjunction with previous reports, this study provides evidence that the sensitivity of XP cells varies towards different physical, chemical and viral carcinogens and mutagens (see ref. 24 for review). Similarly, cells of patients with Fanconi's anaemia have an elevated sensitivity to some but not all mutagenic agents²⁵. Cells of patients afflicted with XP and those with Fanconi's anaemia differ in their response towards the same carcinogens. Such selective sensitivities of certain human groups will make the assignment of carcinogen and mutagen levels that are safe or tolerable to all human beings a difficult, if not impossible, task.

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- ¹ Smith, K. C., and Hanawalt, P. C., *Molecular Photobiology Inactivation and Recovery*, (Academic Press, New York, 1969).
- ² Cleaver, J. E., *Nature*, **218**, 652 (1968).
- ³ Stich, H. F., and San, R. H. C., *Mutat. Res.*, **10**, 389 (1970).
- ⁴ Setlow, R. B., and Regan, D., *Biochem. biophys. Res. Commun.*, **46**, 1019 (1972).
- ⁵ Stich, H. F., San, R. H. C., Miller, J. A., and Miller, E. C., *Nature*, **238**, 9 (1972).
- ⁶ Stich, H. F., and San, R. H. C., *Proc. Soc. exp. Biol. Med.*, **142**, 155 (1973).
- ⁷ Cleaver, J. E., *Int. J. rad. Biol.*, **18**, 557 (1970).
- ⁸ Goldstein, S., *Proc. Soc. exp. Biol. Med.*, **137**, 730 (1971).
- ⁹ Takeba, H., Furuyama, J., Miki, Y., and Kondo, S., *Mutat. Res.*, **15**, 98 (1962).
- ¹⁰ Stich, H. F., San, R. H. C., and Kawazoe, Y., *Mutat. Res.*, **17**, 127 (1973).
- ¹¹ Parington, J. M., Delhanty, J. D. A., and Baden, H. P., *Ann. hum. Genet.*, **35**, 149 (1973).
- ¹² Stich, H. F., Stich, W., and San, R. H. C., *Proc. Soc. exp. Biol. Med.*, **142**, 1141 (1973).
- ¹³ Cleaver, J. E., *Mutat. Res.*, **12**, 453 (1971).
- ¹⁴ Stich, H. F., *Progr. exp. Tumour Res.*, **18**, 260 (1973).
- ¹⁵ Zur Hausen, H., *J. Virol.*, **1**, 1174 (1967).
- ¹⁶ Stich, H. F., Avila, L. R., and Yohn, D. S., *Expl. Cell Res.*, **53**, 44 (1968).
- ¹⁷ McDougall, J. K., *Nature*, **225**, 456 (1970).
- ¹⁸ Martinez-Palomo, A., Le Buis, J., and Bernard, W., *J. Virol.*, **1**, 817 (1967).
- ¹⁹ Stich, H. F., Kalnins, V. I., McKinnon, E., and Yohn, D. S., *J. ultrastruct. Res.*, **19**, 556 (1967).
- ²⁰ Rainbow, A. J., and Mak, S., *Radiat. Res.*, **50**, 319 (1972).
- ²¹ Gilead, Z., and Ginsberg, H. S., *J. Bact.*, **92**, 1853 (1966).
- ²² Aarason, S. A., and Lytle, C. P., *Nature*, **228**, 358 (1970).
- ²³ Lytle, C. P., Aarason, S. A., and Harvey, E., *Int. J. rad. Biol.*, **22**, 159 (1972).
- ²⁴ Stich, H. F., Kieser, D., Laishes, B. A., and San, R. H. C., *Proc. Can. Cancer Conf.* 1973 (in the press).
- ²⁵ Sasaki, M. S., and Tonomura, A., *Cancer Res.*, **33**, 1829 (1973).

the flies are old^{11,12}. Russell¹³ reported an increase in induced monosomy in mice by examining the progeny of matings with sex-linked markers. But many aneuploids may be lethal and the dosages used by Russell were large enough to cause loss of chromosomes by breakage. Recent advances in the technique of culturing mouse oocytes *in vitro* (ref. 14, modified by E. P. Evans and C. E. Ford) have made it possible to circumvent these difficulties by examining meiotic chromosomes in second metaphase. The following experiment was designed to examine the effect of low doses of whole-body irradiation on chromosome segregation during first meiotic division in the oocytes of young female mice.

(C3H×ICR/Swiss) F₁ hybrid females, aged 3 and 6 months, were exposed to 10 r., 20 r. and 30 r. whole body γ irradiation from a ¹³⁷Cs source at a focal distance of 51 cm with a rate of 29 r. min⁻¹. The dosage was kept low to minimise chromosome breakage. During exposure, the mice were held in pie-shaped compartments of a round plexiglass container. Equal numbers of control mice were handled in identical fashion but were not irradiated. The mice and their paired controls were killed and their ovaries removed within one week of exposure to radiation. No gonadotrophic hormones were used to stimulate oocyte maturation or superovulation.

The oocytes were teased out of the ovaries and those containing a germinal vesicle were incubated in foetal calf serum for 18–23 h to obtain cells in second metaphase. They were then transferred into a hypotonic solution of 1% sodium citrate for 25 min and those in which the germinal vesicle had disappeared were prepared for chromosomal analysis. A maximum of five oocytes were carefully placed on a microslide, excessive hypotonic solution was removed and a tiny amount of fixative (3:1 methanol:acetic acid) was dropped on the cells and quickly blown dry under the heat of a 25 W bulb. The chromosomes were stained with Giemsa. The slides were coded, before the cells were scored, by three technicians. The number of cells on each slide was recorded so that all cells or remnants of cells were accounted for during the analysis.

Metaphase II chromosomes were clearly analysable in 1,149 irradiated oocytes and 1,054 controls. All identifiable chromosome breaks occurred in the centromeric region and could have resulted from premature separation of the chromatids or rupture during slide preparation. They were slightly more common among the irradiated cells (Table 1), but a significant increase was observed only after exposure to 30 r. There were no acentric fragments and small chromatid breaks, if present, could not be identified because of the characteristic shape of metaphase II chromosomes.

Six of the irradiated oocytes had an extra chromosome, that is, 21 chromosomes (Fig. 1). All other oocytes on the same slides were found and those with analysable metaphase chromosomes were positively identified as having 20 chromosomes. It is unlikely, therefore, that the extra chromosome in these six oocytes had strayed from other cells. Since metaphase II chromosomes cannot be karyotyped, it is not possible to identify the extra chromosome in these cells. No oocytes with an additional chromosome were found among the controls. The difference between irradiated and control is statistically significant ($P=0.02$, Fisher exact test).

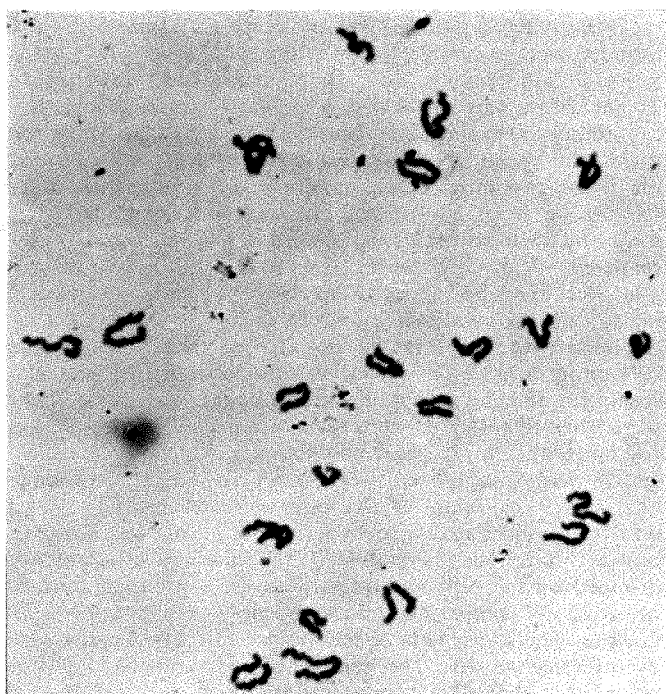
Chromosome loss during slide preparation was most probably the cause of our finding oocytes with less than the haploid number of 20 chromosomes. Cells with fewer than 19 chromosomes have not been tabulated. For each oocyte with 21 chromosomes a complementary cell with 19 chromosomes must have been produced to form the polar body. The reverse should also hold true in the absence of preferential segregation of one type of chromosome complement into the polar body, that is, a similar number of polar body cells with 21 chromosomes could have resulted from the abnormal segregation that produced oocytes with 19 chromo-

Radiation-induced nondisjunction in mouse oocytes

BECAUSE chromosomal trisomy is found with relatively high frequencies in congenital malformations and spontaneous abortions in man, the aetiology of abnormal segregation is a pressing problem. One possible factor is exposure of women to diagnostic X rays^{1–8}. Experiments with *Drosophila melanogaster* indicate that nondisjunction can be induced by exposure of females to X rays^{9,10} especially if

Table 1 Frequencies of numerical and structural aberrations in second metaphase oocytes of mice irradiated *in vivo* compared with nonirradiated controls

Nonirradiated controls											
Dose (r.)	No. of oocytes analysable	Irradiated				No. cells with breaks	Nonirradiated				No. cells with breaks
		No. of chromosomes			No. of oocytes analysable		No. of chromosomes				
		19	20	21			19	20	21		
10	426	14	408	2	2	379	11	363	0	5	
20	368	7	355	3	3	421	14	405	0	2	
30	355	13	330	1	11	254	6	246	0	2	
Totals	1,149	34	1,093	6	16	1,054	31	1,014	0	9	

**Fig. 1** Oocytes in metaphase II with 21 chromosomes from mice irradiated *in vivo*.

somes. Since the latter cannot be distinguished from among the total number of oocytes with chromosome loss, the total frequency of nondisjunctional events can be estimated to be approximately double the number of oocytes with 21 chromosomes, that is, 12, or 1%.

The absence of hypermodal cells among more than 1,000 nonirradiated oocytes in our sample agrees with Russell's conclusion¹³ from phenotypic ratios that spontaneous nondisjunction during first meiotic division in the female mouse is exceedingly rare. On the other hand, examination of metaphase II oocytes of a C₃H inbred strain by Röhrborn¹⁵ gave a spontaneous rate of 2.3% with extra chromatids among 128 metaphase II oocytes obtained from females after mating with vasectomised males and not pretreated with hormones. In a relatively limited sample reported by Yamamoto *et al.*¹⁶, no pure trisomy was observed among foetuses of young mice, irradiated and nonirradiated, but both spontaneous and induced nondisjunction was observed among the progeny of aged mice. These conflicting reports probably reflect strain variations as well as differences in experimental design.

Human epidemiological studies have produced contradictory evidence on the effect of radiation on chromosome segregation. But most with negative results show a slight though insignificant increase in nondisjunction. One notable exception was the report on the effect of the atomic bombs in Japan² but cytogenetic investigations are now beginning to reveal a significant increase in sex chromosome aneuploidies among the children of exposed parents¹⁷. The human situation has emphasised the need for experimental

confirmation. The results of our experiment indicate that nondisjunction can be induced in young female mice by exposure *in vivo* to low doses of radiation.

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- ¹ Uchida, I. A., and Curtis, E. J., *Lancet*, **ii**, 848-850 (1961).
- ² Schull, W. J., and Neel, J. V., *Lancet*, **i**, 537-538 (1962).
- ³ Sigler, T. T., Lilienfeld, A. M., Cohen, A. M., and Westlake, J. E., *Bull. Johns Hopkins Hosp.*, **117**, 374-399 (1965).
- ⁴ Uchida, I. A., Holunga, R., and Lawler, C., *Lancet*, **ii**, 1045-1049 (1968).
- ⁵ Marmol, J. G., Scriggins, A. L., and Vollman, R. F., *Am. J. Obstet. Gynec.*, **104**, 533-543 (1969).
- ⁶ Villumsen, A. L., *Environmental factors in congenital malformations* (F.A.D.L.s Forlag, København, Aarhus, Odense, 1970).
- ⁷ Stevenson, A. C., Mason, R., and Edwards, K. D., *Lancet*, **ii**, 1335-1337 (1970).
- ⁸ Alberman, E., Polani, P. E., Fraser Roberts, J. A., Spicer, C. C., Elliott, M., and Armstrong, E., *Ann. Hum. Genet.*, **36**, 195-208 (1972).
- ⁹ Mavor, J. W., *J. exp. Zool.*, **39**, 381-432 (1924).
- ¹⁰ Traut, H., *Mutat. Res.*, **1**, 157-162 (1964).
- ¹¹ Patterson, J. T., Brewster, W., and Winchester, A. M., *J. Hered.*, **23**, 325-333 (1932).
- ¹² Uchida, I. A., *Can. J. Genet. Cytol.*, **4**, 402-408 (1962).
- ¹³ Russell, L. B., *Science*, **133**, 1795-1803 (1961).
- ¹⁴ Tarkowski, A. K., *Cytogenetics*, **5**, 394-400 (1966).
- ¹⁵ Röhrborn, G., *Humangenetik*, **16**, 123-125 (1972).
- ¹⁶ Yamamoto, M., Shimada, T., Endo, A., and Watanabe, G., *Nature new Biol.*, **244**, 206-208 (1973).
- ¹⁷ Hamilton, H. D., Awa, A. A., Sofuni, T., Neriishi, S., and Honda, T., *Fourth Int. Conf. Birth Defects Abstracts* (Vienna, 1973).

Very long stretches of free DNA in chromatin

THE arrangement of histones along the DNA in chromosomes has been studied using digestion of the chromatin with DNase¹⁻⁴ and titration of the chromatin with polylysine^{5,6} or certain dyes^{7,8} as probes. The precision of these probes is limited by the extent to which DNA partially covered with histones will react with the enzymes or titration agents used^{7,8}. Furthermore, it has been noted that histones are redistributed along or between DNA fibres under conditions arising during the normal course of isolation and handling of the chromatin⁹⁻¹². This phenomenon may have strongly influenced the results obtained previously.

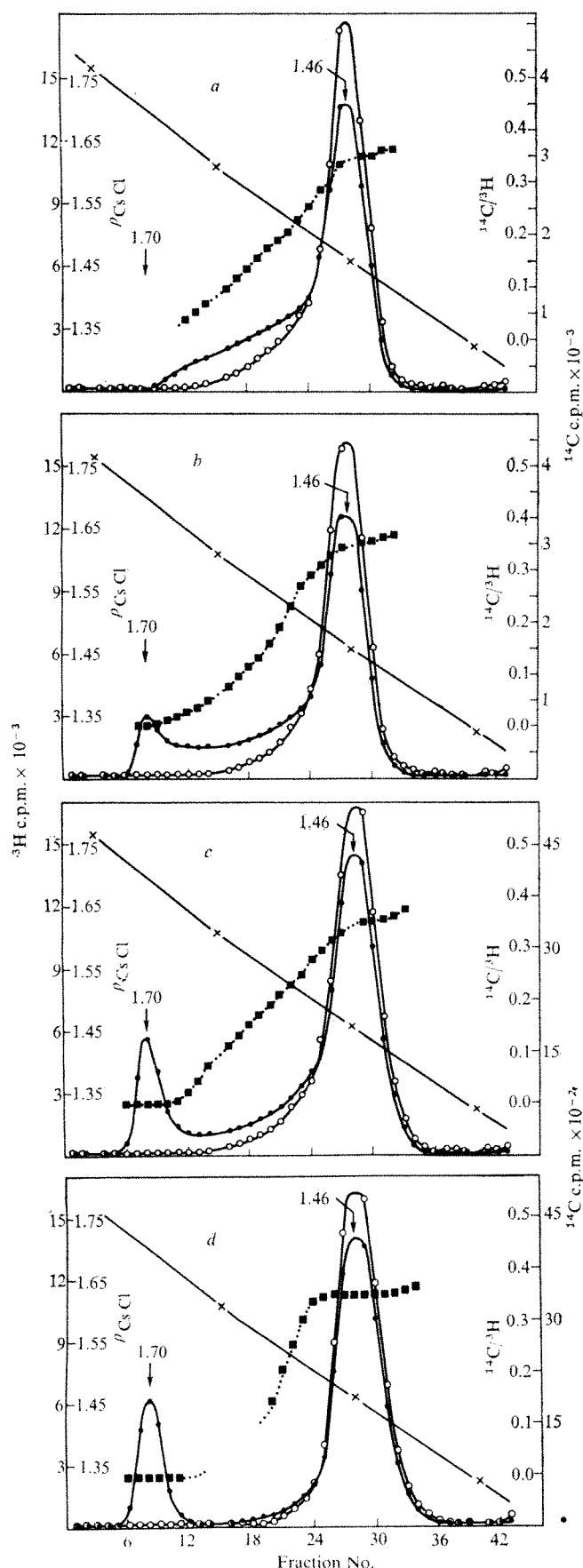


Fig. 1 Long stretches of completely free DNA in DNP-F₁. Mice carrying Ehrlich ascites tumour cells were injected intraperitoneally with a mixture of methyl-³H-thymidine and ¹⁴C-lysine to obtain a double-labelled chromatin¹⁰⁻¹². Less than 0.1% of the total ¹⁴C counts were in DNA and 0.1-0.2% of the total ³H counts were in protein. The specific radioactivity of the ³H-DNA in the ¹⁴C, ³H-chromatin was usually 2×10^4 - 3×10^4 c.p.m. μg^{-1} . The ¹⁴C/³H ratio was usually about 0.6.

We have tried to examine directly the distribution of histones in the chromatin under conditions that minimise the redistribution of histones¹⁰⁻¹². Our method is based on the direct detection and quantitation of the free DNA segments by equilibrium centrifugation of formaldehyde-fixed deoxyribonucleoproteins (DNP) in CsCl gradients¹¹⁻¹⁵. The use of highly labelled chromatin enables work at low DNP concentrations thus minimising aggregation of the DNP^{11,12}.

Double-labelled chromatin (³H-DNA, ¹⁴C-protein) was used throughout our work. Briefly, unsheared chromatin was prepared from mouse Ehrlich ascites tumour cells which were simultaneously labelled *in vivo* with methyl-³H-thymidine and L-¹⁴C-lysine^{11,12}. The purified chromatin¹⁰⁻¹² was washed and suspended in 1 mM MgCl₂, 1 mM Na₂HPO₄, pH 7.8 buffer to a final concentration of 20-50 μg DNA ml⁻¹. Purified total yeast tRNA was added to this suspension to a final concentration of 1 mg ml⁻¹ and the mixture was incubated at 0° C for 3-4 h (ref. 10). The swelled gel was solubilised by treatment in a Dounce homogeniser and the resulting soluble ¹⁴C, ³H-DNP lacking histone F1 and most nonhistone proteins (DNP-F₁) was separated from the tRNA-¹⁴C-protein complexes and from the free tRNA by gel chromatography on Sepharose 2B (refs 10-12). No degradation of histones (as monitored by polyacrylamide gel electrophoresis^{10,11}) could be detected for up to 48-64 h at 0-4° C in either the chromatin gel or the soluble unfixed DNP-F₁.

Soluble ¹⁴C, ³H-labelled DNP-F₁ with an average chain length of about 10 kbases (1 kbase \equiv 1000 bases or base pairs) was fixed with formaldehyde and centrifuged to equilibrium in CsCl (Fig. 1). The majority of the DNP is banded at a density of 1.46 g cm⁻³ with a prominent shoulder at higher densities up to the density of the free DNA (1.70 g cm⁻³) (Fig. 1a). Thus, even in the sample containing high molecular weight DNA one could observe a significant fraction of material which contained much less protein than the main component.

When the formaldehyde-fixed DNP-F₁ is sheared to an average chain length of about 0.5 kbase (Fig. 1d) the DNP is banded in two homogenous peaks with a negligible amount of material of intermediate density. One peak has the density of free DNA (1.70 g cm⁻³) and the other has a density of 1.46 g cm⁻³ (Fig. 1d). The first band (1.70) does not contain any ¹⁴C-label (protein). The second band (1.46) is characterised by a nearly constant ¹⁴C/³H ratio throughout the peak area; thus the width of this band is determined almost entirely by diffusion. It should be noted that no protein is removed from the fixed DNP upon shearing in either formaldehyde-free or formaldehyde-containing buffer¹¹⁻¹⁴.

The average length of the stretches of free DNA was estimated from experiments in which the intensity of shearing was varied (Fig. 1). When less intensive shearing was used only a fraction of the free DNA was separated from the DNP-F₁. Under these conditions some of the material is distributed between the densities of the two bands (Fig. 1b and c; compare d). The fraction of the

The ¹⁴C, ³H-DNP-F₁ was obtained as described in the text, then fixed in 1% formalin¹¹⁻¹⁴ and thereafter sheared to the required average chain length by mild or strong sonication or by passage through the water-cooled French press. Appropriate controls have shown that the results depended only on the final average length of DNA obtained but not on the particular method of shearing used. The samples were centrifuged in CsCl at 45,000 r.p.m. for 70-75 h in the SW50.1 rotor (Spinco) at 10° C (refs 11 and 12).

a, DNP-F₁; equilibrium pattern of the unsheared (~10 kbases) preparation in CsCl. 97×10^3 ³H c.p.m.; 28×10^3 ¹⁴C c.p.m.; ~10 μg of DNP. b, As a but after shearing of the fixed DNP-F₁ to an average length of about 4 kbases. c, As a but after shearing to ~1.2 kbases. d, As a but after shearing to about 0.5 kbase.

●, ³H-DNA; ○, ¹⁴C-protein; X, density; ■ ¹⁴C/³H.

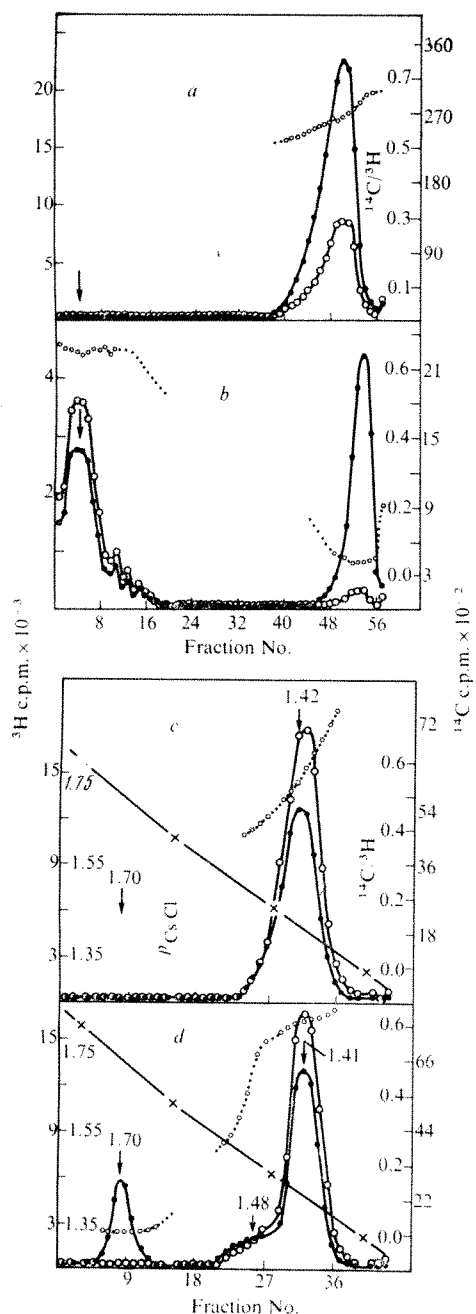


Fig. 2 Free DNA molecules in the sheared chromatin. *a*, Sucrose gradient centrifugation. The ^{14}C , ^3H -chromatin¹⁰⁻¹² was washed in 5 mM TEA-HCl, pH 7.8, then suspended in the same buffer to a final DNP concentration of $10\text{--}20\ \mu\text{g DNA ml}^{-1}$ and thereafter sheared to an average DNA length of about 0.5 kbase by a double passage through the water-cooled French press¹⁰⁻¹². The sheared sample was made 1% in formalin and thereafter was centrifuged through a linear 5–40% (w/v) sucrose gradient in the same buffer (35,000 r.p.m.; 3.5 h at 5°C in the SW40 rotor). *b*, As *a* but before layering on to gradient an equal volume of 0.30 M NaCl, 4 mM MgCl_2 , 5 mM TEA-HCl, pH 7.8 was added to the sheared chromatin. After 15 min at 0°C the sample was fixed in 1% formalin and thereafter centrifuged as above. *c*, CsCl equilibrium pattern of the sheared chromatin fixed in 5 mM TEA-HCl, pH 7.8 (see *a*). *d*, CsCl equilibrium pattern of the sheared chromatin fixed in 0.15 M NaCl, 2 mM MgCl_2 , 5 mM TEA-HCl, pH 7.8 (see *b*). \bullet , ^3H -DNA; \circ , ^{14}C -protein; \blacksquare , $^{14}\text{C}/^3\text{H}$; X, density. Arrows in *a* and *b* indicate the position of a dense sucrose shelf.

To achieve a 90% recovery of the DNP in *d*, CsCl centrifugation was carried out in the presence of 0.5% Sarcosyl NL97. Free DNA was recovered quantitatively either with or without Sarcosyl (data not shown).

material of intermediate density consists of DNA-histone complexes linked to remaining stretches of the free DNA. This material is almost undetectable when shearing is more intensive (Fig. 1*b–d*). The lengths of DNA molecules isolated from CsCl gradients were determined by sucrose gradient centrifugation in the presence of internal sedimentation markers (data not shown). One can estimate that the number average length of stretches of free DNA in the DNP-F1 is ~ 4 kbases. The variability in length of free DNA segments in the DNP-F1 remains unknown. It seems, however, that there are few, if any, segments of free DNA longer than ~ 10 kbases (since no free DNA is present in the unshereed DNP-F1 preparation; Fig. 1*a*) and there are almost no segments of free DNA shorter than 1 kbase (since shearing to ~ 0.5 kbase results in an almost complete separation of free DNA segments; Fig. 1*d*). These estimates would indicate the maximal and minimal values while the actual variability of the chain length of free DNA in the DNP-F1 may be, of course, much lower.

The average length of histone-covered DNA in the DNP-F1 is about four times that of the free DNA (since free DNA constitutes 20–22% of the total DNA; Fig. 1*d* and thus equals about 14–16 kbases. It may be estimated that a stretch of 16 kbases of DNA covered with four histone fractions to a protein/DNA ratio of 1.1 (ref. 14) would be occupied by about 8×10^2 histone molecules (assuming an average molecular weight of 13,500 for the histone). These four histone fractions are nearly uniformly distributed within the histone-covered stretch of DNA, as the fragmentation of DNA to ~ 0.5 kbase (corresponding to ~ 25 bound histone molecules) reveals no heterogeneity in histone distribution within histone-covered 'blocks' (Fig. 1*d*).

It was shown previously, that if the unfolding of chromosomal DNP is carried out in the absence of Mg^{2+} ions all DNA-bound histones may be redistributed¹². This particular type of redistribution is completely blocked in already unfolded DNP preparations. If the unfolding-induced dedistribution of histones is allowed, it leads to a considerable decrease of the average length of free DNA segments in the unfolded, histone F1-depleted DNP¹².

The implications of the above findings will be discussed below (see Fig. 5).

Chromatin which was hydrodynamically sheared in a low (0.005) ionic strength buffer lacking divalent cations, sediments in a sucrose gradient as a single peak with a significant internal heterogeneity (Fig. 2*a*). The same DNP, when fixed with formaldehyde and centrifuged to equilibrium in a CsCl gradient, is banded as a single peak with even higher internal heterogeneity (Fig. 2*c*).

The main result of this part of the work is that addition of approximately physiological concentrations of NaCl and/or MgCl_2 to the sheared, unfixed chromatin preparation results in the appearance of a bimodal distribution of the DNP after centrifugation in either sucrose or CsCl gradient (Fig. 2). For example, after a shift to 0.15 M NaCl, 2 mM MgCl_2 , 5 mM triethanolamine (TEA)-HCl, pH 7.8, a significant (18–19%) proportion of completely free DNA molecules appears in the sheared chromatin (Figs 2*b, d* and 3). The average length of either total or free DNA in the experiment presented in Fig. 2 equals about 0.5 kbase. If the shearing of chromatin is less intensive, producing DNA chains with average length of 2–3 kbases, free DNA molecules are still present in the sheared chromatin after addition of NaCl and/or MgCl_2 but their percentage is decreased to 2–3% of the total DNA (data not shown).

An additional feature of the sedimentation pattern of the sheared chromatin in 0.15 M NaCl, 2 mM MgCl_2 (Fig. 2*b*) is that DNP particles, after liberation of the free DNA, sediment 20–30 times faster than the sheared chromatin before addition of NaCl and/or MgCl_2 (Fig. 2*b*; compare Fig. 2*a*). Electron microscopy has shown that this effect is

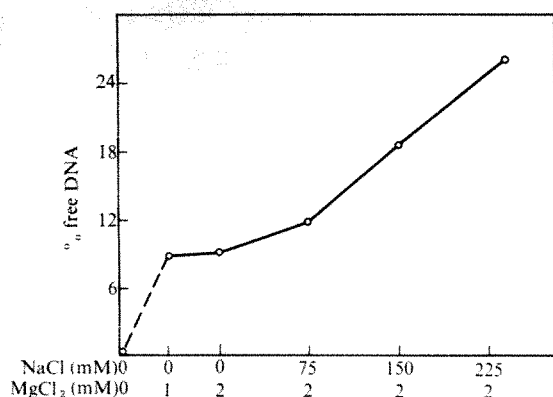


Fig. 3 Percentage of free DNA molecules in the sheared chromatin as a function of the ionic strength of solution. Each point was obtained by calculating the percentage of the free DNA in a CsCl equilibrium pattern of the sheared chromatin (Fig. 2c and d) which was preincubated in 5 mM TEA-HCl, pH 7.8 plus stated concentrations of NaCl and MgCl₂.

due to a collapse of the DNP accompanied by some aggregation of the DNP (unpublished data).

Percentage of free DNA molecules obtained from sheared chromatin is increased from zero in 5 mM TEA-HCl, pH 7.8 to 26% in 0.24 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8 (Fig. 3). At sufficiently high (0.10–0.25 M) NaCl concentrations Mg²⁺ is not necessary for the appearance of free DNA; the same result could be obtained also with NaCl/Na-EDTA mixtures.

After a shift to 0.15 M NaCl, 2 mM MgCl₂ a small fraction (5–7%) of the DNP was reproducibly found cose-dimenting with free DNA molecules in the upper part of the sucrose gradient (Fig. 2b). This slowly sedimenting DNP banded in CsCl at 1.48–1.50 g cm⁻³ and could thereby be separated from the free DNA (Fig. 2d and unpublished data). The significance and origin of this material remains unclear.

Ehrlich ascites tumour cells labelled in the usual manner (see above) were suspended in 0.15 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8 and passed once through a water-cooled French press under a pressure drop of about 1.5 kbar. The preparation was fixed in 1% formalin within a few seconds of shearing and thereafter centrifuged to equilibrium in CsCl gradient (Fig. 4). About 10–12% of the total DNA obtained is banded in the region of free DNA (Fig. 4). Appropriate controls (unpublished) have shown

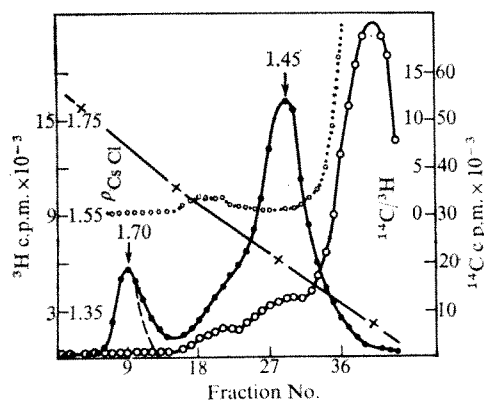


Fig. 4 Free DNA molecules in homogenate of whole cells. *a*, An aliquot of the ¹⁴C,³H-labelled ascitic fluid was immediately diluted ~200 times into 0.15 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8, then sheared to an average DNA length of about 0.5 kbase by a single passage through a water-cooled French press and thereafter fixed in 1% formalin within a few seconds after shearing. The sample was centrifuged to equilibrium in CsCl as described in the legend for Fig. 1. ●, ³H-DNA; ○, ¹⁴C-protein; X, density.

that most if not all free DNA is of nuclear origin. A reproducible, but yet unexplained feature of the CsCl pattern of the whole cell homogenate is the relatively high density of the main DNP peak (1.45 g cm⁻³ compared with 1.41–1.42 g cm⁻³ for purified sheared chromatin; Fig. 2c and d). These data indicate that in homogenates of whole cells under physiological ionic conditions long molecules of the free DNA are present.

At the present time it is unclear whether the very long stretches of completely free DNA discovered in this work have a functional significance with regard to transcription or whether they are structural features of the chromosome irrelevant to transcription. Studies on the renaturation of the free DNA and hybridisation of pre-mRNA to free DNA are in progress and should clarify this question.

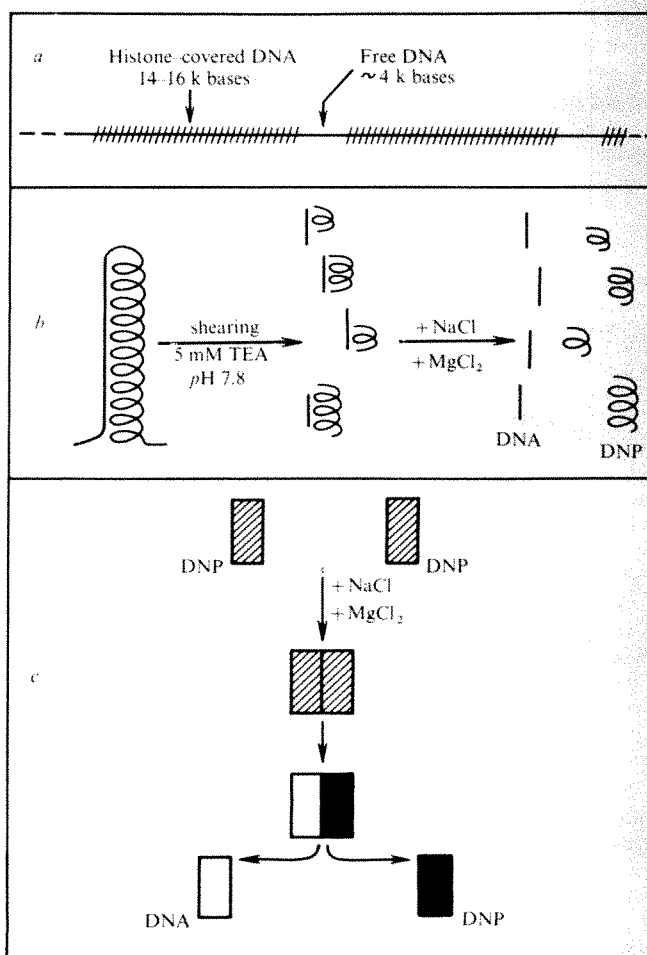


Fig. 5 Possible mechanisms of formation of free DNA molecules in the sheared chromatin. *a*, An experimentally observed mode of arrangement of histones other than F1 along unfolded chromosomal DNA (see Fig. 1 and the text). *b*, Generation of free DNA molecules in the sheared chromatin according to an 'asymmetric hairpin' model of DNA packing in a chromomere (see the text). *c*, Alternative model for the generation of free DNA molecules from sheared chromatin. Each DNP particle contains only one DNA molecule (see text for details).

At present we would like to consider another question: if there are only DNP particles in the sheared, soluble chromatin and no free DNA molecules (in the absence of NaCl and/or MgCl₂; see Fig. 2) what is the mechanism of generation of free DNA molecules on shifting to physiological ionic conditions? There are two explanations (Fig. 5b and c). According to the first model shearing of chromatin in a low (~ 0.005) ionic strength buffer lacking divalent cation produces DNP particles which contain two

(or more) noncovalently linked DNA molecules per particle. Such DNP particles are interpreted as fragments of the 'asymmetric hairpin' (Fig. 5b). One branch of the hairpin is thought to contain tightly supercoiled DNP whereas the other branch is in a relatively extended form. Addition of 1–2 mM $MgCl_2$ and/or 0.1–0.2 M NaCl induces separation of the branches, the final result being the liberation of free DNA molecules (which correspond to the extended branch) and of the supercoiled DNP particles (Fig. 5b). The asymmetric hairpin model of DNA packing in the chromatin was suggested to explain the observed distribution of histones other than F1 along unfolded chromosomal DNA (Fig. 5a) and also to explain a particular type of histone redistribution which occurs only at the moment of DNA unfolding in the chromatin but not after it (ref. 12; see also above). Furthermore, it should be noted that the average length of histone-covered plus histone-free DNA stretches (~20 kbases; see Fig. 5a) is comparable with the average length of transcriptional units in higher eukaryotes^{16–17}. Although the asymmetric hairpin model (Fig. 5b) is formally consistent with the present experimental data, an alternative model (Fig. 5c) now seems more likely. According to this model, each DNP particle in the sheared chromatin contains only one DNA molecule. In the presence of 1–2 mM $MgCl_2$ and/or 0.1–0.2 M NaCl, interactions between different DNP particles, for example, during 'dimer' or aggregate formation (Fig. 5c) induce an 'asymmetric' redistribution of proteins between DNP particles in the dimer or in aggregates of higher order. As a result dissociation of such a complex produces one molecule of free DNA and one or a few molecules of DNA associated with increased amount of protein (Fig. 5c). At present a more detailed model is not possible.

The second model (Fig. 5c) is consistent with all results obtained with sheared chromatin (Figs 2 and 3 and unpublished data) including the dependence of the final percentage of free DNA on the ionic strength of solution (Fig. 3). Work is now in progress to distinguish between the two models (Fig. 5b and 5c).

Although the *in vivo* significance of the long free DNA segments in the chromatin is not yet understood, it is clear that the fine structure of chromatin is strongly dependent on the ionic conditions of the medium. One should be careful when attempting to assess the significance of results of earlier studies on chromatic structure, which were carried out mainly at low ionic strength^{16,17}.

We thank V. V. Bakayev and A. A. Bayev, jun. for collaboration at later stages of the work, Dr V. N. Soyfer for providing ultracentrifuge facilities and N. N. Dobbert for technical assistance.

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- ¹ Murray, K., *J. molec. Biol.*, **39**, 125 (1969).
- ² Clark, R. J., and Felsenfeld, G., *Nature new Biol.*, **229**, 101 (1971); *ibid.*, **240**, 226 (1972).
- ³ Itzhaki, R. F., *Biochem. J.*, **122**, 583 (1971); **125**, 221 (1971).
- ⁴ Marushige, K., and Bonner, J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2941 (1971).
- ⁵ Itzhaki, R. F., and Cooper, H. K., *J. molec. Biol.*, **75**, 119 (1973).
- ⁶ Angerer, L. M., and Moudrianakis, E. N., *J. molec. Biol.*, **63**, 505 (1972).
- ⁷ Li, H. J., and Chang, C., *Biochem. biophys. Res. Commun.*, **47**, 883 (1972).
- ⁸ Billing, R. J., and Bonner, J., *Biochim. biophys. Acta*, **281**, 453 (1972).
- ⁹ Jensen, R. H., and Chalkley, R., *Biochemistry*, **7**, 4388 (1968).
- ¹⁰ Ilyin, Yu. V., Varshavsky, A. J., Mickelsaar, U. N., and Georgiev, G. P., *Eur. J. Biochem.*, **22**, 235 (1971).

- ¹¹ Varshavsky, A. J., and Georgiev, G. P., *Biochim. biophys. Acta*, **281**, 669 (1972).
- ¹² Varshavsky, A. J., and Georgiev, G. P., *Molec. Biol. Rep.*, **1**, 143 (1973).
- ¹³ Ilyin, Yu. V., and Georgiev, G. P., *J. molec. Biol.*, **41**, 299 (1969).
- ¹⁴ Ilyin, Yu. V., Varshavsky, A. J., and Georgiev, G. P., *Molec. Biol., U.S.S.R.*, **44**, 821 (1970).
- ¹⁵ Hancock, R., *J. molec. Biol.*, **48**, 357 (1970).
- ¹⁶ Simpson, R. T., *Adv. Enzymol.*, **38**, 41 (1973).
- ¹⁷ Huberman, J. A., *A. Rev. Biochem.*, **42**, 355 (1973).

Erratum

In the article 'Relative-distance Machian theories' by J. B. Barbour (*Nature*, **249**, 328; 1974) paragraph 7, line 5 should read:

of particles, and their derivatives $\dot{r}_{ij} = dr_{ij}/d\lambda$ with respect

Equation (1) should read:

$$L = \Psi \Gamma \quad (1)$$

where

$$\Gamma = (\sum_{i < j} m_i m_j r_{ij}^2)^{1/2}, i = 1, \dots, N (\dot{r}_{ij} = dr_{ij}/d\lambda)$$

$$\Psi = \sum_{i < j} m_i m_j / r_{ij}$$

Paragraph 7, penultimate line should read:

pendent of the time parameter (a sum like $(\Psi + \Gamma)d\lambda$ would

Equations (2) and (3) and the intervening explanation should read:

$$L = \Psi [\sum_{i < j} m_i m_j (\dot{x}_i^2 - 2\dot{x}_i \dot{x}_j + \dot{x}_j^2)]^{1/2} \quad (2)$$

and the Euler-Lagrange equations

$$\frac{d}{d\lambda} (\partial L / \partial \dot{x}_i) = \partial L / \partial x_i$$

are

$$\frac{d}{d\lambda} \left\{ \frac{\Psi m_i}{\Gamma} \left[\sum_{j \neq i} m_j (\dot{x}_i - \dot{x}_j) \right] \right\} = \frac{\Gamma \partial \Psi}{\partial x_i}$$

We can now specialise both λ ; taking

$$d\lambda = ds = (\sum_{i < j} m_i m_j dr_{ij}^2)^{1/2}, \text{ i.e. } \Gamma \equiv 1,$$

and the coordinate system, taking it such that

$$\sum m_i \ddot{x}_i = 0 \text{ (here } d\lambda = ds)$$

In these special frames, which are distinguished by the uniquely determined relative motion, the equations of motion simplify to

$$M \frac{d}{ds} (\Psi m_i \dot{x}_i) = \frac{\partial \Psi}{\partial x_i} \quad (3)$$

In the last line, for ψ read Ψ

Equation (4) and the next line should read:

$$m_i d\dot{x}_i/dt = (1/M\Psi) \partial \Psi / \partial x_i \quad (4)$$

where $\gamma = 1/M\Psi$ is a gravitational constant that is determined.

Page 329, paragraph 5, line 2 should read:

linear in the \dot{r}_{ij} s to L (gyroscopic-type forces) or multiplying L

Equation (5) should read

$$m_i d\dot{x}_i/dt = (1/M\Psi) \partial \Psi / \partial x_i + (1/M\Phi) \partial \Phi / \partial x_i \quad (5)$$

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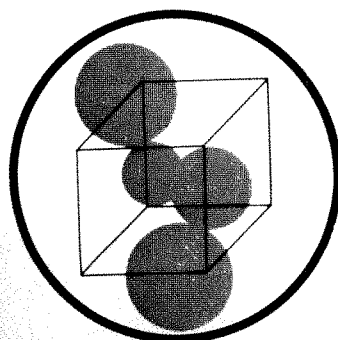
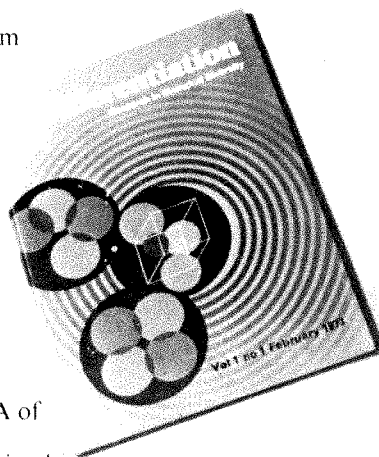
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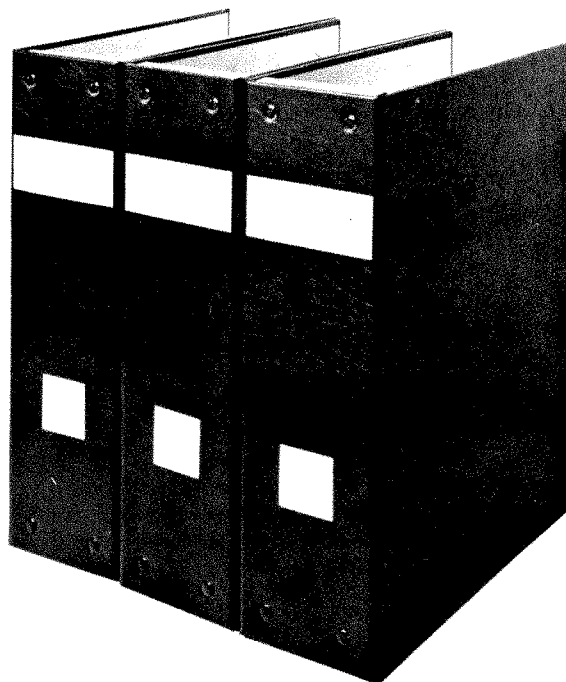
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reviews

Since India met Eurasia

Ecology and Biogeography in India
Edited by M. S. Mani. (Monographiae
Biologicae, Vol. 23.) Pp. xix+773.
(Junk; The Hague, 1974.) 190 guilders.

INDIA (including Pakistan, Ceylon (now Sri Lanka), Nepal, Sikkim, Bhutan and Burma) provides a particular fascination to biogeographers, for it is the arena where a southern continent, detached from the disintegrating Gondwana continental block in the vicinity of south-east Africa and Madagascar apparently before the Maestrichtian towards the end of the Cretaceous period, finally traversed the Tethys Sea and collided with the southern shores of Eurasia perhaps in the Oligocene. The Indian Peninsula and Ceylon is the surviving fragment, but the Laccadive, Maldive, Chagos and Mascarene archipelagos represent submerged mountain summits of a larger part that collapsed as the great scarp of the western Ghats rose, consequent on the underthrusting of the northern margin of this continental plate beneath the southern margin of Eurasia; the other consequences of this underthrust have been the creation of Ganges-Indus plains and the massive Tertiary orogene that lifted the Himalayas.

It may be supposed that two tropical Cretaceous faunas and floras, having evolved in isolation, met then for the first time: an essentially insular and probably largely oceanic rain forest biome from the south, and a much more diversified continental array in the north among which were nevertheless the once vast but already retreating rain forests which stretched as far as western Europe in the Eocene, whose heirs now clothe the hills of western Malesia and south-east Asia. Subsequently the uplift of the Himalayas has created whole new environments for colonisation and, with the Pleistocene glaciations, has radically altered regional climate, while man has for long and increasingly destroyed or impoverished the Indian environment.

It is the biogeographers' task to explain the distribution of modern biomes in terms of past events, and to trace the origin, migration and diversification of their component taxa. His only direct evidence is palaeontological, which in India is almost completely lacking between the lower Cretaceous and Miocene, and in the Peninsula

decidedly scant thereafter. He must therefore fall back on an unavoidably speculative approach; but, by careful analysis of the phylogeny and ecology of selected groups, whose ecology, behaviour and structure prevent rapid and distant dispersal in relation to their distribution, deductions from repeating patterns can be made. Here he is aided by the rich and distinctive biomes of the Mascarenes, including Madagascar, which contain representatives of many groups that are unable to traverse any but the shortest breaks in their chosen habitat, and which provide valuable comparison with those of the Andaman and Nicobars to the north-east and of northern continental origin.

Professor Mani's massive compendium largely fails to do this: it suffers from inadequate integration of the contributions from different authors, and overreliance on evidence from coincidence of modern distribution patterns alone, presented by lists of species whose ecology, and phylogenetic relationships where they exist, are either not discussed or are overgeneralised. The five introductory chapters on physiography, geology, climate and soils present valuable detail, but lack integration; as one example in many, a section on the microclimate within selected Indian crops is in no way integrated with the theme of the book. The editor's own chapters in particular suffer from lack of maps to summarise the mass of geographical detail and place names quoted. The five chapters on vegetation are particularly thin on ecology and contain many errors of detail. Ceylon is said to contain few endemic genera and species, yet in fact almost one third of the angiosperm species are endemic; *Impatiens* and *Apostasiaceae* are wrongly stated not to occur in Malesia; *Shorea* is described as a characteristic genus of the western Ghats forests, yet the only species in fact occurring, *S. roxburghii*, is extremely local and at the edge of its range there. The absence of tropical Amentiferae—Fagaceae, Juglandaceae, Myricaceae and Hamamelidaceae—and the extreme poverty of Ericaceae and Coniferae is a salient feature of the peninsula and Ceylon demanding phytogeographic discussion, but there is none; the single native peninsular conifer is merely cursorily mentioned as *Podocarpus latifolius* in one

chapter, *Decussocarpus latifolius* in another. Nowhere does the contrasting biogeography and ecology of forest and grassland in the peninsular mountains receive the interpretation it deserves, and indeed these mountains are stated to lack altitudinal zonation which is quite incorrect.

The editor is a zoologist, and the main emphasis is zoological, the five chapters on regional biogeography being in fact entirely zoogeographical. This is reasonable and must depend on availability of authors (though their titles are misleading), but once again the integrated approach is lacking. The book's introduction stimulates the reader with controversial statements dogmatically presented but he is ultimately disappointed to find that, though they are often repeated, their substantiation is never realised: where is the evidence that the original Gondwana flora and fauna were evolutionary "stagnant" or "degenerating" when the Asiatic land connection became established? Indeed, though extensive descriptions of subsequent migrations in and out of the peninsula are included, where in the whole book are the taxa of acclaimed Gondwana origin, upon which this discussion must depend, convincingly identified by reasoned argument? One is led to suspect that, in spite of disclaimers, the evidence lies merely in the evocative but probably irrelevant relict distribution patterns of a wide range of species of clearly different affinities (and origins?) in peninsular biomes that have become fragmented in Pleistocene to recent times.

Yet the evidence is there for the searching. An integrated approach would, for instance, compellingly suggest that the mighty dipterocarp trees, whose present centre of distribution is in the western Malesian rain forests which they so distinctively dominate, are extraordinarily successful immigrants whose origin must be sought in the so-called degenerate and stagnant Gondwana biomes. The distinct distribution of interfamilial links such as the genus *Axinandra*, as well as the isolated and distinctive Nepenthaceae, demand discussion in relation to their ecology. Four chapters are in fact provided on the biogeography of specific animal groups; among them P. K. Sen-Sarma's treatment of the termites, and K. C. Jayaram's of the freshwater fishes, amphibians and reptiles, con-

taining ideal taxa for biogeographical analysis (unlike the butterflies of D. J. Holloway, which are likely to be of too recent origin to have existed in the peninsula before it became disconnected from Africa). Centres of origin should be traceable, but the discussion never reaches a sufficiently detailed level to convincingly do so. Professor Hilary Cruz's work on the ecology of the extraordinary endemic agamid genera in the hill forests of wet zone Ceylon could, for instance, have provided the basis for such a discussion but his work is not mentioned.

The biogeography of the Indian region is not, of course, that of the peninsula alone; separate chapters are devoted to the Indo-Gangetic plain, and the Himalayan region. But though much information is presented as in the rest of the book, these chapters are marred in the same manner. The chapters on the impact of man, and the ecology of 'tribal' man (by P. Lal) are welcome and useful, as are those on recent faunal impoverishment (A. K. Mukherjee), the ecology of vertebrates of the Indian desert (I. Prakash) and the mammals of Assam (G. V. Kurup).

The book is well designed and printed, though spelling errors, particularly in scientific names, are not infrequent.

P. S. ASHTON

Anatomy of experiments

Design of Experiments: A Realistic Approach. By V. L. Anderson and R. A. McLean. Pp. xvii+418. (Statistics: Textbooks and Monographs.) (Dekker: New York, February 1974.) \$19.75.

I HAVE long contemplated the value of a book that would discuss in detail a few experiments in which statistical concepts were vital to the design, the analysis and the interpretation. The declared objective of the book under review is "to express rather complex ideas on how and why scientific investigators should design experiments". Is this the book I want?

Certainly it has many merits. The authors employ a wide range of examples to illustrate the theory and practice of experimental design, choosing these largely (but not entirely) from manufacturing industries of which they obviously have much experience. For the teacher, the examples analysed fully and others outlined as problems for the student will prove useful sources of ideas. Nevertheless, I was disappointed to find attention concentrated on questions that can be answered in terms of currently available theory of linear models for observations. On the structure of analysis of variance and on variance components,

the presentation is good. Little is said about reasons for choosing particular designs. The interpretation of results remains on the statistician's desk, and is not carried to the field of application. Computational methods and problems are not mentioned: even the existence of standard programs and the desirable features of computer input and output are neglected. The anatomy of analysis of variance is thoroughly displayed, but the developmental and operational physiology of experimentation remains unexplained.

The book proceeds through standard designs, from completely randomised to lattices and factorials, in a manner at times refreshingly different from other texts. Without entering deeply into combinatorial problems, clear accounts are given of the partitioning of sums of squares. The dependence of analysis on randomisation and of significance tests on variance components is well emphasised, and the emphasis on the inference space is often illuminating. The text is enlivened by many comments that reflect the authors' familiarity with the particular examples, increasing my regret that they did not choose to make that familiarity a distinctive feature at the expense of some of the more conventional algebra. Had they done so, they might have let the reader see the severe limitations of the Newman-Keuls and other multiple comparison tests which so seldom represent any realistic model of data: they might also have illustrated the full interpretation of analyses of transformed data. The final chapter on response surfaces is an interesting brief survey, tantalising because too short to allow critical comparison of methods and objectives.

D. J. FINNEY

Plant becomes soil

Biology of Plant Litter Decomposition. Edited by C. H. Dickinson and G. J. F. Pugh. Vol. 1: Pp. xiv+1-244+46. Vol. 2: Pp. x+245-775+75. (Academic: London and New York, March 1974.) Vol. 1: £7; Vol. 2: £10.50.

RECYCLING of elements bound in living matter is essential to the continuation of life in our, at present, virtually closed global ecosystem. Since plants form the bulk of the world's biomass and, at least on land, the bulk of this biomass is recycled by the decomposition of plant litter rather than by consumption by herbivores, there can be no doubt as to the importance of the subject matter of these volumes. The excellent introductory chapter on 'Litter—Interface of Animate/Inanimate Matter' gives a broad and stimulating view of the whole field of decomposition studies. Some later chapters, by the nature of their narrower

topics, seem by comparison more mundane in their approach to the ecological issues. Following the introduction, the work is in three parts.

In the first part, plant litter decomposition is treated on the basis of different types of plant litter. Thus there is a chapter on lower plant litter, so important in the transition from rock to soil, followed by chapters on decomposition of herbaceous litter, angiosperm tree leaf litter, coniferous leaf litter, wood and roots. Finally, in part I is a chapter on the nature and decomposition of digested plant litter in the form of animal dung and in the faecal pellets from certain soil fauna. It seems artificial to exclude from consideration in the work the decomposition of plant material which has actively been detached from plants by herbivores or by man for feeding to his herbivorous livestock. Such lines of thought, however, lead to the large, but dubiously separable areas of biodeterioration and ruminant nutrition.

In part 2, the chapters survey different groups of organisms involved in plant litter decomposition ranging from bacteria, actinomycetes, terrestrial fungi, aquatic fungi, protozoa, nematodes, oligochaetes, microarthropods, macroarthropods and molluscs, to aquatic crustaceans. The emphasis in these chapters varies: some provide a brief introduction to the classification of the group whereas others assume the reader's familiarity with the group and devote attention directly to the ecological significance of the organisms in litter decomposition.

This approach to ecological problems of plant litter decomposition seems less thought provoking for the general reader than is the approach through litter types or the chapters in part 3 where decomposition of plant litter is discussed in relation to the environment in which decomposition takes place. These last chapters cover decomposition both on the surface of soil and in the depth of soil, in fresh water and in marine environments. The two final chapters discuss special environmental problems of the breakdown of agricultural crop debris and urban waste. Peat and coal are economically important deposits of partly decomposed plant debris. Surprisingly, these are largely ignored in the environmental chapters, though a forthcoming review on peat, by one of the contributors, may account for part of this omission.

The editors have performed a considerable service in bringing together and indicating the relevance of some 2,000 references. I suspect that the work will be appreciated as a source book mainly by students, though researchers in this field will glean grains of information.

R. C. CODNER

Amoral concepts of biology

Philosophy of Biological Science. By David L. Hull. Pp. xi+148. (Prentice-Hall: Englewood Cliffs, N. J. March 1974.) \$6.95.

In this book, Professor Hull sets out at once to explain to students of philosophy what he regards as the most important laws and concepts of modern biology, and to initiate biologists into a philosophical approach to their problems, therefore assuming in his readers little prior knowledge of either discipline. Hull freely acknowledges the difficulties he has experienced in attempting this in lecture courses addressed to mixed classes of biology and philosophy students, and admits that the aim is a large one for so small a book. It can at least be said that he has achieved his initial aim of making the exposition intelligible to relatively unprepared readers. Students taking courses of the type that Professor Hull delivers will doubtless find this book useful, even though his bibliography is short, highly selective and almost exclusively American.

The extent to which modern professional biologists look to philosophy for guidance in their scientific thought and activity, or to which modern philosophers look to biology as a testing ground for their views on the methods and logic of science, are probably both rather small. One has the impression that biologists tend to develop an interest in philosophy after their main scientific work has finished, generally looking for systems of thought which would justify *post facto* the ways in which they have been thinking. Most philosophers of science, at least within the dominant positivist schools, take the Comtean view, of physics as the paradigmatic science and of biology as a relatively immature and secondary study. It is doubtful whether Professor Hull's book will have any significant influence in changing this situation; neither philosophers nor biologists will find in it any very compelling new insights. Like another recent author in this field, Michael Ruse, Hull seems concerned mainly to provide a respectable articulation of the current American 'conventional wisdom' on the subject. His method of exposition consists in posing a series of philosophical questions about biology, which he proceeds to discuss serially, usually without reaching any very conclusive answers.

Hull's exposition has some very notable omissions—for example, though he is a member of the editorial board of the journal *Systematic Zoology*, and has contributed to it and other journals a series of articles on the theory of biological classification, this topic receives no specific attention in

the book—although most experienced botanists, zoologists and bacteriologists would admit the centrally important role of classification in their sciences (a role with no real parallel in physics), and Michael Ruse devoted two whole chapters to it. Admittedly, one important question posed—but not finally answered—by Hull, does involve the theory of systematics; this question is, to what extent are taxa individuals, with names analogous to proper names, and therefore not permissible in what Hull would recognise as 'laws'? Furthermore, though evolutionary theory is considered at some length, Hull makes no reference to the scientific nature and status of geology—of which it would be quite possible to consider the biological sciences as subordinate parts—nor does he consider the epistemological problems posed by the palaeontologists' claim to knowledge of the organisms of the remote past.

Pride of place in Hull's exposition of biology is accorded to classical and molecular genetics, as domains of what he would accept as scientific 'law'. Yet no reference is made to the profound critique of this conception of genetics by a very eminent geneticist, as expressed in the article "Axiom and Process in Genetics" by C. D. Darlington (*Nature*, 234, 521–5; 1971). Darlington drew attention, among other things, to the nature of the genotype as the embodiment of race history, and to the dual nature of time, as cyclic and as unidirectionally progressive. Almost the only reference to the nature of time in Hull's book is the offhand remark "Both space and time are now viewed as organisational properties of material bodies"—one of the few ontological assertions he makes. Even the second law of thermodynamics, which might call into question this assertion as far as time is concerned, is mentioned only in dismissing the claim by some authors that organisms can violate it.

Professor Hull's omissions have I think a definite tendency—that of excluding from consideration anything which might raise ontological or moral problems. Recent Anglo-Saxon schools have generally equated philosophy with epistemology, dismissing ontology as "metaphysics" and treating ethics as not properly part of academic philosophy. Hull's failure to consider the moral problems posed by modern biology, and especially by modern medical research, is perhaps a little old-fashioned; there is a rapidly growing general consciousness of these moral issues, not least among students. The issues concern, not merely the ethics of experimentation on human beings, but also the proper relations of man to non-human organisms.

The shortcomings of this book are

shared, to a greater or less extent, by most recent treatments of the subject, and should be attributed less to the author than to the general intellectual climate in which he writes. The essential virtue which Professor Hull can claim is that he at least poses a number of profoundly important questions, the serious pursuit of which by students might lead them far beyond his own exposition—which, after all, is an end that any teacher should be proud to have achieved.

R. A. CROWSON

Spoonful of saccharin

Sensory Processes: The New Psychophysics. By Lawrence E. Marks. Pp. x+334. (Academic: New York and London, February 1974.) \$17.50; £8.25.

PEOPLE who use saccharin to sweeten their tea may have noticed a surprising thing: halving the concentration of sugar in a solution reduces its sweetness far more than halving an equally sweet (although much weaker) concentration of saccharin. This is one of many examples that demonstrate differing relationships between sensory magnitude and physical stimulus. How does one measure sweetness, brightness, pitch, odour? It has been shown, particularly in the pioneering work of the late S. S. Stevens, that asking subjects to assign numbers to sensation strength leads to a power function with an exponent dependent upon the stimulus and sensation considered. Lawrence Marks draws a clear distinction between this, the "new" psychophysics, and sensory physics, the "old" psychophysics, in which the observer is simply a detector of threshold, masked threshold, or null point, with measured quantities all in the physical domain. He describes and attempts to interrelate the various psychophysical procedures such as fractionation, category rating, and magnitude estimation and discusses the influences of extraneous factors. The senses are each considered under the headings of sensitivity, temporal and spatial factors, and qualitative aspects. Although one detects a certain antipathy towards the "old" psychophysics it is a carefully reasoned and comprehensive account and shows great concern for validation of the approach. Unfortunately his rather detached attitude coupled with the large number of references makes difficult reading in parts and a certain amount of repetition is inherent in the organisation he has adopted.

Although the new psychophysics gives insights into sensory processes not obtainable in any other way the field has a certain contrived air to it. We do not generally use our senses, or num-

failed, is not that it uses abstract models and traces interdependence between variables, but that it has sometimes been pseudo-planning, confined to a ceremonial superstructure, without being geared to where the action is.

The authors argue for the central importance of the annual budget. Though it is obviously true that no plan can be implemented unless it is integrated into the annual budget, there is a danger of mistaking a necessary, though minor, condition for the strategic one. Budgets are, at best, annual public expenditure plans. A focus on fiscal magnitudes, though essential for proper public accounting, obscures and evades the real activities. Links between fiscal (or even financial) expenditures and results are tenuous, especially in underdeveloped countries. There are no fixed coefficients between money expenditure and land reform, population policy, incomes policy, education, public health, nutrition. Foreign exchange budgeting, manpower budgeting, raw material budgeting are just as important as fiscal budgeting and even they do not exhaust the range of necessary policies. Proper public accounting is necessary to ensure, negatively, that public money is not spent extravagantly or corruptly, but it cannot ensure, positively, that it is spent according to social priorities and that the necessary complementary actions are taken. Budgeting is to planning what bookkeeping is to business management: without it, management is impossible; but with the best book-keeper in the world, a firm can go bankrupt. These points are not enough stressed by the authors, who see the plan essentially as a many-year public capital budget.

Much is made of the need for redundancy. In a piece of exquisite jargon, the authors write: "Broadly speaking, we can regard social poverty as a lack of functional redundancy" (Page 49). But there is a vast difference between reserves (which serve a purpose) and redundancies. A more analytical and quantitative approach would have made the distinction clear. It is now well known that in poor societies, not only unskilled labour, but also capital and technically trained professional manpower are redundant: but, alas, they are not reserves.

The authors make a large number of entirely fair and commonsensical criticisms of planning. "If we were asked to design a mechanism for decisions to maximise every known disability and minimise any possible advantage of poor countries, we could hardly do better than comprehensive, multi-sectoral planning" (Page 293). The need for unavailable information, for political stability, for consistent aims are cited as unattainable condi-

tions. But perhaps the most serious criticism of planning is omitted, viz. that its very success, measured by coherence and consistency, becomes an obstacle to adaptation and innovation. Plans introduce an additional rigidity into societies already inflexible. Plans, for this reason, in spite of their declared intentions, are elements strengthening conservatism.

The conclusion is not, however, reliance on *laissez-faire* and the free play of market forces. The authors rightly point to the need for a combination of contingency planning, continuous budgeting and rolling planning, so that there can be adequate and speedy responses and adaptations to unforeseen events, both favourable and unfavourable.

The authors treat planners and planning as part of the social and political environment which they are supposed to plan. Planning the planners is not an invitation to an infinite regress but a reminder that there must be continuous mutual adaptation between plan objectives and social constraints.

PAUL STREETEN

Details of sense organs

The Ultrastructure of Sensory Organs. Edited by I. Friedmann. (North-Holland: Amsterdam and London; American Elsevier: New York, 1974.) Dfl.90; \$32.70.

THIS collection of four essays is less general than the title implies; only vertebrates are described. Of their sense organs only taste buds (R. G. Murray), the organ of Corti (H. Engström and H. W. Ades), the vertebrate retina (R. F. Dunn) and the olfactory mucosa (P. P. C. Graziadei) are included. The preface indicates the intention of the articles to summarise "the immense and rapid progress that has been made in electron microscopy" for "anatomists, physiologists, pathologists and clinicians". Only Engström and Ades make some attempt to meet the latter two classes of busy people part way. Anatomists are best treated in that all the articles provide descriptive anatomical accounts while remaining substantially innocent of physiological commentary or synthesis of the two approaches.

Thus the essays have to be judged not as summaries of the contributions ultrastructural studies are making to understanding of the mechanisms of action of the organs treated, but as summaries of anatomical information available up until about 1970-71. Most of the summaries are competent but, perhaps because of editorial direction, they all seem of rather even emphasis; so that exciting new developments and the relative importance, or accuracy, of different and differing observations are

hard to discern. Perhaps the main point of the articles was to provide a review of the literature together with an atlas of ultrastructure. There are numerous electron micrographs including a fair number, especially of the retina, that have not previously been published. For the non-specialist in a particular sense organ the essays provide a reasonably readable guide to the literature of the past twenty years. There are useful summary tables of data, for example in the article on the retina. But for the readership suggested, and given that the articles are purely anatomical, a greater use of summary diagrams, perhaps at the expense of some of the electron micrographs, would have been more helpful to ready comprehension. The essays are probably most useful for someone beginning an interest in the structure of a particular sense organ. Most people are likely quickly to prefer the more detailed and often more synthetic articles of the currently appearing *Handbook of Sensory Physiology*.

B. B. BOYCOTT

Computers in mathematics

Computer Approaches to Mathematical Problems. By Jurg Nievergelt, J. Craig Farrar and Edward M. Reingold. Pp. xii + 267. (Prentice-Hall, Englewood Cliffs, 1973.) \$8.95.

AFTER an introductory chapter on languages, including some remarks on the parenthesis notation and the polish notation, there is a chapter on combinatorial computing. This includes a discussion of block designs, latin squares, scheduling and tiled rectangles; and also an account of various graph algorithms, for shortest paths, spanning trees and sorting. There follows a chapter on the use of computers in game playing and decision making and another chapter on random numbers and their use in Monte Carlo methods and in simulation. Computing in number theory is next considered and there is a review of the position in the computing of $\sqrt{2}$, e and π . For example, in 1967 the value of π was computed to 500,000 decimal places. Finally there is a section on the use of computers in calculating large prime numbers. The largest known Mersenne prime, by 1971, is $2^p - 1$ where $p = 19937$. The book closes with a chapter on logic and computers.

This book will be of particular interest to the mathematician interested in the use of computers in pure mathematics. But in this connection, it is a little surprising to find no reference to the use of computers in the theory of finite groups. It will also be relevant for students of operational research mathematics.

L. S. GODDARD

Comprehensive mineralogy

Modern Mineralogy. By Keith Frye. Pp. ix+325. (Prentice-Hall: Englewood Cliffs, NJ, 1974.) £6.50.

MANY new mineralogy textbooks have appeared during the past decade; most have been devoted to specialised subjects and these have tended to replace the earlier type, which aimed at providing for the whole of a student's requirements up to the level of the first degree. With advance of knowledge and the need to explain quite sophisticated techniques even in an elementary book, it is no longer possible to provide a comprehensive text at an adequate level without making the volume unduly expensive, cumbersome and forbidding. Specialised books necessarily contain a much fuller treatment than the average student requires at an early stage but the rapid escalation in book prices has now reduced their attraction for purchase by students. To some extent this has restored the need for a new type of comprehensive text and this book is a valiant attempt to meet just this need. It is selective, it omits much of the conventional crystallographic introduction, and its stated purpose is to provide a sound physically-based approach through familiar atomic theory and crystal structures in a logical sequence, and to introduce the student to present-day concepts of mineral formation and equilibrium. How well does it measure up to these rigorous requirements?

There are seven chapters and a 25-page tabular appendix of data on 150 common minerals. Chapter one is a fairly conventional crystal-chemical introduction that considers the nature of chemical bonds, close packing, ionic radii, simple model structures, defects and solid solution. It is pleasant to note the attention paid here to chemical and structural defects, to dislocations and to the evolution of grain boundaries. Chapter 2 provides a systematic account of the crystal structures found in minerals; again it follows a conventional treatment except where the model-structures theme is developed attractively by way of stuffed derivatives to explain many common rock-forming silicates. There follows in chapter 3 an all-embracing exposition of crystal symmetry, including lattices, point groups, the stereographic projection, crystal morphology, twinning and mineral habits, in 60 glorious pages. Logic seems to have quite deserted the author at this stage, for crystal structures have already been explained and many of the topics introduced (such as space groups) are certainly not needed for what follows. Chapters 4 and 5 are shorter; they are concerned with physical properties and the interaction of radiant energy

with crystalline material. Topics dealt with here include mechanical behaviour, radioactivity, surface properties (but not pyro- and piezo-electricity) colour and lustre, atomic absorption, justification of the optical indicatrix, and the principles of X-ray diffraction. Although this is attractively presented, as in chapter 3 it is difficult to understand why so many principles are explained, albeit briefly, when no attempt is made to show how to use them. This middle part of the book is rather like Hamlet without the Prince of Denmark!

Chapter 6 is probably the best part of the entire book; it is excellent. Beginning with a concise and lucid explanation of the phase rule, it passes on to consider phase diagrams. It deals mostly with crystallisation and melting relations in binaries and ternaries, but regrettably does little on 3-phase triangles or on working out detailed courses of crystallisation. It then introduces isothermal and isobaric sections (used in the final chapter) and then passes on to a brief consideration of a quaternary system with a vapour phase. The final chapter spans an even vaster canvas—the whole of petrology! It begins with an enunciation of the principles of geochemistry and mineral formation and then proceeds to consider the classification and petrogenesis of igneous, sedimentary, and metamorphic rocks as well as some aspects of ore deposits. Although much of the treatment here is skilfully conceived as a consummation of all that has gone before, the familiar symptoms of extreme compression and a plethora of quite difficult new concepts, criticised earlier, here becomes a veritable disease. This must render the chapter virtually useless to an elementary student as a first reading. The repeated misuse of 'lattice' for 'structure' here is deprecated. I cannot see any value in the appendix, which is not sufficiently comprehensive to be of much use for identification yet takes up valuable space.

The diagrams are clear and well drawn but a few minor errors were noted (Fig. 6.13a). Separate author, mineral name and subject indexes are provided.

The author deserves full credit for producing an original, thoughtful and always interesting book which omits much of the irrelevant and otiose morphology still on offer in many. I do not wish to be pejorative but far too much has been attempted, the result being a book that is not really adequate as a crystallography, mineralogy or petrology text. It does not explain how to determine a point group, analyse a stereogram, use a polarising microscope or obtain information from an X-ray photograph. It does not explain the detailed reading of phase diagrams or

how to describe a rock. Nevertheless it contains much information of value not easily found elsewhere and sections can be strongly recommended as further reading by more mature students and as a source of good ideas for elementary teaching. The author has demonstrated his ability to explain difficult ideas clearly, but the book would have fulfilled its rôle as a mineralogy text better if the final chapter and appendix had been omitted, the space saved being used to explain practical aspects of optics including some mention of reflected light, to expand the X-ray section, and to say more on solid solution in rock-forming minerals.

I. D. MUIR

Microscopy for all

The Encyclopedia of Microscopy and Microtechnique. Edited by Peter Gray. Pp. xi+638. (Van Nostrand Reinhold: New York and London, January 1974.) £16.25.

IN recent years methods of microscopy and microtechnique have expanded at such a rapid rate that it is almost impossible to remain abreast of current developments in all but a small area of specialisation. This not only makes the provision of extensive reference books essential, it renders the task of their compilers almost impossible. The present book is, as the editor states in the preface, an attempt to cater for the needs of all who use the microscope, not only in the medical and biological sciences, but also among the "atmospherologists, bakers, chemists, metallurgists, textile workers, the manufacturers of paints and those who examine paintings". With such an implied wide-ranging coverage in mind, the need for interdisciplinary communication (especially with regard to such a basic tool as microscopy) is clearly apparent and the attempt is praiseworthy. In practical terms, however, it seems that it is not entirely successful. One wonders what the textile worker or paint manufacturer who uses the microscope will gain from the inclusion of a long description of the morphology and systematics of the annelids.

An attempt to cover the whole spectrum of possible microscope usage must fall far short in some fields and it seems that the basic fault of this book lies here. Had the coverage been limited to the instrument and to basic preparative techniques, the value of the book to all users of the microscope would have been increased and the topics could have been covered in more detail.

Most of the articles in this book (provided by about 180 specialists) are short, only one or two pages in length,

and so cannot provide more than a very superficial coverage of the subject. There is, however, a list of references after most articles which serves as an introduction to sources which provide more detailed information. As might be expected, there is a considerable variation in the standard of the individual articles. Some provide an excellent thumb-nail sketch of the subject whereas others contrive to say very little indeed. The production quality is good and there are surprisingly few misprints for such a large book (although some inconsistencies have crept in, Abbe's name being rendered variously as Abbé or Abbee for example). The index is an essential feature of any reference book and in this case it is perfectly adequate.

S. BRADBURY

Raman analysis

Applications of Laser Raman Spectroscopy. By Stanley K. Freeman. Pp. xi+336. (Wiley: New York and London, January 1974.) £9.75.

THIS new book on the applications of Raman spectroscopy is the most significant modern volume to survey the analytical value of laser Raman spectroscopy. It includes a compendium chapter on the principles behind the Raman and related effects and others on experimental technique, a long section on qualitative analytical applications and concludes with chapters on synthetic and biopolymers and the value of Raman methods in pollution studies.

Molecular vibrations are normally studied by infrared absorption and Raman scattering. Since the vibrations characteristic of a complex molecule are themselves complex, these two techniques can, in principle, provide excellent fingerprints of analytical value. Infrared spectroscopy has been used routinely in this way for more than thirty years but Raman spectroscopy has not. Forty years ago Raman spectroscopy was thought to have immense analytical potential but it became clear in the later 1930s that experimental limitations severely restricted the versatility of the method. The relatively recent advent of the laser and sophisticated commercial spectrometers have improved the situation dramatically with the result that Raman spectroscopy is now playing an invaluable part both as an analytical tool (in the broadest possible sense), in structural chemistry, solid state physics and many other diverse areas.

In his book, Freeman selects some of the analytical applications of Raman spectroscopy and discusses these in considerable detail. Since other books exist covering infrared and Raman

spectra of crystals, and reviews abound in other detailed areas, he quite rightly spends most of his time on the area in which he is best known and which is worst surveyed elsewhere: the use of Raman spectroscopy for functional group identification in organic analysis. In this area, the book is unique and contains a wealth of otherwise unpublished data and the significant results of years of experience. In fact, it can be truthfully said to be the logical successor to the classic books in this field by Hibben and Kohlrausch, both published before 1940!

In the chapters on polymeric and biological compounds the coverage is again chemical and analytical rather than structural and little space is spent in considering the niceties of vibrational theory and structural consequences thereon. But in the context of the book as a whole, this is entirely relevant. The section on pollution monitoring and Remote Raman techniques is also valuable in that it surveys a field which is very diversely covered in the literature.

My initial reaction to Freeman's book was most unfavourable since the title is misleading. Nowhere convenient does the author point out that to him "applications" mean analytical ones, thus many will be disappointed by the narrow coverage and extravagant claims on the dust cover. Further, the production quality is reminiscent of a cheap paperback. But once the first chapter is digested and the general tenor of the coverage grasped, the book endears itself to the practicing chemical vibrational spectroscopist.

P. J. HENDRA

Aluminium analysed

Analytical Chemistry of Aluminum. By V. N. Tikhonov. Translated by J. Schmorak. Pp. x+303. (Analytical Chemistry of the Elements.) (Halsted, Wiley: New York, Toronto and Chichester; Israel Program for Scientific Translations: Jerusalem, January 1974.) £9.55.

THIS latest volume in the series of monographs dealing with the analytical chemistry of the elements, and sponsored by the USSR Academy of Sciences through the Vernadskii Institute of Geochemistry and Analytical Chemistry, is concerned with aluminium (*sic*).

The contents follow the general pattern established in previous volumes. A short preliminary chapter details relevant information about the occurrence, the physical and chemical properties of aluminium and its compounds, particularly the properties of complexes of the element with organic compounds which have found use as

analytical reagents. Chapter 2 contains an extensive account of chemical and physicochemical methods for the determination of aluminium, in which gravimetric, titrimetric, photometric and fluorimetric methods are given comprehensive coverage, rather less space being devoted to polarography, radioactivation and spectroscopic methods. Chapter 3 deals with the separation of aluminium from accompanying elements by precipitation with inorganic and organic reagents, by extraction, by a variety of chromatographic methods and by mercury cathode electrolysis. Chapter 4 treats the determination of aluminium in natural and industrial materials such as minerals, ores and industrial concentrates, soil, water and organic substances, metals and alloys. Chapter 5 brings the text to a conclusion with a description of methods for the determination of impurities in high-purity aluminium. The bibliography of work cited in the text contains over 1,300 entries. As with the earlier volumes in this series, the translation has been expertly done.

As the third most abundant element in the Earth's crust, aluminium is present in most natural and fabricated materials. Although its chemistry and hence its analytical chemistry is not particularly complicated, a vast literature on methods for its determination, particularly in the presence of interfering elements, now exists. Surprisingly, no attempt has been made to review this information since 1942, when Fischer contributed a monograph on the subject in the *Handbook of Analytical Chemistry* by Fresenius and Jander. The present text deals mainly with the literature up to 1968 with occasional later references, none, of course, beyond 1971, the year of publication of the original Russian version, and covers all known methods for the determination of aluminium.

There can be no doubt about the value of this type of monograph to the practising analyst. Many of the problems associated with the analysis of aluminium—the ignition temperature of aluminium oxide, the pH for precipitation of aluminium 8-hydroxyuolate, the difficulties of the complexometric titration, the volatilisation of aluminium for AAS, the best photometric reagent for a particular purpose—all are given thorough and realistic treatment. Although this book is essentially a review of existing literature, perhaps a major benefit is its presentation of Russian work not readily accessible to analytical chemists in the Western World. As a comprehensive and authoritative source of information on the analytical chemistry of aluminium, it must assume the premier role.

W. I. STEPHEN

Science in the media

Two contrasting looks at the place of science reporting in the media—one from each side of the Atlantic—have recently combined to provide an insight into the delights and pitfalls of science journalism, while showing how different these are in different countries. In Britain, the BBC Radio 3 programme "The Communicators" looked at science reporting in its issue of July 27. Here, the emphasis was squarely on the difficulties involved in getting a science story into the British national press at all.

The situation in this respect has deteriorated over the years, as the long serving science correspondent of the Daily Express recalled. With shortage of newsprint and a feeling that the public is to some extent disenchanted with hard science, it is all too easy for science journalists to fall into the trap of overdramatisation, at least in the eyes of the scientists. Several scientists interviewed in the programme expressed their concern about sensational reporting—one was distressed by a comment from one of the people making a documentary about typhoid that "we are in this game to entertain the public, not to educate them".

Such concern about sensational and inaccurate reporting is also felt on the other side of the Atlantic, to judge from an 'occasional paper' on *Science in the Newspaper* which has recently been published by the American Association for the Advancement of Science. But in many other respects the situation in the USA seems far different from that in Britain, giving the lie, perhaps, to any suggestion that sensational reporting arises because of the difficulty of getting any science story into print.

Science in the Newspaper itself is an odd mixture; contributions from award winning writers discussing their craft rub cheek by jowl with 'scientific' investigations of science reporting, complete with jargon and footnotes. Whether by accident or design, this neatly emphasises one point: the scientists certainly do need help in communicating their ideas to anyone outside a restricted circle of specialists.

Perhaps the most worrying contribution describes the result of a survey of what scientists think about science in the newspaper. The scientists had been asked to check for errors published in stories about their own work, and in more than 25 per cent of the cases they noted such damaging errors as misquotations, misleading headlines, omission of relevant information and excessive brevity.

But are the criteria scientists apply too strict, bearing in mind the audi-

ence for which the articles were intended? When one respondent objected that in a report of the drop out rate of college students the first sentence said that more than half of the college students 'failed to obtain degrees' when it should have been qualified to add 'within four years', the criticism seems valid; but, we are told, the report in question did actually contain the important qualifications in its next sentence. That was not good enough for the scientist whose work was being described, but I would find it difficult to see his objection as one to worry the reporter who made the 'error'.

The point is, of course, that newspaper readers, by and large simply speak a different language from scientists, and that the journalist is literally in the position of interpreter. So many of the irritations felt by scientists are on the same level as the quibbling among multinational organisations about exactly how a French word in an agreement, say, should be translated into German.

This interpretative role is made abundantly clear in the most heartening article in *Science in the Newspaper*. The *National Enquirer* is an American weekly that was until a few years ago, we are told, a 'scandal sheet'. But now the *Enquirer* has changed its image, become a 'family newspaper' and just about quadrupled its circulation, to 4 million. And in that large circulation paper, science has a vital role.

In one recent 48 page issue mentioned, five per cent of the space was given up to science or medical stories. The treatment of these stories is dramatic, and successful in reaching a wide audience who respond with floods of letters. Not all the scientists whose work makes the pages of the *Enquirer* are pleased—Dr Sol Spiegelman, of Columbia University's Institute of Cancer Research, is quoted in *Science in the Newspaper* as being particularly unhappy about the treatment he received from the *Enquirer's* reporters.

But you can't please all the people all the time, and if a paper like the *Enquirer* can, by and large, encourage its readers to accept science as a valuable part of modern society which deals with problems that are relevant to the man in the street, the science journalists are doing their job. The success of the *Enquirer's* science policy ought to make some British popular newspapers rethink their attitude to science; the scientists might not always approve of the result, but then, non-scientists do not always approve of the activities of scientists.

The message from both of these slightly narcissistic looks in the mirror

emphasises a feeling which already exists in both the USA and the UK. Science today must be relevant to the problems of today, and what is more it must be seen to be relevant.

JOHN GRIBBIN

Easy listening science

Over the past few weeks Saturday morning listeners to BBC Radio 4 have been having a taste of up to the minute reports from the scientific world. "Science Now", as its title suggests, has been keeping its audience abreast of the latest developments in science and techniques involved in anything from building a skyscraper from the top downwards to the possible hazard to the public from the development of new strains of mutant cells.

The BBC's radiophonic workshop provides a snappy piece of introductory music which sets the tone for a tightly packed programme of comment, interview and discussion. The presenter, Brian J. Ford, manages to maintain the tempo throughout, and it is this which seems to make the programme such easy listening.

Each thirty minute programme can cover a range of scientific topics acceptable both to working scientists and to the man in the street with an interest in science. When 'test tube babies' hit the headlines recently, "Science Now" covered the subject and brought out the implications in non-sensational terms. As Brian Ford said, the work represents "an interesting development, but not by any means a terrifying prospect".

In the same programme the question of how massive the Universe is and the eventual slowing down of its expansion, was discussed. But as seems inevitable in any science programme today, medical items receive a good share of attention. Audience response indicates the understandable popularity of medicine, but it does seem a little unfortunate that this sometimes serves to divert a large proportion of the very limited air time available for science (see *Nature*, 250, 362; 1974) away from 'hard science'.

Still, a complete edition of "Science Now" was devoted to applied science and included oceanography, crop irrigation, building methods, cancer research and an exhibition to celebrate the discovery of oxygen 200 years ago.

Where the programme differs from some of its predecessors is in the amount of comment provided by the presenter, either with or without contributions from a second party. This is a significant and worthwhile development, since often the caution of a

scientific paper, which might be the peg for an item, masks an exciting development. Equally, of course, Ford has scope to comment about developments, such as the 'test tube baby' story, where exciting publicity has masked the reality of a small and cautious scientific step, forward.

Given that such a programme must, to a large extent, reflect the personal approach of presenter and producer, "Science Now" is doing a good job. It seems unfortunate, however, that it is broadcast only once a week, at a time when many would be listeners must be involved with the ritual of

weekend shopping and/or sport. Although much of the same ground is covered by *New Scientist* or the national press, it would still be good to have a second opportunity to hear the programme, perhaps on a weekday evening.

SALLY OWEN

obituary

Chu K'o-Chen

CHU K'O-CHEN, one of China's most distinguished scientists, Vice President of Academia Sinica, died last February at the age of 84. When I first met him in 1944 he was President of Chekiang University, then in exile in the little town of Mei-t'an in Kweichow Province. One could soon glimpse the qualities which endeared him to generation after generation of Chinese scientists—a real lover of the things of the intellect and of Nature—small of stature, kindly, judicious and compassionate.

His original training in geography, meteorology and astronomy was gained at the University of Illinois in 1917 and subsequently at Harvard. After he returned to China he became Director of the National Meteorological Institute of Academia Sinica at Nanking. Later on, after the Second World War, he was called from Chekiang University to become one of the Vice Presidents of the newly reconstituted National Academy of Sciences. Apart from his early publications in geography and the

techniques of meteorology, he acquired a life-long interest in phenology (historical meteorology). Thus it came about that Chu K'o-Chen led a whole group of scientists in the study and analysis of China's climatic changes over more than 5,000 years.

Another great interest of Chu K'o-Chen was the history of astronomy in Chinese culture. He published a number of papers on such subjects as the origin of the twenty-eight lunar mansions (*hsiu*) and the development of calendrical science. It was natural, therefore, that he should exercise a beneficial influence on such studies in China, and he was one of the founders of the Institute of the History of Science some years ago in Peking, where it occupies the lovely old buildings of a former Manchu prince's palace.

Many Western scientists who have worked in China have been greatly indebted to Chu K'o-Chen for his kindly understanding and never failing help. Two pictorial reminiscences of him come especially to my mind. First at Ts'un-yi, giving a warm welcome to

some wandering European scientists in 1944 with the aid of the delicious *pao-tzu* (stuffed dumplings) made in that place and cooked traditionally on beds of pine needles. Secondly, at the International Congress of the History of Science at Florence in 1956, Chu K'o-Chen led a distinguished Chinese delegation. When we all went out to Vinci to pay our respects at the farm where Leonardo was born, and to visit the castle of the little town with its museum of Leonardo's inventions, we, as an international convention, received an unexpectedly enthusiastic welcome from the *podestà* and the people of Vinci. They regaled us with a splendid lunch and with an al fresco supper where they pressed upon us flasks of the excellent local wine. The Chinese and Japanese delegates, having come from so far away to pay homage to Leonardo, were particularly appreciated by the *cittadini*, and the sight of Chu K'o-Chen and his colleagues leaving for the bus with as many flasks as they could possibly carry remains one of my happiest recollections in the world of international science and its history.

Announcements

Awards

Alasdair Muir Breckenridge has been awarded the **Paul Martini Award 1974** by the Medizinisch Pharmazeutische Studiengesellschaft eV, Frankfurt.

Chaim Leib Pekeris has been awarded the **1974 Vetlesen Prize in Earth Sciences** by the University of Columbia.

Appointments

Bernard Salvage has been appointed to a personal chair of high voltage engineering at **Heriot-Watt University**.

W. A. Cramond has been appointed Principal and Vice-Chancellor of the **University of Stirling**.

Errata

In the article "Elongation factors for chloroplast and mitochondrial protein synthesis in *Chlorella vulgaris*" by O. Ciferri and O. Tiboni (*Nature new Biol.*, **245**, 209; 1973) some of the references in the text were wrongly numbered. The following corrections should be made: in Table 1 and Fig. 1, 3 should be 6; 4, 7; 5, 8; 6, 9; 7, 10; in Fig. 2, 9 should be 12; in the text on pages 210 and 211, 10 should be 13; 11, 14; 12, 15; 13, 16; 14, 17; 15, 18; 16, 19; 17, 20; 18, 21; and 19, 22.

In the article "Dietary preference and diseases of age" by M. H. Ross and G. Bras (*Nature*, **250**, 263; 1974) an error appeared in Table 1. The third number down in the penultimate column should read 77" not 7".

Corrigenda

In the article "Early visual adaptation in goldfish retinal ganglion cells" by A. J. Afanador and A. J. Adams (*Nature*, **250**, 346; 1974) the last line of the legend to Fig. 2 should read " 0 corresponds to $7.2 \times 10^{15} \text{ s}^{-1} \text{ cm}^{-2}$ ".

The following corrections should be made to the article "Platelet contractile regulation in an isometric system" by I. Cohen and A. de Vries (*Nature*, **246**, 36; 1973): line 41, $0.2 \text{ g min}^{-1} \text{ cm}^{-2}$; line 43, 1.8 g cm^{-2} ; Fig. 1 "... expressed in g cm^{-2} "; Fig. 2 ordinate Tension (g cm^{-2}) legend, "... expressed in g cm^{-2} "; Fig. 2 ordinate Tension (g cm^{-2}); Fig. 4 legend, Clot contraction model.

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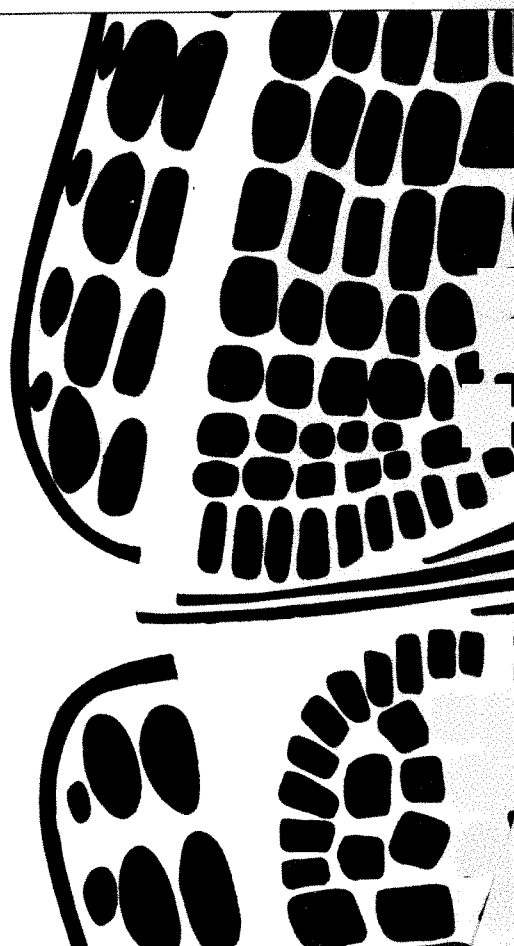
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Apply on forms obtainable from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT. Quote reference 74/20. (630)

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New South Wales

Australia

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Applications closing September 15, 1974, are invited from suitably qualified medical practitioners for the position of Staff Specialist In Charge of the Intensive Care Unit at Sydney Hospital.

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Under certain circumstances The Board may give consideration to two part time appointments to this post. Further enquiries may be directed to The Director of Anaesthesia.

Sydney Hospital is a 460 bed teaching hospital of the University of Sydney. It conducts a wide range of medical, surgical and special surgical services and is accredited for post graduate training in general and special medicine and surgery.

The Director of Anaesthesia will be visiting the United Kingdom in August/September. Applications in writing, stating age, training, experience and the names of three referees should be directed as follows:-

1st copy to Dr F. R. Berry, C/- Bank of New South Wales, 9-15 Sackville Street, London.

2nd copy by airmail to The General Medical Superintendent, Sydney Hospital, Box 1614, GPO, Sydney, 2001, New South Wales. (610)

nature

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Department of Biomedical Engineering,
Institute of Orthopaedics (University of London),
Brockley Hill, Stanmore, Middlesex HA7 4LP.

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The post is grant aided and will be for three years in the first instance. The commencing salary will be £3636 rising to £3990 (plus London Allowance). Further details can be obtained from Professor John T. Scates, to whom application should be made with curriculum vitae and two referees by 14th September 1974. (717)

CHARING CROSS HOSPITAL MEDICAL SCHOOL (UNIVERSITY OF LONDON)

The Animal Unit requires a RESEARCH ASSISTANT to collaborate with the Veterinarian in charge in the establishment of a quality control laboratory. The post would be suitable for a graduate or similarly qualified person with a background in microbiology or a relevant biological science. Starting salary not less than £1,350 depending upon experience.

For further information please contact: Head of Animal Unit, 55 Aspenlea Road, London W6 9HH. Telephone 01-385 7709. (628)

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A vacancy exists for an Immunologist on the scientific staff of the M.R.C. Virology Unit. Applicants should be experienced in immunological techniques and able to take responsibility for the immunological side of investigations with herpes virus. A considerable interdisciplinary effort, using a combination of biochemical, genetical and virological techniques is already under way and it will be the candidate's role to add immunological know-how and bring an immunological attitude of mind to the combined approach. The Immunologist would have the full time services of a technician.

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Applicants should send a Curriculum Vitae giving full details of qualifications and experience, and the names of three referees to Professor J. H. Subak-Sharpe, Institute of Virology, University of Glasgow, Glasgow G11 5JR by September 20, 1974. (728)

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The first is for one year for research on the Biosynthesis of Fungal Antibiotics at a salary of £2,247 per annum. F.S.S.U.

The second is for two years for research on the Biosynthesis of a Novel Fungal Metabolite at an initial salary of £2,247 rising to £2,412 per annum. F.S.S.U.

Further details may be obtained from Dr. N. J. McCorkindale, Department of Chemistry, The University of Glasgow, Glasgow, G12 8QQ.

In reply please quote Ref. No. 3520 M. (724)

UNIVERSITY OF WARWICK RESEARCH TECHNICIANS

The Department of Biological Sciences has vacancies for a permanent appointment and also one to three year contract posts for technicians in the Microbiology and Virology Research Laboratories. Applicants should have experience in microbiology and/or biochemistry and possess appropriate qualifications. Salary on the Technician Grade 3 scale £1,650 by £54 to £1,920 p.a. plus threshold payments. Applications in writing to the Academic Registrar, University of Warwick, Coventry CV4 7AL, quoting Ref. No. 1/D/74 by August 21, 1974. (725)

DUDLEY AREA HEALTH AUTHORITY

Applications are invited for the post of BASIC GRADE BIOCHEMIST in the Group Biochemistry Laboratory at the Guest Hospital, Dudley. The successful applicant would be expected to contribute significantly to the routine service offered by the department as well as to the expansion of the service. Whitley Council conditions of service apply. Candidates may visit the department by arrangement with Mrs. P. A. Jones, Principal Biochemist, Telephone Dudley 53304 Ext. 7.

Applications, quoting names and addresses of two referees to: Hospital Secretary, The Guest Hospital, Tipton Road, Dudley. (722)

KINGSTON POLYTECHNIC RESEARCH ASSISTANT SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES

to study ultrasonically induced damage in biological material and the mechanisms by which this occurs.

Applicants should be honours graduates in a physical science or biology. You would be encouraged to register for a higher degree. Salary £1,427 to £1,537 (under review).

Application forms and further details from Appointments Officer, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel. 01-549 1366. (743)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY DEPARTMENT OF CHEMISTRY POSTDOCTORAL RESEARCH

(Ref: CH/123/A1)

Applications are invited for an S.R.C. Postdoctoral Assistant to join a research group concerned with the dynamical properties of liquids and solutions. The project involves a study of molecular motion in liquids by computer simulation of molecular dynamics. Applicants should have experience in a related area of chemistry, physics or mathematics and also experience with computer programming. The appointment will be for up to two years with a minimum starting salary of £2,118 per annum, plus F.S.S.U.

Applications, including a curriculum vitae and the names of two referees, should be sent to the Registrar, U.M.I.S.T., Manchester M60 1QD to arrive not later than September 7, 1974. (719)

MEDICAL RESEARCH COUNCIL VIROLOGY UNIT INSTITUTE OF VIROLOGY UNIVERSITY OF GLASGOW

Applications are invited from post-doctoral graduates in biology, cell biology, biophysical, genetics or microbiology to join a research group concerned with human adenovirus genetics and oncogenicity. Experience in virus genetics or animal cell culture is not a prerequisite, but would be an advantage. The post is tenable for a period of three years from October 1, 1974 and the salary will start at an appropriate point on the Grade II scale (expected range from October 1974 — £2,019 to £3,636 per annum) F.S.S.U.

Applicants should send a Curriculum Vitae giving full details of qualifications and experience, and the names of three referees to Professor J. H. Subak-Sharpe, Institute of Virology, University of Glasgow, Glasgow G11 5JR by September 20, 1974. (727)

UNIVERSITY OF NEWCASTLE UPON TYNE MICROBIOLOGICAL CHEMISTRY RESEARCH LABORATORY

Applications are invited for the position of Research Associate in this unit which is concerned with chemical and biochemical aspects of bacterial cell walls and membranes. Applicants should possess a Ph.D. or equivalent qualification. The work involves a study of the mechanism of biosynthesis of teichoic acids and related compounds in bacterial membranes under the direction of Professor J. Baddiley, F.R.S. Experience in most aspects of biosynthesis, enzymology or microbial biochemistry would be appropriate. The position which is supported by the Science Research Council is for three years and is in the salary range £2,118 to £2,412 with F.S.S.U. and is available from October 1 or as soon thereafter as convenient.

Applications should be made to Professor J. Baddiley, Microbiological Chemistry Research Laboratory, The University, Newcastle upon Tyne NE1 7RU, giving full particulars and the names of two referees. (731)

DORSET AREA HEALTH AUTHORITY

EAST DORSET HEALTH CARE DISTRICT

SENIOR CHIEF TECHNICIAN

IN BIOCHEMISTRY

A vacancy exists for a senior chief technician in the above department (situated at Poole General Hospital).

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Further information and job description can be obtained from Dr. J. H. Johnstone, Consultant Biochemist, Poole 5100 ext. 474 or Mr. Butcher, Principal Chief Technician, Bournemouth 35201 ext. 351.

Application forms etc. can be obtained from the District Administrator, District Office, Royal Victoria Hospital, Shelley Road, Boscombe, Bournemouth, Dorset BH1 4HX. Closing date August 31, 1974 (716)

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(Ref N)
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21 Mincing Lane
London EC3R 7QY



(748)

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Applications or requests for further information should be sent to Dr. T. Dickinson, Department of Physical Chemistry, The University, Newcastle upon Tyne NE1 7RU. (732)

MEDICAL RESEARCH COUNCIL

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Application are invited for post of Senior Technician (Animal) in charge of Isolation Area of the Laboratory Animals Centre, which includes Germ-Free Unit, quarantine room, sheep and goats and experimental animals. This interesting and varied post requires a Fellow of I.A.T. with experience in germ-free technology, a wide knowledge of animal husbandry techniques and the management of staff. Ability to liaise with staff of all levels essential.

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Apply to Chief Technician's Office, Medical Research Council, Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4HF. (721)

UNIVERSITY OF NEWCASTLE UPON TYNE ELECTROCHEMISTRY RESEARCH LABORATORIES INORGANIC/STRUCTURAL CHEMIST

A post-doctoral Research Associate is required to work on the preparation and characterisation of ionically-conducting solids. The successful applicant would join an existing group investigating the electrochemical properties of solid electrolytes. A wide range of experimental facilities are available.

The appointment is for two years commencing in the Autumn 1974 and is in the salary range £2,118 to £2,412 depending on age and experience.

Applications or requests for further particulars should be sent to Dr. T. Dickinson, Department of Physical Chemistry, The University, Newcastle upon Tyne NE1 7RU. (733)

UNIVERSITY OF NEWCASTLE UPON TYNE ELECTROCHEMISTRY RESEARCH LABORATORIES

JUNIOR RESEARCH ASSOCIATE

A Junior Research Associate is required to work on a variety of novel electroanalytical devices. The successful applicant will join an existing group under the direction of Dr. J. V. Dobson which is developing electrochemical techniques for the analysis of gases and solutions.

Preference will be given to applicants possessing some knowledge of electronics and being prepared to learn to operate simple machine tools and assist in the experimental work.

The appointment is for an initial period of one year, as from autumn of 1974, but is renewable for at least a further two years. A salary of £1,569 by £81 is payable.

Applications or requests for further information should be sent to Dr. T. Dickinson, Department of Physical Chemistry, The University, Newcastle upon Tyne NE1 7RU. (735)

UNIVERSITY OF DUNDEE

Department of Biological Sciences

Applications are invited from Honours graduates in Botany, Biology, Agriculture or a related discipline for a post as

RESEARCH ASSISTANT

for work on an investigation of the contribution of Myrica to the nitrogen economy of natural ecosystems.

The project is supported by a N.E.R.C. grant and the post is available for three years. The salary will be in the region of £1,500 per annum.

Further information is available from Dr J. I. Sprent of the Department of Biological Sciences. Applications, quoting reference Est/51/74, and including the names of two referees, should be sent to: The Secretary, The University, Dundee DD1 4HN as soon as possible. (744)

UNIVERSITY OF NOTTINGHAM

FACULTY OF AGRICULTURAL SCIENCE

Applications are invited from suitably qualified graduates for the position of **DEMONSTRATOR IN ANIMAL PHYSIOLOGY** in the Department of Physiology and Environmental Studies.

Duties include assistance in practical and tutorial classes in animal physiology. The person appointed will be required to participate, under the supervision of Dr. T. B. Mepham, in a research project on the control of metabolism in the lactating mammary gland, using isolated perfused gland preparations. Where appropriate the person appointed will be expected to register for a higher degree.

The salary will be within the range £1,611 to £1,758 per annum. Applications by letter to the Staff Appointments Officer, University of Nottingham, University Park, Nottingham, quoting the names of two referees. Closing date, August 31. (736)

THE CITY UNIVERSITY

DEPARTMENT OF CHEMISTRY

S.R.C. RESEARCH TECHNICIAN

A Technician is required in Organic Chemistry to assist in an investigation into the 'Mechanism of Aromatic Sulphonation in Aqueous Sulphuric Acid', a project supported by the S.R.C. for three years from October 1, 1974.

Initial salary up to £1,704 per annum plus £228 London Allowance depending on age, qualifications and experience.

Application with names of two referees, as soon as possible, to: Dr R. G. Coombes, Department of Chemistry, The City University, St John Street, London EC1V 4PB. (739)

North East London Polytechnic

Department of Applied Biology

Applications are invited for the post of:

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The successful applicant will be responsible for the first year of the two year part-time MSc course (CNA). He will also be required to teach on the BSc Applied Biology course (CNA). Applicants should possess a good honours degree in a suitable specialism (Pharmacology, Physiology, Biochemistry or Pharmacy), and have had extensive post-graduate experience.

Salary scale:

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Further details and application form from:

The Academic Staffing Officer,
Ref. S/CF 131
North East London Polytechnic,
Forest Road, London E17 4JB.
Tel: 01-527 2272.

for return by: August 30, 1974. (742)

Department of Agriculture and Fisheries for Scotland
Royal Botanic Garden, Edinburgh

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London

Microbiologist/Chemist

■ To run small unit concerned with evaluation and development of traditional and industrial fermentation processes, particularly those relevant to nutrition in tropical countries.

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□ Application forms (for return by 9 September 1974) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB.

Science group
CIVIL SERVICE

(713)

Veterinarian for Mastitis Research

Applications are invited for an established post in the Veterinary Research Laboratories of the Department of Agriculture at Stormont.

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The successful candidate will be specially assigned to work on mastitis in cattle. Collaborative research opportunities are available and educational and field extension work will be required.

The salary scale is:

Veterinary Research Officer (Grade II)—£2,852 to £3,610

Veterinary Research Officer (Grade I)—£3,610 to £4,365

Grading and starting salary will be related to qualifications and experience. And a cost of living supplement is payable.

Please write or telephone for an application form and further information, quoting Ref. SB 215/74/N to Civil Service Commission, Clarendon House, Adelaide Street Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms must be returned to arrive not later September 10, 1974.

(782)



**NORTHERN IRELAND
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UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

Applications are invited from suitably qualified persons for the following posts:-

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Grade 4. £1,848 to £2,163 per annum.
To be responsible for technical support to medical and science undergraduate Biochemistry/Microbiology classes and provide some support for related research. Ref: 275/T/NA.
- LABORATORY TECHNICIAN—PHARMACOLOGY**
Grade 3. £1,650 to £1,920 or Grade 4. £1,848 to £2,163 per annum, according to qualifications and experience.
To provide technical support for Science undergraduate Pharmacology/Mammalian Physiology classes and research. Ref: 276/T/NA.
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To provide a departmental photographic service, to produce slides, and to prepare diagrams, graphs and illustrations. Ref: 277/T/NA.
- LABORATORY TECHNICIAN—RADIOISOTOPIC SERVICES**
Grade 3. £1,650 to £1,920 per annum.
To be primarily responsible for radioisotopic facilities in the Analytical Services Unit. Experience of instrumentation and/or chemistry/biochemistry. Ref: 279/T/NA.
- ELECTRON MICROSCOPY TECHNICIAN**
Grade 2B. £1,524 to £1,794 per annum.
To join a unit providing Electron microscopy services. Some experience of E.M. work and/or histology. Ref: 281/T/NA.

Cost of living allowance currently £2.40 per week payable in addition to salary shown.

Posts 1-4: Related experience, an appropriate qualification (minimum O.N.C. or equivalent) and enthusiasm are required for these established appointments. Graduates in appropriate disciplines are welcome to apply for posts 1-3.

Posts 5-6: Minimum qualification for these posts O.N.C. or equivalent in appropriate subjects.

Applications giving details of age, qualifications and experience and the names of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH, quoting appropriate reference number. (730)

ROYAL POSTGRADUATE
MEDICAL SCHOOL
JUNIOR TECHNICIAN

required in a Biochemistry Laboratory. Post suitable for candidate attempting entrance to university in 1975. Other candidates holding GCE "O" levels in Maths, English and two Science subjects will also be considered. Day release available for further study. Salary according to age and qualifications. Applications to the Secretary, RPMS, Hammersmith Hospital, DuCane Road London W12 0HS, quoting ref. no. 2/146N. (712)

ROYAL POSTGRADUATE
MEDICAL SCHOOL
JUNIOR TECHNICIANS
(2 Posts)

required from October 1, 1974 to assist with research into muscle diseases. Posts suitable for School Leaver holding a minimum of GCE "O" level passes in English, Maths and two science subjects.

Salary according to age and qualifications. Applications to the Secretary, RPMS, Hammersmith Hospital, DuCane Road, London W12 0HS, quoting ref. no. 6/254N. (711)

NEW ZEALAND
DEPARTMENT OF SCIENTIFIC AND
INDUSTRIAL RESEARCH

The Applied Biochemistry Division of the Department of Scientific and Industrial Research, Palmerston North has a vacancy for a mycologist-fermentation scientist in the carbohydrate research group. The group is involved in research on the production of mould extracellular enzymes and their use in the degradation of wood waste, and would also aim to contribute to a more fundamental study of the lignin-carbohydrate relationship in plant cell walls.

Qualifications: Ph.D with 2-3 years research experience, and with a sound knowledge of fungi.
Salary: Up to NZ\$9,339 p.a. dependent on qualifications and experience.

Passages: Fares for appointee and his immediate family, will be paid.

Incidental expenses: Up to NZ\$120 for a single man and NZ\$800 for a married man can be claimed to cover the cost of taking personal effects to New Zealand.

Application forms and general information are available from the High Commissioner for New Zealand, New Zealand House, Haymarket, London, SW1Y 4TQ, with whom applications will close on September 16, 1974.

Please quote reference P/T 116 when enquiring. (714)

NATIONAL INSTITUTE FOR
BIOLOGICAL STANDARDS
AND CONTROL

HAMPSTEAD, LONDON NW3 6RB

Medical or post-doctoral biochemistry graduate needed in Division of Hormones and Blood Products to help in setting up W.H.O. and other reference standards for certain substances important in medicine, including peptide hormones, which are estimated by radioimmunoassay.

The work involves wide collaboration with scientists in a variety of disciplines. There are excellent opportunities for research.

Appointment on M.R.C. salary scale for non-clinical scientific staff £1,830 to £3,708 plus London Weighting £162 p.a. University or N.H.S. superannuation.

Further information available on request to the Institute.

Applications, addressed to the Director of the Institute, should arrive by the end of September 1974. (745)

MEAT RESEARCH INSTITUTE
BACTERIOLOGIST

required to study the origin, spread and survival of salmonellae and other food-poisoning organisms which may contaminate carcass meat, and improve methods for their demonstration and recovery. The successful applicant will also be expected to participate in an established programme of research on factors which inhibit u.v. growth of bacteria.

Salary range: Scientific Officer £1,592 rising to £2,675; Higher Scientific Officer £2,461 rising to £3,371. There is a non-contributory superannuation scheme.

Applicants will be expected to have a first or upper second class honours degree. Starting salary according to qualifications and experience but for entry to the H.S.O. scale, applicants must have at least two years postgraduate research experience.

Application forms, Secretary, Meat Research Institute, Langford, Bristol BS18 7DY. (741)



Pharmaceuticals
Division

Information
Scientist

Applications are invited for a Science Graduate to join a team providing current awareness and information retrieval services to research scientists. The applicant should have a sound knowledge of organic chemistry. A reading knowledge of German would be an advantage.

Mechanised information systems are in operation.

Previous experience is not essential, but the successful applicant must be prepared to meet the challenge of mechanisation in information work and also show a keen interest in the pharmaceuticals industry.

Pharmaceuticals Division is attractively situated in rural North Cheshire within easy reach of main road and rail routes. Conditions of service, career opportunities and assistance given to married men in moving home are designed to attract and retain staff of high calibre.

Please write, asking for an application form to:

Miss K E Webster, Personnel Officer
ICI Pharmaceuticals Division
Mereseid, Alderley Park,
Nr. Macclesfield, Cheshire.

UNIVERSITY OF LONDON
CHARING CROSS HOSPITAL
MEDICAL SCHOOL
DEPARTMENT OF BIOCHEMISTRY

Applications are invited from graduates in science or medicine for a research post (17 months initially but probably continuing) to join a group working on the possible use of lipid vesicles (liposomes) in drug administration and in enzyme replacement therapy in disease. The applicant should be under 30 years; salary according to age and qualifications but on the lectureship scale. Experience in the field of tissue culture would be an advantage. The group is based in the new laboratories of Charing Cross Hospital Medical School in Fulham.

Applicants should send a curriculum vitae and the names of two referees as soon as possible to Professor Brenda E. Rymann, Department of Biochemistry, Charing Cross Hospital Medical School, Fulham Palace Road, London, W6 8RF.

(718)

THE GRASSLAND RESEARCH
INSTITUTE

MURLEY, MAIDENHEAD BERKSHIRE
SCIENTIFIC OFFICER/HIGHER
SCIENTIFIC OFFICER

is required in the AGRONOMY DEPARTMENT to assist with husbandry investigations on the yield and quality of grass and forage crops, with particular relevance to the efficiency and nitrogen usage and/or the management of grass/legume mixtures.

Minimum qualifications:—Pass degree or equivalent in agriculture or a relevant science and preferably experience of field and laboratory investigation on grassland plants.

Salary: Scientific Officer £1,592 to £2,675 per annum; Higher Scientific Officer £2,461 to £3,371 per annum, plus cost of living supplement.

At least 5 years post graduate experience will be required for appointment in the higher grade. There is a non-contributory Superannuation Scheme.

Applications to the Secretary by September 30, 1974, giving curriculum vitae and the names of three referees. Reference 2/1/2.

(740)

UNIVERSITY COLLEGE GALWAY
JUNIOR LECTURESHIP IN BOTANY

Applications are invited for the above post. Salary scale £2,478 by 99 (10) to £3,468 p.a. plus Family Allowances. Applications will be particularly welcome from candidates with a specialised knowledge of Irish vegetational history.

The closing date for receipt of applications is **September 12, 1974**. Prior to application, further information should be obtained from the Registrar of the College.

(738)

UNIVERSITY OF LIVERPOOL
DEPARTMENT OF BIOCHEMISTRY
EXPERIMENTAL OFFICER

Applications are invited for the above post (financed by the Science Research Council) to work with a group on some aspects of Affinity Chromatography. Applicants should hold a good honours degree in Chemistry or Biochemistry, and have experience in either nucleotide chemistry or enzymology. The appointment will be on the scale £1,764 to £2,553 per annum, and it is hoped that the successful applicant will take up the post by October 1, 1974.

Application forms may be obtained from the Registrar, The University of Liverpool, P.O. Box 147, Liverpool L69 3BX. Quote ref. RV/N/276175.

(766)

UNIVERSITY OF SYDNEY
LECTURESHIP/SENIOR
LECTURESHIP IN BIOCHEMISTRY

Preference to candidates with teaching and research experience in fields of metabolism of animal tissues.

The position advertised is a permanent one, but it may be filled at the Lectureship level for three years in the first instance, with possibility of permanency after that time or in certain cases return fares.

Salary ranges—Lecturer: \$A9,002 to \$A12,352 p.a.; Senior Lecturer: \$A12,643 to \$A14,724 p.a. Applications, including curriculum vitae, list of publications and names of three referees, by September 16, 1974 to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information is available. Further information also available from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

(764)



AUSTRALIAN ATOMIC ENERGY COMMISSION
RESEARCH ESTABLISHMENT

Lucas Heights—near Sydney

Deputy Controller Safety

An experienced safety specialist is required to fill the position of Deputy Controller Safety within the Safety Section of the Australian Atomic Energy Commission Research Establishment, Lucas Heights, N.S.W., Australia. The Safety Section covers a broad spectrum of safety activities including:—

- radiological protection,
- critically safety,
- reactor safety,
- non-ionising radiation protection,
- industrial safety.

This is a senior position and the appointee will be expected to have had a number of years of relevant safety experience and to have a strong interest in safety management. He will be required to assist in the management of the Safety Section, to undertake specific work in one or more of the above mentioned safety activities and to maintain a broad interest in the others.

The minimum qualifications required are a University degree or diploma of a standard acceptable to the Commission. It is anticipated that an appointment will be made within the grade of Scientific Services Officer, Class IV, \$A13,299 to \$A14,202, but an appointment to a higher grade will be considered for an applicant who is better qualified and more experienced than envisaged in this advertisement.

Application forms may be obtained by writing to the Counsellor (Atomic Energy), Australian High Commission, Canberra House, 10-16 Maltravers Street, London WC2R 3EH.

(754)

HERIOT-WATT UNIVERSITY
DEPARTMENT OF PHYSICS

Applications are invited for a Post-Doctoral Research Assistantship beginning October 1974. The research programme, directed by Dr. D. L. Weaire, is the study of structural models of Amorphous Solids, and involves the use of computational methods.

The appointment is funded by the Science Research Council, and is for a period of three years.

Commencing salary not more than £2,118 per annum.

Applications, consisting of a brief curriculum vitae and the names of two referees, should be sent to Professor Harpe, Department of Physics, Heriot-Watt University, Riccarton, Currie, Midlothian, EH14 4AS. (757)

HERIOT-WATT UNIVERSITY
DEPARTMENT OF CHEMISTRY

Post-Doctoral Research Assistantship

Applications are invited for a Post-Doctoral Research Assistantship, sponsored by the Science Research Council, to study photochemistry in the vacuum ultraviolet region in collaboration with Dr. P. John. The appointment is tenable for two years, commencing October 1, 1974. The salary will be in the range £2,247 to £2,412, plus F.S.S.U.

Applications consisting of a brief curriculum vitae and the names of two referees, should be sent to Dr. P. John, Department of Chemistry, Heriot-Watt University, Riccarton, Currie, Edinburgh EH14 4AS. (758)

NORTHERN RIVERS COLLEGE OF ADVANCED EDUCATION LISMORE, N.S.W., AUSTRALIA

The Northern Rivers College of Advanced Education is a multi-purpose institution located in Lismore, on the far north coast of New South Wales.

The college is temporarily housed in the form of Lismore High School Building, adjacent to the business section, whilst new buildings are to be erected on a site of over 100 acres at Lismore Heights on the outskirts of the city.

The College has three schools; Teacher Education, Business Education, and recently the Department of Cultural and Scientific Studies has been upgraded to a School.

Applications are now invited for the following positions:-

SCHOOL OF CULTURAL AND SCIENTIFIC STUDIES

PRINCIPAL LECTURER

The successful applicant will be expected to encourage the development of programmes within the School, continue negotiation on the possibilities of transfer courses in Engineering and possibly Science, with a large metropolitan tertiary institution and maintain service courses for the School of Teacher Education and the School of Business Education.

The applicant, as well as having the academic background of leadership in tertiary work, should also have the appropriate qualifications in the Physical Sciences or allied fields. It is expected, although not essential, that a Doctoral Degree would be the minimum academic qualification.

SALARY: \$A15,744 range \$A16,389

SCHOOL OF TEACHER EDUCATION

PRINCIPAL LECTURER

EARLY CHILDHOOD EDUCATION

This position requires a person with appropriate academic qualifications and training and experience in Early Childhood Education, with an understanding of developmental needs of children in child care centres as well as in pre-school centres. It is expected that applicants training and experience should cover the childhood area from 2 to 8.

The successful applicant would be working mainly within the School of Teacher Education, contributing where appropriate to present courses and would be involved in planning and innovating the educational area related to children of early age.

It may also be expected that such a lecturer would initiate the planning of a proposed new school related to this age group to be established in due course.

SALARY: \$A15,744 range \$A16,389

SCHOOL OF CULTURAL AND SCIENTIFIC STUDIES

SENIOR LECTURER

A vacancy will exist at the beginning of 1975 for a Senior Lecturer with academic qualifications in Government, Public Administration, Management and Urban Affairs. This Senior Lecturer will be required to assist the Head of the School in co-ordinating the work and development of the School and to co-operate with other schools by providing courses for their students.

Assistance in the development of the academic reputation of the College will also be required.

SALARY: \$A12,643 range \$A14,724

SCHOOL OF BUSINESS EDUCATION

SENIOR LECTURER

DATA PROCESSING

The successful applicant would need minimum qualifications, Degree or Diploma in a relevant area with experience in programming several languages. Lecturing experience is desirable and marketing as a major area would be an advantage. It is anticipated that the initial semester unit for 1975 will provide for over 60 students and the applicants will be required to develop courses in programming and system analysis.

A Wang Mini Computer configuration will be used initially, with a Main Frame P.D.P. 11/45 planned within the new building programming in the tri-ennium.

SALARY: \$A9,002 range \$A12,352.

The successful applicants will be appointed as employees of the College. Conditions of employment are similar to those enjoyed in Universities and Colleges of Advanced Education, and include four weeks' recreation leave annually and sick leave, with a staff Housing Scheme available.

Provision exists for the granting of study leave after suitable periods of service, and access is available to the N.S.W. State Superannuation Scheme, subject to certain conditions.

Assistance is available to persons residing outside Lismore N.S.W. towards removal expenses.

Applications stating age, qualifications, experience, marital status, and the names and addresses of three referees, together with a recent photograph, should be lodged with the undersigned by Thursday, September 14, 1974.

Postal applications should be sent to P.O. Box 157, Lismore, N.S.W. 2480.

I. D. K. WREN

SECRETARY.

(768)

CENTRAL PUBLIC HEALTH LABORATORY

Honours Science Graduate required by the Standards Laboratory for Serological reagents for work with viral diagnostic reagents.

Applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT. (749)

SEALE-HAYNE COLLEGE LECTURER IN ANIMAL PRODUCTION

Applications are invited for the above appointment. The salary will be based on the Lecturer Grade I or II Scales. Further details may be obtained from and applications should be sent to: The Principal, Seale-Hayne College, Newton Abbot, Devon. (750)

UNIVERSITY OF LEICESTER DEPARTMENT OF PHYSICS

Applications are invited for a Postdoctoral research appointment in theoretical physics, available from October 1, 1974. The successful applicant will work on the theory of the electronic structure of liquid metals under the supervision of Professor J. L. Beeby. Previous experience in many-body theory or solid state theory is desirable. The salary will be in the range £2,118 to £2,580. For application forms or further details please write to: Professor J. L. Beeby, Dept. of Physics, University of Leicester, Leicester LE1 7RH. (752)

SENIOR BIOCHEMIST

A leading British pharmaceutical research organisation has a vacancy for a SENIOR BIOCHEMIST with a few years postdoctoral experience to initiate and direct research projects in the areas of pharmacokinetics, drug metabolism, molecular pharmacology and enzymology in association with the design and development of new medicines. Persons with appropriate experience are invited to apply in writing for an Application Form from The Secretary, Biorex Laboratories Limited, Biorex House, Canonbury Villas, London N1 2HB. (759)

RESEARCH ASSISTANT required to work on radioimmunoassay in the Department of Obstetrics and Gynaecology. The post is funded by the International Planned Parenthood Federation and the successful applicant would continue the work for the Federation which is carried out in the departmental laboratories. The balance of the work planned would enable a suitably qualified person (1st or 2nd class honours degree) to register for a Ph.D. in the University of London. Salary £1,797 to £2,259 plus London Weighting. Apply in writing with curriculum vitae to the Secretary, University College Hospital Medical School, University Street, London, W.C.1. (753)

UNIVERSITY OF LONDON KING'S COLLEGE DEPARTMENT OF PHYSIOLOGY

Applications are invited for two posts of lecturer in Physiology. The Department of Physiology is engaged in teaching medical, dental and science students and has a rapidly expanding research programme especially in the areas of membrane transport and electrophysiology. Applicants should have an active research interest either in one of these areas or in some other aspect of basic physiology including such interdisciplinary fields as genetic, development, or behavioural physiology. A medical qualification although desirable is not essential.

Salary Scale £2,118 to £4,896 per annum plus £213 London allowance p.a. The starting salary will be at an appropriate point on the scale F.S.S.U. benefits will be payable.

Application forms and conditions of appointment are available from the Registrar, University of London, King's College, Strand, London WC2R 2LS and should be returned to him by September 21, 1974. Quoting reference N16/8. (755)



STATE OF KUWAIT Kuwait University Administration Department

NOTICE

Applications are invited for the following posts for the academic year 1974/1975:-

1. Laboratory Technician at the Geology Museum of the Faculty of Science with a B.Sc. degree and not less than three years experience in this field.
2. Chief Laboratory Technician (male, married) required to take charge of teaching and research laboratories of an expanding Department of Zoology. The successful candidate will be highly experienced and preferably a Fellow of the Institute of Medical Laboratory Technicians (F.I.M.L.T.).
3. Chief Animal Technician (male, married) required to take charge of small Animal Unit in the Department of Zoology. The candidate should be highly experienced and preferably a Fellow or Associate of the Institute of Animal Technicians (F.I.A.T. or A.I.A.T.).
4. Senior Technician or Technician required to join a newly established Immunology Unit in the Department of Zoology. Experience in general immunological and/or tissue culture techniques preferred.
5. Laboratory Technician in educational methods for the Department of Education with a degree from a recognized college or institute majoring in technical education (painting and handicraft) and educational methods. Applicants must have a good command of English.
6. Supervisor in audio-visual methods for the English Language Center. Applicants must be qualified with a diploma or M.A. degree in T.E.F.L. as well as majoring in educational methods with enough experience.

Salaries commence at a minimum of K.D. 200/- per month (tax free), according to qualifications and experience. K.D. = \$2.90.

Applications should be addressed to Kuwait University, Kuwait, with photostatic copies of the academic qualifications and experience, including all details pertaining to the post applied for, present employment and salary, aside from full address of each applicant.

Deadline for all applications is September 5, 1974.

(765)

POSTDOCTORAL RESEARCH ASSISTANT IN ORGANIC CHEMISTRY

Applications are invited from research workers with experience in organic chemistry for a post-doctoral research assistantship. The appointment is for three years, salary on M.R.C. scale (£2,058 by £165 to £2,388). The work is in collaboration with Dr. A. J. Kirby and Dr. D. M. Blow and will involve the synthesis of phosphonate analogues of oligopeptides for evaluation as enzyme inhibitors. Applications, with curriculum vitae and the names of two referees, to Dr. S. G. Warren, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. (767)

UNIVERSITY OF IBADAN NIGERIA

Applications are invited for the following posts in the Faculty of Medicine:

LECTURER IN NUTRITIONAL BIOCHEMISTRY

in the Department of Biochemistry. Applicants should hold a Ph.D. in the general field of Human Nutrition.

Salary scales: Lecturer (Proclinical) N2,760 to N4,830 p.a. (£1 sterling = N1.46). The British Expatriates Supplementation Scheme is unlikely to be applied to this appointment. Family passages; various allowances; superannuation scheme; biennial overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, not later than September 12, 1974 to the Registrar, University of Ibadan, Ibadan, Nigeria. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. (772)

UNIVERSITY OF READING

Research Assistant required in Department of Microbiology to work on the preparation and properties of a ribosomal vaccine derived from Bordetella Pertussis. The project will involve a study of both humoral and cellular responses to the vaccine. Salary from £1,616 or the equivalent of a Research Council Studentship if the candidate is accepted for a higher degree. The project is supported by Medical Research Council funds. Applicants, who should be graduates with a 1st or 2nd Class Honours degree in Microbiology or a related subject, should apply to Dr A. I. Tiffin, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ, giving a brief curriculum vitae and the names of two referees. (Ref. MM41). (777)

ROTHAMSTED EXPERIMENTAL STATION

HARPENDEN, HERTS. AL5 2JQ

PLANT PATHOLOGIST

required to measure the prevalence, dispersal and effects of splash dispersed pathogens, particularly those attacking cereals in reduced cultivation systems.

Appointment in grade of Higher Scientific Officer £2,461 to £3,371 or Senior Scientific Officer £3,157 to £4,441 (minimum qualifications: 1st or upper 2nd class honours degree and not less than 2 years' appropriate post-graduate experience, or 4 years for S.S.O.). There is a non-contributory superannuation scheme.

Applications, with names of two referees and quoting Ref. No. 234 to the Secretary by September 9, 1974. (779)

Guy's Hospital

Department of Clinical Physics
and Bioengineering

Electronic Engineer or Physicist—Senior Grade

Applications are invited from engineering and physics graduates with not less than four years postgraduate experience in applied electronics research. The successful applicant will be expected to become involved in the development of new measurement techniques in a variety of clinical situations. A knowledge of digital circuit and/or computer methods would be an advantage. Excellent facilities in new department. Salary Scale £2,964 to £3,843 plus London Weighting plus Threshold Allowance.

Electronics Technician/ Engineer Medical Physics Grade III

Applications are invited for the post of Electronics Technician/Engineer to join a small team engaged upon the maintenance, repair, and calibration of a wide range of electromedical equipment.

Candidates should have an O.N.C., H.N.C. or equivalent in electronics with at least three years experience in maintenance and servicing of electronic instruments. Salary Scale £1,719 to £2,211 plus London Weighting plus Threshold Allowance.

Application Forms available from the Personnel Officer, Guy's Hospital, St. Thomas Street, London SE1 9RT. Telephone 01-407 3662 Ext. 68. (770)

UNIVERSITY OF READING TECHNICIAN (Grade 5)

required for Media and Glassware Preparation Unit in the Plant Science Laboratories. Experience in the preparation of complex media and administrative competence required. Applicants should have H.N.C./Degree in Microbiology or related subjects. Salary scale £2,007/£2,382 p.a. Apply in writing quoting Ref: T.N.69 to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. (776)

CONSERVATION OFFICER SUSSEX TRUST FOR NATURE CONSERVATION

has vacancy at a starting salary of £1,800 to £2,200 p.a. Car ownership essential. Applications by September 7 to, and for further information from: The Hon. Secretary Woods Mill Henfield, Sussex. (783)

UNIVERSITY OF NEWCASTLE UPON TYNE TEMPORARY LECTURESHIP IN PSYCHOLOGY

Applications are invited for the above post, tenable from a date as soon as possible until July 31, 1977, in the Department of Psychology. The department is especially interested in persons whose interests are in the field of Personality; but suitable candidates with other interests should not be dissuaded from applying.

It is expected that an appointment will be made in the lower part of the scale £2,118 to £4,896 according to age, qualifications and experience. Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne NE1 7RU with whom applications (3 copies) together with the names and addresses of three referees should be lodged not later than September 9, 1974. Please quote reference N. (787)

WELSH NATIONAL SCHOOL OF MEDICINE

(University of Wales)

DEPARTMENT OF MEDICAL
MICROBIOLOGY

RESEARCH ASSISTANT

required in the above Department for an investigation into the biochemistry of meconium in connection with Cystic Fibrosis. Candidates should have a Degree in Biochemistry or an allied science. The appointment will commence in October, 1974 and will be for three years; salary on the scale for Junior Scientific Officers (£1,497 to £2,259). Applications, with a brief curriculum vitae and the names and addresses of two referees to the Registrar, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN from whom further particulars are available. (788)

UNIVERSITY OF GLASGOW

DENTAL HOSPITAL AND SCHOOL

RESEARCH ASSISTANTSHIP IN DENTAL BIOCHEMISTRY

Applications are invited for a RESEARCH ASSISTANTSHIP IN DENTAL BIOCHEMISTRY, tenable for a period of up to four years from October 1974.

Candidates should have an Honours Degree in Biochemistry, Microbiology or an allied subject. There will be an opportunity to apply to register for a higher degree.

The work will be mainly concerned with the synthesis of glucans by extracellular bacterial enzymes.

Salary scale £1,500 by £75 to £1,725. F.S.S.U.

Applications, including curriculum vitae and names of two referees, should be sent to Dr J. A. Beeley, University of Glasgow Dental School, 378 Sauchiehall Street, Glasgow G2 3JZ, from whom further information may be obtained.

In reply please quote Ref. No. 3527M. (792)

UNIVERSITY OF ZAMBIA

Applications are invited for the post of (a) PROFESSOR or (b) SENIOR LECTURER IN CROP PROTECTION in the School of Agricultural Sciences. Candidates should possess a higher degree, extensive teaching experience to undergraduate and postgraduate students, extensive research preferably related to problems of tropical agriculture and experience in academic administration and extra-curricular activities. Appointee will be expected to teach the basic course in Crop Protection which consists of lectures and practicals in Entomology, Plant Pathology and Nematology. For this reason, a broad background of training and experience is necessary. Research interests can be in any Crop Protection discipline, but applicants should be able to carry out field orientated investigations leading to the protection and increased production of Zambian agricultural crops.

Salary scales: Professor K7,400 to K7,800 p.a. Senior Lecturer K5,600 to K6,600 p.a. (£1 sterling = K1.53). The British Government may supplement salary in range £954 to £1,152 p.a. (sterling) for married appointees or £78 to £204 p.a. (sterling) for single appointees (normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; various allowances; superannuation and medical aid schemes; regular overseas leave. Detailed applications (2 copies), including a curriculum vitae and naming 3 referees, should be forwarded by air mail, not later than September 5, 1974 to the Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia. Applicants resident in U.K. should also send 1 copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further particulars of these appointments may be obtained from either address. (791)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF MICROBIOLOGY

TUTORIAL ASSISTANTSHIP

Applications are invited for a S.R.C. Postgraduate Tutorial Assistantship for work on the synthesis of inducible, membrane-bound, respiratory enzymes in bacteria. Preference will be given to candidates with biochemical qualifications. The appointment will be for two years only in the salary range £1,500 to £1,581 per annum.

Applications, giving a brief curriculum vitae and the names and addresses of two referees, should be forwarded to The Registrar, University College, P.O. Box 78, Cardiff CF1 1XL. Please quote ref. 0627. Closing date September 13. (790)

UNIVERSITY OF ABERDEEN

RESEARCH ASSISTANT IN PHYSIOLOGY

Applications are invited for above post in **Invertebrate Physiology** to work on either invertebrate behaviour under high pressure or the cytology of invertebrate neurones under high pressure. The post offers wide opportunities for research to a suitable graduate; no experience of high pressure methods is required.

Salary in range £1,494 per annum by 87 to £1,668. Appointment is supported by the S.R.C. for three years.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by August 30, 1974. (785)

GLASSHOUSE CROPS RESEARCH INSTITUTE

requires

SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER

in

MICROBIOLOGY DEPARTMENT

for work on nutrition and growth of mushrooms. Applicants should have degree or equivalent qualification in microbiology, botany or biochemistry. Practical experience in Microbiology or biochemistry an advantage.

Salary within scale £1,592 to £2,675 (S.O.) or £2,461 to £3,371 (H.S.O.) according to qualifications and experience. Contributory superannuation scheme with additional allowance of 5½% of gross salary to offset contributions.

Further particulars from Secretary of the Institute, Worthing Road, Rustington, Littlehampton, Sussex, to whom applications giving full biographical details and names and addresses of two referees should be sent by September 12. Please quote N84. (784)

UNIVERSITY OF LEEDS ASTBURY DEPARTMENT OF BIOPHYSICS

Applications are invited from suitable qualified physical chemists or physicists for a Postdoctoral Research Assistantship to investigate the Light Scattering Rayleigh Line-width of Gelling Systems. The appointment will be for one year in the first instance at a salary of £2,118 to £2,247 with F.S.S.U., depending upon age and experience. Candidates should have an interest in the general field of polymers. Applications giving a brief Curriculum vitae, etc., and naming two referees, should be sent as soon as possible to Dr D. B. Sellen, Astbury Department of Biophysics, The University, Leeds LS2 9JT. (775)

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Advertisement No. 10/74

It is proposed to appoint Chief Editor in the Publications and Information Directorate, New Delhi.

Job requirements:— It is a top management post in the editorial field of Science & Technology. The duties involve overall charge of editing and production of scientific and technical journals published by the C.S.I.R. (b) building up the background information for the publication of the "Wealth of India" series; and (c) publication of monographs, reports of seminars and conferences and other scientific and technical literature.

Qualifications and Experience: Candidates should have appropriate qualifications in Science/Technology and proven literary excellence and experience of editorial work of high standard. Candidate should give evidence of competence in management of publications and their production.

Salary/Conditions of Service: The salary scale attached to the post is Rs. 1,600 by 100 to 1,900 (likely to be revised). Initial pay will be fixed according to merits. The person selected will be appointed on contract for a period of six years, which would be confirmed after an initial period of two years of satisfactory service. Other conditions of contract will be supplied on request.

Age limit: Below 50 years relaxable in special cases. Scientists/Technologists interested may obtain a standard proforma for sending their curriculum vitae from the Chief (Administration), Council of Scientific & Industrial Research, Rafi Marg, New Delhi-1. They can also obtain a brochure on the aims and objects and latest annual report of the Directorate. Completed curriculum vitae proforma must be received in this office on or before 21.8.1974.

"Canvassing in any form and/or bringing in any influence, political or otherwise, will be treated as a disqualification for the post."

(786)

THE INSTITUTE OF ORTHOPAEDICS (University of London)

Royal National Orthopaedic Hospital
234 Great Portland Street,
London W1N 6AD

Applications from graduates in either medicine or science are invited for the whole time post of

RESEARCH ASSISTANT in the Department of Morbid Anatomy

The appointment is for one year in the first instance, and is renewable annually. Salary on the appropriate scale for lecturer (£3,135 to £4,041 Clinical; £2,118 to £3,462 Non-Medical) according to qualifications and experience. Superannuation under F.S.S.U.

The post will provide opportunity for research in the general field of bone structure and bone pathology, under the supervision of Prof. H. A. Sissons. For a medically-qualified pathologist it will also provide training and experience in orthopaedic pathology. Further information can be obtained from Prof. H. A. Sissons.

Applications, together with the names of two referees, should reach the Secretary, at the above address, by September 30 1974. (798)

Soil Scientist

Applications are invited for a permanent post in the Agricultural and Food Chemistry Research Division of the Department of Agriculture. The successful candidate may be required to undertake teaching duties in the Faculty of Agriculture, The Queen's University, Belfast.

The successful candidate will join a team engaged in research and specialist advisory work in chemical, physio-chemical and physical properties of soils and soil/crop relationships.

The appointment may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level, and the qualifications required are as follows:

SSO Over 25 and under 32 years of age with a first or second class Honours Degree in Chemistry, Biochemistry, Agricultural Chemistry or Soil Science and at least 4 years postgraduate experience preferably in physio-chemical or pedological aspects of Soil Science.

HSO Under 30 years of age with an Honours Degree as above and at least 2 years relevant postgraduate experience.

SO Under 27 years of age with an Honours Degree as above.

Salary scales: SSO £3,157 to £4,441
HSO £2,461 to £3,371
SO £1,592 to £2,675

Entry point to the relevant salary scale will be related to qualifications and experience, and a cost of living supplement is payable.

Please write or telephone for an application form, quoting Ref. SB 216/74/N to the Secretary, Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26). Completed forms must be returned not later than September 10, 1974. (781)



**NORTHERN IRELAND
CIVIL SERVICE**

UNIVERSITY OF LEEDS DEPARTMENT OF ANIMAL PHYSIOLOGY AND NUTRITION

Applications are invited for a post of RESEARCH ASSISTANT to undertake work on the mode of action in sheep of the growth stimulating effect of long daylength. The work will involve radioimmunoassay of hormones in plasma samples taken from sheep kept in different daylengths and following various surgical and hormone treatments.

Salary equivalent to a Research Council postgraduate award.

Application forms and further particulars from the Registrar, The University, Leeds LS2 9JT (please quote 41/14/D). Closing date August 27, 1974. (774)

THE UNIVERSITY OF LANCASTER DEPARTMENT OF BIOLOGICAL SCIENCES RESEARCH ASSISTANT

Applications are invited from graduates with interests in natural product chemistry, protein chemistry and/or biophysics to investigate the structure of certain scleroproteins of molluscan origin. The appointment is supported by the Science Research Council for a period of two years, commencing October 1, 1974, on the salary scale £1,404 to £1,509 plus F.S.S.U.

Further particulars may be obtained (quoting reference L.840/C) from the Establishment Officer, University House, Bailrigg, Lancaster LA1 4YW to whom applications (three copies), naming three referees, should be sent not later than August 31, 1974. (778)

UNIVERSITY OF SUSSEX SCHOOL OF BIOLOGICAL SCIENCES

Postdoctoral Research Assistants

required for project on:—

- The biochemical basis of plasma membrane modifications associated with cell transformation.** Experience with membrane enzymes, enzyme isolation and purification of glycolipid biochemistry would be an advantage. The project is supported by the Cancer Research Campaign, it is for one year in the first instance and is renewable. Salary will be on University Lecturer scale up to £3,400 a year according to experience and qualifications.
- The changes in the biochemistry of the cell nucleus induced by chemical carcinogens.** Experience with enzymes or with chromatin would be an advantage. The project is supported by the Cancer Research Campaign, it is for one year in the first instance and is renewable. Salary will be on University Lecturer scale up to £3,000 a year, according to experience and qualifications.

Applications for both posts with the names of three referees to Dr S. Shall, School of Biological Sciences, University of Sussex, Brighton BN1 9QG. (805)

AUSTRALIA

PUBLIC SERVICE OF VICTORIA MINISTRY FOR CONSERVATION

DIRECTOR OF ENVIRONMENTAL STUDIES

REF. NO. (Z/02)

Yearly Salary: \$A19,302.

Duties: To direct the activities of the Environmental Studies Group of the Ministry. To represent the Ministry at policy level in the formation of integrated environmental studies and in the planning and conduct of those studies.

To plan and implement the actions necessary to co-ordinate the activities of Government departments, Universities and other groups participating in such studies.

To prepare appropriate reports and advice

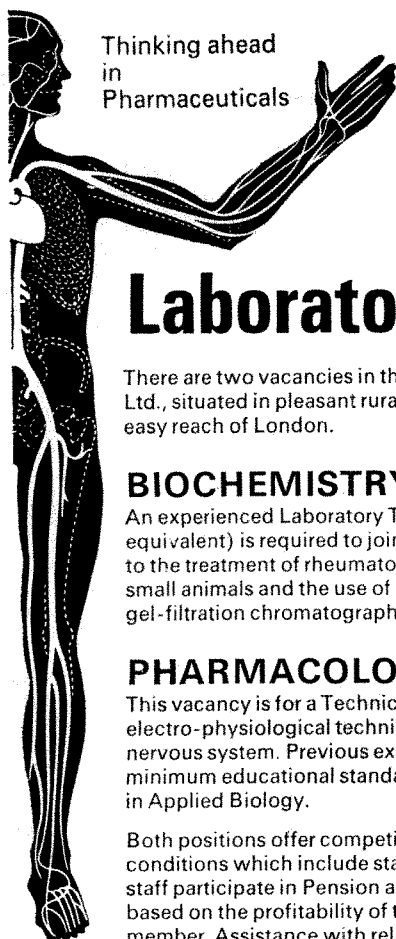
To participate in the application of research findings to multi-resource planning for environmental management.

Qualifications: A higher degree in the physical or biological sciences or in engineering, or equivalent

qualifications, and evidence of experience in more than one discipline; proven ability to initiate and supervise an integrated set of inter-disciplinary investigations required to understand the resources of a region and the constraints to be exercised in their use and management.

The applicant must have had experience or a close relationship with Government, University and Industry.

Applications quoting reference number (Z/02), should be addressed to the Secretary, Public Service Board of Victoria State Public Offices, No. 1 Treasury Place, Melbourne, 3002, Australia, by not later than 9.30 a.m. on Wednesday, September 25, 1974, together with statements of experience and qualifications and date and place of birth. (800)



Thinking ahead
in
Pharmaceuticals

Laboratory Technicians

There are two vacancies in the Biology Division of Allen & Hanburys Research Ltd., situated in pleasant rural surroundings in Ware, Hertfordshire, within easy reach of London.

BIOCHEMISTRY DEPARTMENT

An experienced Laboratory Technician (with H.N.C. in Applied Biology or equivalent) is required to join a team of biologists working on new approaches to the treatment of rheumatoid arthritis. The work will involve the handling of small animals and the use of biochemical laboratory techniques. Experience of gel-filtration chromatography would be a particular advantage.

PHARMACOLOGY DEPARTMENT

This vacancy is for a Technician to work with a senior graduate in developing electro-physiological techniques for evaluating drug effects on the central nervous system. Previous experience is not essential but we do require a minimum educational standard of two 'A' levels (one in Biology) or an H.N.C. in Applied Biology.

Both positions offer competitive commencing salaries and good working conditions which include staff canteen and sports and social club. Members of staff participate in Pension and Life Assurance schemes and a bonus scheme based on the profitability of the Glaxo Group of Companies of which we are a member. Assistance with relocation expenses will be given, where appropriate.

Please apply in writing to:



Ms. E. W. Smith,
Assistant Personnel Officer,
Allen & Hanburys Research Ltd.,
Ware, Herts. SG12 0DJ.



Allen & Hanburys

MAKERS OF FINE PHARMACEUTICALS

(793)

CHARING CROSS HOSPITAL MEDICAL SCHOOL (University of London)

Applications are invited from medical or other suitably qualified graduates for the post of LECTURER in the DEPARTMENT OF PHYSIOLOGY, 1974-75 session. Apply with full details of qualification and experience to the Secretary, Charing Cross Hospital Medical School, Brandenburgh House, 116 Fulham Palace Road, London W6 9HH, quoting reference PH/L. Closing date August 31, 1974. (799)

The Marie Curie Memorial Foundation

THE CHART, OXTEAD RH8 0TL

1. RESEARCH ASSISTANT FOR THE METABOLIC UNIT with a degree in Nutrition or Biochemistry and an interest in clinical research in cancer. Experience of drug metabolism would be an advantage.
2. RESEARCH ASSISTANT FOR THE BIOLOGICAL CHEMISTRY UNIT with Grad. R.I.C., or B.Sc. (Hons) in chemistry, with an interest in organic chemistry. Current projects involve the design, synthesis and evaluation of new anti-tumour agents and metabolism of anti-cancer drugs.
3. RESEARCH ASSISTANT FOR THE IMMUNOCHEMISTRY UNIT with a degree in biochemistry to investigate the role of acid hydrolases in tumour invasion and metastatic spread. Experience in immunochemical and biochemical analytical techniques would be an advantage.

Salaries are based on the Whitley Council Scale with superannuation. Successful applicants may register for a higher degree after an initial probationary period. Applications including the names of two referees and indicating the post sought should be sent to the Secretary at the above address. (802)

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Haematology/blood group serology.

TECHNICIANS/JUNIORS

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Applications in the first instance to the Senior Chief Technician. Tel. 01-856 5555. (801)

**IMPERIAL COLLEGE
UNIVERSITY OF LONDON
LECTURESHIP
IN PETROLEUM GEOLOGY**

Applications are invited for a Lectureship in the Department of Geology under the direction of Professor W. D. Gill, for the coming session. Requirements are a good degree and experience in the Petroleum Industry.

Salary depending on qualifications and experience in the scale £2,118 to £4,896 plus £213 London allowance. Superannuation under ESSU scheme.

Applications with curriculum vitae and names of referees to Professor W. D. Gill, Department of Geology, Imperial College of Science and Technology, London SW7 2BP. Closing date August 31, 1974.

(797)

**FELLOWSHIPS AND
STUDENTSHIPS**

**UNIVERSITY OF LEICESTER
DEPARTMENT OF BIOCHEMISTRY**

Applications are invited for a Postdoctoral Fellowship for a project financed by the Nuffield Foundation for work with Dr M. J. Morgan on "The Regulation of Carbohydrate Metabolism in Animal Cell Culture." Experience in biochemical genetics and cell culture techniques would be an advantage.

The appointment would be for one year in the first instance, renewable for up to three years. Starting salary £2,118 p.a. with F.S.S.U. membership.

Applications, including curriculum vitae and the names of two referees should be sent to Dr M. J. Morgan, Department of Biochemistry, School of Biological Sciences, University of Leicester, Leicester LE1 7RH.

(723)

**THE QUEEN'S UNIVERSITY
OF BELFAST
RESEARCH STUDENTSHIP
IN ORGANIC
CRYSTALLOGRAPHY**

Applications are invited for a research studentship involving the application of X-ray crystallographic and computing techniques to the study of organic and organometallic compounds. The successful applicant should have an honours degree or G.R.I.C. and will be expected to proceed to a Ph.D. degree. Applications, with the name of one referee, should be sent as soon as possible to Dr J. F. Malone, Chemistry Department, Queen's University, Belfast, BT9 5AG.

(726)

**ROYAL HOLLOWAY COLLEGE
(University of London)
EGHAM HILL, EGHAM, SURREY
RESEARCH STUDENTSHIPS**

Applications are invited from suitably qualified graduates in Chemistry, Physics or Biochemistry for Research Studentships in Biophysical Chemistry and Surface Energetics. Successful applicants will be expected to register for a higher degree. Applications with a curriculum vitae and the names and addresses of two referees should be sent to Dr. P. J. Gardener, Department of Chemistry. (N) (729)

OPTICAL FIBRE COMMUNICATIONS

Communication by optical fibres is a rapidly expanding new technology which is being widely taken up by industry. The Laser research group at Southampton, comprising some 15 research workers, has been engaged in this field for some years with support from Science Research Council, industry and elsewhere. We have already announced world records for low transmission loss and high bandwidth in our fibres and wish to expand the present research team. Applications are therefore invited for a number of research fellowships, including a Pirelli Fellowship for work in collaboration with industry, at salaries linked to the scale for Lecturers in the range £2,118 to £3,462 plus threshold payments. Persons with an interest in any aspect of the subject are eligible. Applications giving details of education, experience and the names of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH. Please quote reference No. 231/R.

(734)

**European Molecular Biology Organization
(EMBO)**

**Short- and long-term fellowships
in molecular biology**

The European Molecular Biology Organisation intends to award to scientists working in laboratories within the European area both short-term (from a few days to several weeks) and longer-term fellowships for collaborative research or advanced training in molecular biology.

The Short-term Fellowships are to support visits to other laboratories for the purpose of carrying out experiments with special techniques or of other forms of scientific collaboration or advanced training, and especially to support developments arising at short notice.

The Long-term Fellowships will usually be for a period of one year, but applications for renewal will be considered. They will be awarded upon individual application, at the "junior" level to promising young research workers who may then spend prolonged periods in other laboratories working under the guidance of leaders in the field of molecular biology. At the "senior" level, they may be awarded to enable established research workers to gain experience in new approaches and new problems. Upon application by European Institutions fellowships at the "senior" level may also be awarded to specialists who can assist in the initiation and development of research programmes in the sponsoring Institution. Such "sponsored" awards may be made to established workers at all stages of their career beyond the postdoctoral stage.

Application forms and further details may be obtained from Dr J. Tooze, Executive Secretary, European Molecular Biology Organisation, 6900 Heidelberg 1, Postfach 1022.40, Germany.

(521)

**UNIVERSITY OF NOTTINGHAM
DEPARTMENT OF CHEMISTRY
S.R.C./C.A.S.E. STUDENTSHIP**

Applications are invited for a three-year studentship to work on the preparation and some reactions of novel inorganic fluorinating agents. The student will receive training in vacuum line techniques, chromatography and spectroscopy.

Supervisors—Dr. M. F. A. Dove (Nottingham) and Dr. G. Fuller (I.S.C. Chemicals Ltd.). Candidates must have the equivalent of a 1st or upper 2nd class Honours chemistry degree.

Applicants should send their curriculum vitae and the names of two referees to Dr. M. F. A. Dove, Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD.

(737)

**UNIVERSITY COLLEGE DUBLIN
DEPARTMENT OF ZOOLOGY**

Post Doctoral Research Fellowship in Paleolimnology under the sponsorship of the National Science Council.

Applications are invited for a Post-doctoral Research Fellowship to investigate the Post Glacial development of Loughs Ennell and Owel, Co. Westmeath. The position is tenable for one year with a possible extension to a second year.

Applicants must hold a doctorate or be about to submit. A knowledge of the taxonomy of the Chironomidae and Cladocera and techniques of coring and sediment chemistry are essential.

Salary to a suitably qualified candidate will be £2,100. The work will be based in Dublin and an allowance for field expenses will be paid.

Applications should be forwarded to the undersigned giving details of qualifications not later than Friday, August 30, 1974.

The Secretary,
Zoology Department,
University College,
Belfield,
Dublin 4.

(746)

**UNIVERSITY COLLEGE DUBLIN
RESEARCH FELLOWSHIP IN
POPULATION ECOLOGY**

A postdoctoral fellow is required for a project sponsored by the National Science Council on the ecology and control of insect pests in peatland. Applicants must have an honours degree in Zoology, postgraduate research experience in insect population ecology and should preferably have some experience of peatland ecosystems. Applications, with curriculum vitae, to Dr M. F. Ryan, Department of Zoology, University College, Belfield, Dublin 4, before August 23, 1974.

(747)

**CHELSEA COLLEGE
UNIVERSITY OF LONDON
CAMBRIDGE UNIVERSITY**

POST-DOCTORAL RESEARCH FELLOW

required from October 1, 1974 to study the excretion of nicotine and its metabolites in smokers of various age groups, in various pathological conditions and exposed to various environmental influences. The appointment will be for one year in the first instance with the possibility of renewal for a further two years. Salary £2,280 per annum. F.S.S.U. Superannuation Scheme. Apply with full curriculum vitae to Dr. J. W. Gorrod, Department of Pharmacy N, Chelsea College Annexe, 271 King Street, Hammersmith, London, W.6, as soon as possible.

(756)

**BREWING INDUSTRY RESEARCH
FOUNDATION
NUTFIELD, SURREY
MICROBIAL PHYSIOLOGIST**

A postdoctoral Microbial Physiologist is required in the Department of Microbiology and Fermentation to participate in wide ranging studies of the metabolism of yeast during fermentation.

The appointment will be made as a Research Fellow for a period of up to three years at a starting salary to be determined but on the scale £2,299 by £80 to £2,619 by £107 to £3,047. Threshold payments will be made additionally to basic salary.

Applications, including a curriculum vitae, should be sent to the Administrative Manager, Brewing Industry Research Foundation, Nutfield, Redhill, Surrey RH1 4HY.

(780)

**UNIVERSITY OF ABERDEEN
UNIT FOR RESEARCH ON ADDICTIVE
DRUGS**

A S.R.C. Studentship (Co-operative Award in Science and Engineering, C.A.S.E.) will become available on October 1, 1974. It is tenable for 3 years in co-operation with the Pharmaceutical Division of Reckitt and Colman Ltd., Hull, and is open to First and Upper Second Class Honours Graduates in Pharmacology, Biochemistry or Physiology. The research project is concerned with the action of newly-synthesised morphine-like compounds on neurotransmitter release and on behavioural patterns in normal and morphine-dependent animals.

Applications (3 copies) to be submitted to Professor H. Kosterlitz, Unit for Research on Addictive Drugs, University of Aberdeen, from whom further particulars may be obtained, Marischal College, Aberdeen AB9 1AS, not later than September 6, 1974.

(789)

MRC

Medical Research Council

Travelling Fellowships 1975-1976

The MRC invites applications from established workers of at least Registrar status, or with postdoctoral experience in the biomedical field in the case of non-medical graduates, for Travelling Fellowships available for a year from October 1975. The awards may be held at any centre abroad (although certain awards are tenable only in the USA).

The closing date for application is 21 October 1974.

Application forms and further details may be obtained from:

Medical Research Council, (Grants and Training Awards Section),
20 Park Crescent, London, W1N 4AL. Telephone: 01-636 5422 (Extensions 240 or 242).

(761)

IRELAND

FELLOWSHIP IN MARINE SCIENCE

The Pfizer Chemical Corporation, Ringaskiddy, Co. Cork, is sponsoring a Fellowship in Marine Science tenable at University College, Cork, under a scheme administered by the Fisheries Division of the Department of Agriculture and Fisheries.

Applications are invited from suitably qualified persons to undertake a study of some aspects of the ecology of Cork Harbour.

QUALIFICATIONS:-

ESSENTIAL—At least a Second Class Honours Grade 1 University degree in Zoology.

DESIRABLE—Experience in some aspect of Marine Biology. The initial starting stipend will normally be within the range of £1,600 to £1,800 per annum but a candidate with special qualifications may be appointed at a higher starting stipend.

Application forms and Conditions of Service attaching to the Fellowship may be obtained from:

The Secretary,
Department of Agriculture and Fisheries,
Fisheries Division,
Agriculture House,
Kildare Street,
Dublin 2.
IRELAND

Closing date for the receipt of completed application forms is 4.00 p.m. on Friday, September 6, 1974. (773)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

Research School of Physical Sciences
RESEARCH FELLOW/SENIOR
RESEARCH FELLOW

DEPARTMENT OF NUCLEAR PHYSICS

The Department, which has an academic staff of eighteen headed by Professor J. O. Newton, carries out work in nuclear structure physics. At present, main research activities are in the broad areas of heavy-ion interactions, direct interactions and nuclear spectroscopy.

The Department operates two major accelerators. The 14UD Pelletron Tandem Accelerator (14 MV terminal) was installed in 1973 and research work with it has recently commenced. It is intended that research with the 14UD will be mainly in the heavy-ion field.

The EN tandem (6MV terminal) is provided with a negative-ion cyclotron injector enabling acceleration of proton and deuteron beams with energies up to 38 and 26 MeV respectively. In addition the EN has a polarised ion source as well as the regular gas and lithium exchange source.

Closing date: **September 13, 1974.**

SALARIES: Salary on appointment will be in accordance with qualifications and experience within the ranges: Senior Research Fellow \$A13,163 to \$A15,348 p.a.; Research Fellow \$A9,002 to \$A12,269 p.a.; current exchange rates are approximately \$A1: 67p: \$US1.49.

Tenure: Senior Research Fellow and Research Fellow normally for three years in the first instance with the possibility of extension to a maximum of five years.

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain further particulars from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF before applying. (795)

SHEFFIELD POLYTECHNIC

Research Fellow in Biological Sciences

Applicants should be experienced in the biochemistry of microorganisms, with particular reference to biological recycling of industrial and town wastes and effluents.

The initial appointment will be for three years with the possibility of extension for a further two years.

Salary Scale: Senior Lecturer Grade: £3,525 to £3,915 (bar) to £4,212.

Application forms and further details obtainable from the Staffing Officer, Sheffield Polytechnic, Halfords House, Fitzalan Square, Sheffield, S1 2BB, to whom completed forms should be returned within fourteen days. (762)

UNIVERSITY OF ADELAIDE

Applications are invited for the following appointment:

SENIOR TEACHING FELLOW IN PHYSICAL AND INORGANIC CHEMISTRY

The Senior Teaching Fellow, who should have completed a Ph.D. degree, will devote about half his time to teaching and the other half to research. He will be responsible for supervising arrangements for some undergraduate practical courses and will initiate new course developments; he will be encouraged to undertake research with one of the existing research groups in the Department, which has good modern facilities. A document detailing research interests within the Department and describing undergraduate class arrangements is available on request (September 13, 1974).

Salary Scales: Senior Teaching Fellow \$A7,545 by \$A292(2) by \$A291(3) to \$A9,002, with superannuation provision.

Further particulars about this post and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London WC1H 0PF.

Applications should be sent in duplicate and giving the information listed in the Statement that will be supplied, to the Registrar, The University of Adelaide, North Terrace, Adelaide, South Australia, 5001. (796)

UNIVERSITY OF LEEDS

DEPARTMENT OF PHYSICAL CHEMISTRY

Applications are invited for two research posts to work with Professor P. Gray and Dr A. A. Clifford.

EXPERIMENTAL OFFICER: work on the measurement of the viscosity and thermal conductivity of the freons in the gas phase. Applicants should be graduates with good practical ability. Salary on the scale £1,752 to £2,376 (rates applicable from October 1974).

RESEARCH FELLOW: work on the measurement of the diffusion rates of hydrogen atoms in gases using the technique of resonance fluorescence. Applicants should hold a Ph.D. degree and have experience of research in a related field. Salary on the scale £2,118 to £2,412 (rates applicable from October 1974).

Appointment will be for one year in the first instance, with possible renewal for a further year. Applications to Dr A. A. Clifford, Department of Physical Chemistry, The University, Leeds LS2 9JT by August 23. (760)

SHEFFIELD POLYTECHNIC
DEPARTMENT OF CHEMISTRY
AND BIOLOGY

RESEARCH STUDENTSHIP IN "OPTICAL METHODS OF INDUSTRIAL ANALYSIS"

Applications are invited for this post, sponsored by Anacon (Instruments) Ltd., to commence in September 1974. Candidates should hold, or expect to obtain this year, a good honours degree or equivalent (such as GradRIC) in chemistry, chemical engineering or biochemistry, or an appropriate joint honours degree. Other qualifications such as FIMLT could also be considered. The successful candidate will be expected to register for a higher degree.

The project will involve study of applications of near-infrared and ultraviolet spectroscopy and refractometry to analytical problems, particularly in the context of on-line analysis, in a wide range of industrial processes (e.g. in the food, chemical and oil refining industries). The student will spend periods of work at the Anacon factory and at appropriate industrial plant, thereby gaining experience of taking a project from the laboratory through to the eventual application on the plant.

The post is for two years in the first instance, renewable for a further year. The value of the Studentship will be £800 per annum (plus all fees), but this may be supplemented by a limited amount of part-time teaching in the Department to afford approximately £1,100 per annum.

Application forms may be obtained from the Staffing Officer, Sheffield Polytechnic, Halfords House, Fitzalan Square, Sheffield, S1 2BB, to whom completed forms should be returned as soon as possible. (763)

UNIVERSITY OF LEICESTER

Department of Engineering

Applications are invited for a

Research Fellowship

for SRC-supported research into **high current-density electrochemical machining with molten salt electrolytes.**

Applicants should have at least two years postgraduate experience or a higher degree.

The post is for 2 years, with salary in the range £1,925 to £2,605 p.a. and superannuation benefits payable.

Further details and application forms may be obtained from the Head of the Engineering Department, The University, Leicester LE1 7RH (Ref. RF3).

Informal enquiries to Mr H. E. Freer or Dr A. C. Baxter.

(803)

LECTURES AND COURSES

"Combustion Fundamentals"

An introductory course of 14 post-experience lectures will be held at IMPERIAL COLLEGE from Monday October 14 to Friday 18, 1974.

Further information from Professor F. J. Weinberg, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7 2BY. (751)

FOR SALE AND WANTED

FOR SALE

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Please contact: D. J. Lovell, E.M. Unit, Guy's Hospital Medical School, London SE1. Telephone: 01-407 7600 ext 547. (804)

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MARINE POLLUTION BULLETIN

Marine Pollution Bulletin is published monthly and sets out to cover all aspects of the fight for life of lakes, estuaries, seas and oceans. It includes news, comment, reviews and research reports not only on the threats of noxious substances to marine life but also on the management and productivity of the marine environment in general. It publishes accounts of new and proposed research programmes as well as the results of those in progress.

Recent research reports include:

Distribution of Caesium-137 in British Coastal Waters, D. F. Jefferies, A. Preston and A. K. Steele. **Export of Lead from Salt Marshes**, M. Banus, I. Valiela and J. M. Teal. **International Scope of Marine Pollution Damage**, D. P. Tihansky. **Effects of Red Mud on Marine Animals**, R. A. A. Blackman and K. N. Wilson. **Pollution Problem of the Golden Horn**, M. Karpuzcu.

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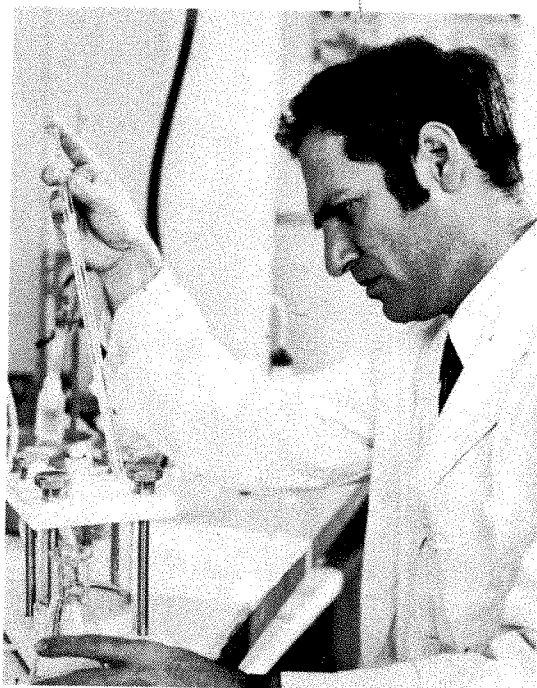
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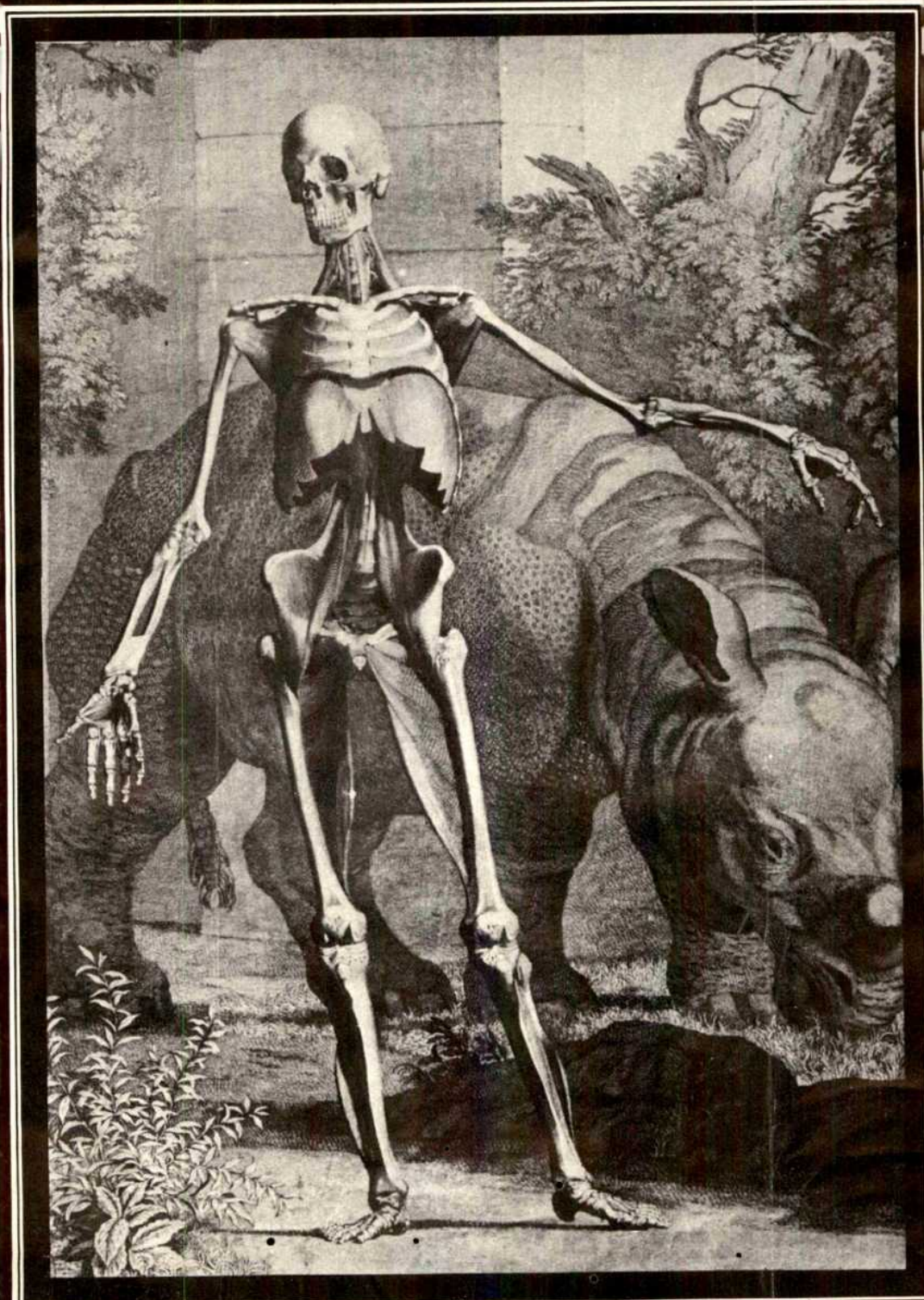
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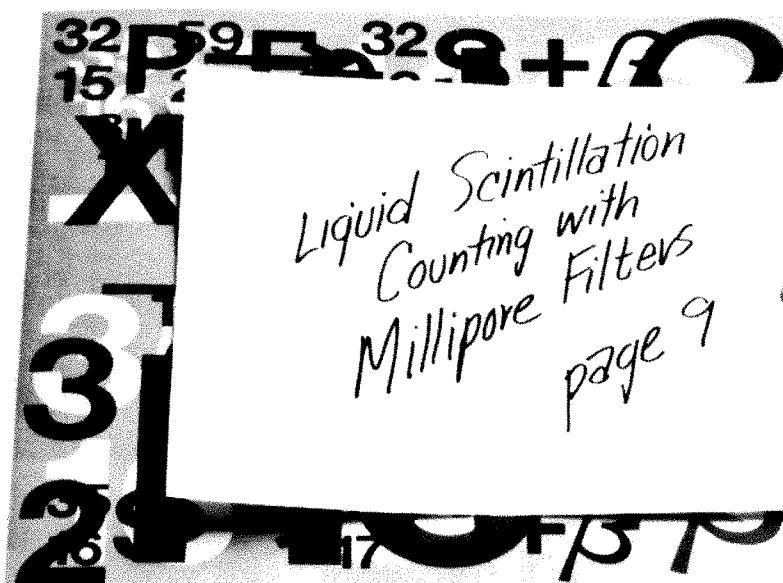
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tion of illustrations for science, re-
viewed on page 691

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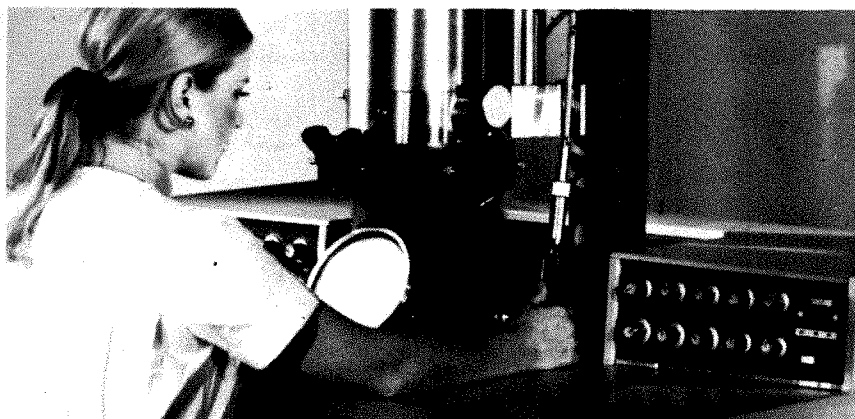
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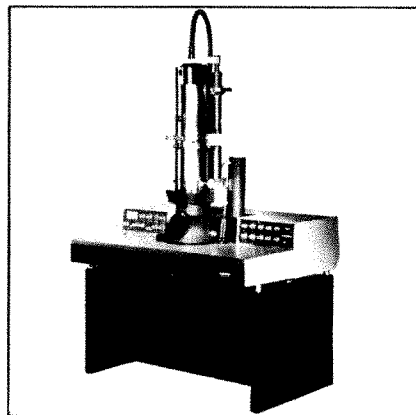
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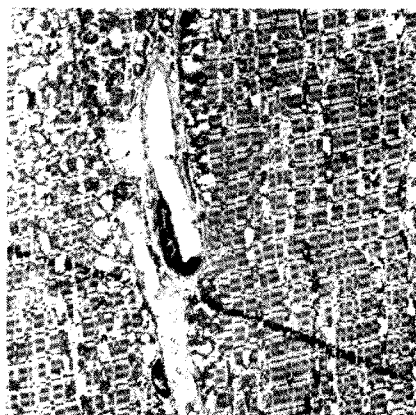


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A fuller guide appeared in *Nature* (246, 238; 1973).

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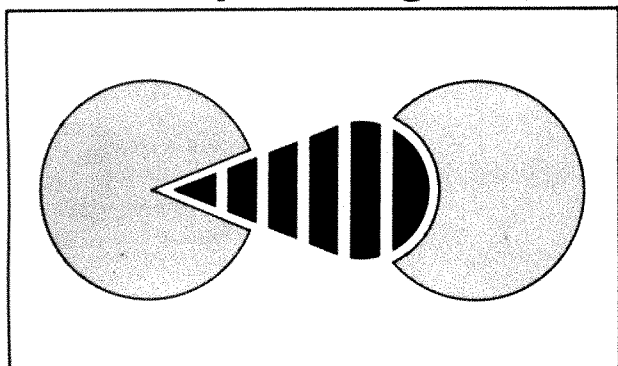


On Saturday evening a lecture on the Transit of Venus was delivered by M. Faye, before a very large audience at the Cercle du Nord, a magnificent building, richly fitted up. The accomplished astronomer referred mostly to the arrangements at the French stations, deeply regretting that all civilised nations had not been united into a kind of federation for working in combination at a problem of such magnitude; he hopes that it will be so in 1882. He insisted upon the importance of photography, which has been used to such good purpose by the Americans, and he trusts that in future times photography will take the lead in such observations. He gave interesting details as to the Yokohama station, to which a Japanese prince educated in France will be attached as a photographer. The consequence will be that M. Jannsen and his associates will be admitted into the interior sea of Japan, where, up to the present moment, not a single foreign vessel has ever sailed.

From *Nature*, 10, 345, August 27, 1874.

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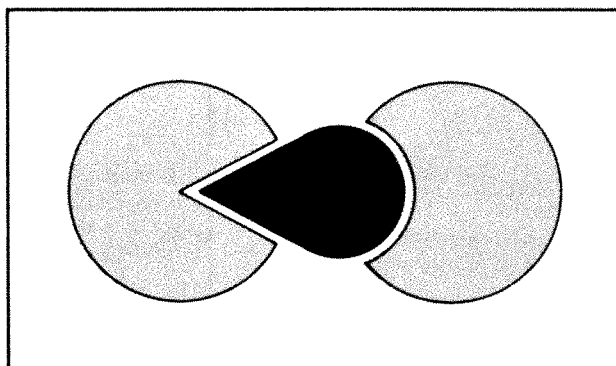
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Mr Benn's mixture of good and bad

BRITISH industry is in a woeful mess; there is little disputing that and small comfort in the knowledge that nowhere in the world is industry doing outstandingly well at present. Nor is the malaise a thing of the recent past. For many years the danger signals have been flying, as Britain's growth has become sluggish and as industrial investment has fallen off.

A long period of decline will require a long period of recovery; certainly no tactical change of course that a single budget could effect will help much and this is the importance of Mr Benn's proposals in *The Regeneration of British Industry*, just published as a White Paper (Cmnd 5710). Nothing less than an immense strategic rethink is necessary and the time when industrialists could simply ask to be left alone is well past. The crudity, so far, of the advertisements of Aims of Industry—a group of employers dedicated to free enterprise—suggests that there are many who still do not appreciate that what is desperately needed now is discussion and not rhetoric. Lack of business confidence, which is what the decline in investment is all about, cannot be treated by urging support of the status quo.

That, at least, is certainly far from Mr Benn's intention. Government and industry, the document argues, have dealt too remotely with each other; and industry has seen the government's function as one of regulation. Now it is time for partnership, and this is being proposed in two fairly distinct ways.

First, there will be Planning Agreements with major firms to ensure harmony with national needs and objectives and to provide a better basis for governmental aid. The agreement will be a rolling three-year look at company plans, and if necessary will provide discretionary financial assistance. The agreement will be drawn up by management 'in close consultation with trade union representatives' who will be provided with all necessary information for effective consultation. The government sees this as a major advance in industrial democracy. In return for companies opening up their plans to Whitehall, they will have the benefit of "the government's views on the likely development of the economy".

The second proposal is for a National Enterprise Board (NEB) and it is through this that the government will channel large scale investment in exchange for equity. It will also take over the government's holdings in companies such as Rolls-Royce (1971) Ltd, assist industrial reorganisation, start new ventures and extend public ownership into profitable manufacturing industry by buying up to 100% of the equity. Criteria for acquiring a profitable company will include the danger of its passing into "unacceptable" foreign control, and the

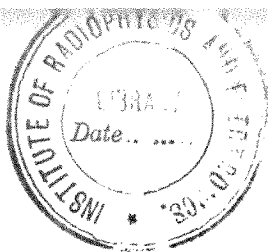
stimulation of competition. The NEB may also take over an ailing company for reasons of regional employment or "industrial policy". The board will ensure that enterprises under its control fully involve employees in decision making. Mr Benn is restrained by the requirement that all major deals require government approval and the Cabinet, much less to the left on average than Mr Benn, will presumably moderate his undoubted vigour.

The timing of the document makes it quite obviously an election manifesto since nobody seriously believes there will not be an election before the White Paper can be put to the vote. And it is this reminder of the fluctuations of politics which casts some doubts on the two schemes. If a Labour government guarantees a three-year planning agreement, will a subsequent Conservative government dismantle it? Surely this would provide for even less investment confidence and so there is a need for the two major parties to hammer out something which they can each live with. The document, as it now exists is more moderate than was originally planned. Even so, the thought of trade unions looking at the books and helping to make decisions will still terrify most Conservative Members of Parliament and may make the whole programme unacceptable if Labour is still a minority government when legislation is being considered.

This would be most unfortunate. If there is one thing that desperately needs attention in Britain, it is the them-and-us divide which permeates industry and most other things too. Industrial democracy, common in many other countries, has to come soon and Mr Benn is right to push for it.

The other compulsory match for industrial management is with government, and this is a dubious affair indeed. The problem is that government has no evidence to show that it has any unusual skills or knowledge to offer. No doubt there will be some bright people recruited into the NEB and Planning Agreement section of the Department of Industry but on the whole staffing will be accomplished by shuffling the pack of civil servants. Is there any evidence that they will be a desirable commodity for industry? And the political masters; well they are much the same as those who were going to harness the technological revolution in 1964, and it is the failure to do that which has contributed much to Britain's present depressed state. No doubt the agreements will allow civil servants to pass on the minister's thinking in their offices instead of their clubs, but vigorous industry is more likely to be able to shape the minister's thinking by its successes than to be shaped itself because of its failures.

If the planning agreement concept suggests the gaining of doubtful benefits from Whitehall, the NEB investment and takeover scheme is worse. The reward for running a profitable company is to find not only the unions but also the government wanting a large piece of the action. No self-respecting manager will stay with a firm in which success is recompensed by loss of responsibility. If there is so much talent in Whitehall, it should surely be used to revive less successful industry.



The changing relationship between science and technology

J. Langrish of the Manchester Business School examines the way in which the relationship between science and technology has changed in the past and is about to change again.

IN recent years, the belief that technological change is somehow based on scientific advance has been increasingly challenged. Price¹, for example, has claimed: "The naive picture of technology as applied science simply will not fit the facts. Inventions do not hang like fruits on a scientific tree. In those parts of the history of technology where one feels some confidence, it is quite apparent that most technological advances derive immediately from those that precede them".

Technological change has been increasingly seen as the adaption of existing technological concepts in response to demand. Schmookler², for example, has attempted to show that the variation in inventive activity between different American industries is explicable in terms of the variation in demand, concluding that economic growth determines the rate of inventive activity rather than the reverse. None the less, governments have continued to invest in scientific research at a level much greater than other activities which can also claim cultural and prestige benefits. Science receives much more money than the Arts because it is believed to be useful. When science is not used, it is claimed that there is a communication barrier; the alternative possibility that it is intrinsically of no use is not examined.

One of the major problems in looking at the usefulness of science is a matter of definitions. What is Science? What is Technology? To avoid this problem, we must ask questions such as, what is the effect of university research and research in government laboratories on industrial change, or on improvements in health and agriculture.

A study³ of 84 British technological innovations showed that "demand pull" occurred more frequently than "discovery push" and that out of 158 key technical ideas made use of in innovations, 56 originated within the innovating firms and of the remaining 102, 7 came from a British university and 17 from a British government labora-

tory (including Research Associations).

The apparent conclusion that ideas from British universities had little effect on British innovation was challenged in a variety of ways. One of these was the claim that although industrial innovation may be based on industrial research, the day to day progress of industrial research depended on university research in ways that would not be revealed in looking at 'key ideas'. It was pointed out, for example, that industrial chemists spend time reading the results of university chemical research.

To examine the relative contribution of university research in the literature cited by industrial researchers, seven review articles written by British industrial chemists were examined⁴. The reviews contained 567 references to other publications (including patents) and the institutional origins of 396 of them were identified. Only 23 out of the 396 references were to the work of British universities and of these, 7 stated that some industrial finance had been involved in the research. British government institutions seemed to be more interesting to the industrial reviewers in that 45 of the references were involved. Table 1 compares the relative importance of the institutional origins of the 102 "key ideas" with the origins of the 396 references and also the origins of 452 abstracts to be discussed later.

These results suggest that British university research has little impact on British industry. Why then does the British government continue to finance university research? One possible reason is that university research only occasionally produces results of economic benefit but when it does the benefits are so large that they outweigh the total costs of all university research for several decades. An example of this kind in the present century would be the research that led to atomic power. A more fruitful source of such examples is the last half of the last century.

There is little doubt that German university research in organic chemistry, for example, provided the foundations for the German synthetic dyes industry and subsequent advances in pharmaceuticals, synthetic rubber and plastics. Similarly the electrical industries can be seen as being based on nineteenth century academic research.

At the time of the First World War Britain realised that Germany had advanced industrially by using the results of scientific research, and in 1917 the Department of Scientific and Industrial Research was established. Since then, the British government has ploughed increasing amounts of money into scientific research in the belief that what was good for Germany in the last century should be good for Britain now.

But times have changed. Reliance on the German example does not seem to have been justified. One possible explanation is that the relationship between science (university research) and technology (industrial practice) has changed since the last century. To test this hypothesis, abstracts produced by the *Journal of the Society of Chemical Industry* were examined. For some eighty years, this Journal produced abstracts of the world's literature that might be of relevance to industrial chemists. Even in the last century, large teams of abstractors were used, reducing the possible bias of an individual. The abstracts were classified according to industrial subject matter, and those classes which might be considered to rest on organic chemistry were chosen for examination.

The original publications selected by the abstractors as being worth the attention of industrial chemists were consulted and wherever possible, institutional origins identified. Initially, the years 1884, 1917, 1935 and 1952 were examined but the difference in institutional origins between 1884 and 1917 was so great that the year 1899 was added to the investigation.

Table 2 shows the institutional sources of abstracts in randomly selected editions of the Journal for the given years. (A small proportion of abstracts in all years defied attempts to identify origins. The figures given are for identified sources.)

Teaching establishments are included under the category of university as are research laboratories and institutions attached to universities. The 'government' category includes Research Associations, government-financed industrial laboratories and military establishments.

Several of the abstracts were to patents some of which caused difficulties in the assignment of origins. Early patents usually give the name and pro-

Table 1 Institutional origins of literature cited in British industrial research

Origins	% of 102 ideas	% of 396 references	% of 452 abstracts (1952)
UK Industry	22	10	19
UK University	7	6	1
UK Government	19*	11	1
Foreign industry	40	40	68
Foreign university	3	21	5
Foreign government	9*	12	6

*Government and military combined

Table 2 Change with time of institutional sources of abstracts in *Journal of Society of Chemical Industry* for industrial areas connected with organic chemistry

Year of journal	1884	1889	1917	1935	1952
Institutional source	%	%	%	%	%
US industry	—	4.8	14.5	38.1	53.8
UK industry	8.8	17.3	6.2	9.3	19.0
European industry	23.5	44.7	52.4	24.1	13.6
Other industry	—	1.0	0.7	0.5	0.9
Total industry	32.3	67.8	73.8	72.0	87.4
US government	—	1.0	1.4	1.9	3.6
UK government	—	1.0	—	0.5	1.1
European government	5.9	1.9	2.1	3.4	1.3
Other government	—	1.0	2.1	0.5	1.1
Total government	5.9	4.8	5.5	6.3	7.2
US university	3.0	1.0	1.4	1.9	2.6
UK university	—	7.7	6.2	0.8	0.7
European university	58.8	18.7	13.1	15.3	0.2
Other university	—	—	—	3.7	2.0
Total university	61.8	27.4	20.7	21.7	5.5

Table 3 Change with time of institutional source of publications referred to by British industrial chemists in reviews of 'polyolefin' chemistry

Date of review	1957	1961	1967
	%	%	%
Institutional source	(n=28)	(n=71)	(n=102)
UK industry	3.5	12.0	12.0
US industry	41.5	46.0	69.5
European industry	5.0	7.0	
Total industry	50.0	65.5	81.0
UK university	—	—	2.0
US university	7.0	—	12.0
European university	43.0	28.0	
Total university	50.0	28.0	14.0
UK industry-university collaboration	—	—	3.0
US industry-university collaboration	—	6.5	2.0
European industry-university collaboration	—	—	
Total industry-university collaboration	—	6.5	5.0

fession of the inventor. In most cases the profession enabled a classification to be made, such as 'professor'—university; 'merchant'—industry. But descriptions such as 'gentleman' or 'subject of the Czar' had to remain unclassified unless the inventor could be identified from other sources. Papers by joint authors from different types of institutions were scored $\frac{1}{2}$ each.

It can be seen from Table 2 that there has been a marked change in the relative importance of the institutional origins from European university (mainly German) to European industry and then to American industry as being the major contributors selected by the abstractors.

Although some of the change may be due to changes in abstractors or journal policy, the large decline in the relative importance of university publications from 61% in 1884 to 6% in 1952 and the steady growth in the importance of American industry from 0 in 1884 to 54% in 1953 can be assumed to be the result of a change in the relationship between university research in organic chemistry and those industries which use organic chemicals.

The data for 1952 are included in Table 1 for comparison with the studies previously mentioned. The data are based on an examination of 544 abstracts of which 59 could not be found, 6 had insufficient information and 27 were unclassifiable patents, leaving 452 identified origins. The three different studies give different percentage inputs but it should be noted that in all three studies:

- Foreign industry was the major source of inputs

- UK industry was the second most frequent source in two studies and the fourth most important in the other

- UK university was either the least important source or next to the least.

The dramatic change which has taken place since the days when German university research was providing results of direct relevance to the German dyestuffs industry may be explained by two alternative hypotheses:

(1) Industry has taken over its own research. The massive growth in industrial research since German industry employed teams of chemists to search for new dyes may explain the decline in apparent relevance of university research. However, university research has

also grown enormously since the last century. (An interesting account³ of the growth of British industrial research has been given by Sanderson.)

(2) A new branch of science is only useful in its early days. The early days of astronomy as a science were linked with economically important attempts at improving navigation but astronomy has hardly been useful since then. (Even earlier, astronomy was used to maintain the power of priestly castes.) The early days of atomic power involved theoretical physicists; since then the theoretical physicists and the designers of nuclear power stations have moved further apart.

The early days of organic chemistry involved the production of new chemical substances and new ways of making them. Since then the university chemist has been more concerned with understanding the results of early research; the industrial chemist has been concerned with using the early

knowledge and is not too interested in later theoretical developments.

One of the seven review articles referred to earlier was about 'polyolefins' and only 14 out of 102 identified references were to university research in spite of the fact that the present industrial importance of such materials as polypropylene started with the non-industrial research of Ziegler and Natta who may be considered to have founded if not a new branch of science, at least a new stem entitled stereospecific polymerisation.

It was decided, therefore, to test the above hypothesis by looking at earlier reviews of 'polyolefins' by industrial chemists. Table 3 shows that the relative importance of university contributions dropped from 50% in 1957 to 14% in 1967, although the absolute number of university contributions remained constant.

Another point of interest is that British industry began publishing items of interest to the reviewer before British universities. This was not because British universities ignored stereo-

specific polymerisation. It was because universities concentrated on the scientific problem of describing and explaining the mechanisms involved and the structure of the polymers produced whilst industry concentrated on obtaining manufacturing processes and improving properties such as light resistance. From a common origin, the discovery of stereospecific polymerisation, academics and industrialists have moved in different directions.

The relationship between university research and industry may well be a function of the degree of development of the area concerned. Once a new area has been established, the aim of science is to understand; the aim of technology is to make it work, and industry has been very successful at making things work without too much reliance on understanding.

Industry makes use of the trained manpower supplied by universities. It also uses new techniques such as chromatography, developed in universities. But the new products and processes of industry seem to depend on a combination of existing technological concepts, economic pressures and empirical research with scientific understanding not being very relevant.

This does not mean that university research is a waste of money; the situa-

tion may be changing. Concern about such matters as resource depletion, ecology, pollution, fire risk, health hazards and quality of working life coupled with the increasing scale of industrial operations may mean that industry will have to pay more attention to understanding what it is doing.

In the study of 84 innovations mentioned earlier, the only example of theoretical work in a British university assisting British industry was in the area of steel frames for building construction. A concern for human safety together with a shortage of steel led to the use of Baker's theory of plastic flow to design buildings which are safe but use less steel.

It could be that in the future, many sections of industry are going to require an increasing reliance upon theoretical research aimed at understanding, as the empirical approach, which has been so successful, joins the quest for economic growth as a thing of the past.

It would seem, therefore, that there are both macro and micro effects leading to changes in the relationship between science and technology.

At the micro level, the relevance of a particular part of science has depended on its degree of development. At the macro level, the relationship

seems to move in cycles, with the first half of the nineteenth century involving little reliance upon scientific theory (although the scientific technique of methodical observation was made use of) the second half of the nineteenth century being characterised by a series of inputs from research concerned with exploring scientific problems, the first half of the present century being a triumph of industrial methodical empiricism and the future requiring an increasing dependence on understanding.

I thank the Department of Education and Science and the Programmes Analysis Unit for funding parts of this research and Trevor Parkinson for his considerable ingenuity in tracking down the institutional origins of some of the more obscure references.

¹ de Solla Price, D. J., in *Factors in the Transfer of Technology* (edit. by Gruber and Marquis), (Massachusetts Institute of Technology, 1969).

² Schmookler, J., *Invention and Economic Growth* (Harvard University Press, 1966).

³ Langrish, J., Gibbons, M., Evans, W. G., and Jevons, F. R., *Wealth from Knowledge* (Macmillan, London, 1972).

⁴ Langrish, J., *Chemistry in Britain*, **8**, 330 (1972).

⁵ Sanderson, M., *Science Studies*, **2**, 107 (1972).

international news

A WORKING party under the chairmanship of Lord Ashby, Master of Clare College, Cambridge is being set up by the Advisory Board for the Research Councils to "make an authoritative assessment of the potential benefits and potential hazards of techniques which allow the experimental manipulation of the genetic composition of microorganisms".

At the same time the Medical Research Council (MRC) has made public the fact that they have asked their staff members not to embark on experiments in bacterial manipulation vetoed in the recent National Academy of Sciences (NAS) statement.

The letter circulated to directors of those MRC units which have the capacity to carry out work in this field asked that no experiments of type I and II in the NAS statement be undertaken in the near future until the working party has reported. This covers work on recombinant drug resistance plasmids that do not occur

Improper plasmids?

by Eleanor Lawrence

naturally, and the linking of animal virus DNA to plasmid DNA and its subsequent introduction and replication in a bacterial cell.

Directors were also asked not to initiate research on linking animal DNA to plasmid or bacterial DNA, which, according to the NAS statement, "should not be undertaken lightly." Experiments of this kind have already been carried out in the United States.

According to a spokesman for the MRC, the council supports no programmes actually involving research of the kind mentioned by the NAS, although the separate techniques are being studied in various laboratories.

He estimated that about 50 laboratories in Britain have the capacity to carry out genetic manipulation of the sort vetoed by NAS, but that until now there had been a self-imposed holding back from this type of experiment, which has been technically possible for some time.

The establishment of a working party to clarify the issue is generally welcomed, as the part of the NAS statement referring to drug resistance plasmids is felt by some to be ambiguous; it could, for example, be interpreted as banning experiments necessary for important public health work. Other workers also feel that the ban on introducing animal genes into bacterial cells could hold up work on new ways of producing important substances such as insulin.

The working party will consist of "high-level scientists" and the Advisory Board for the Research Councils hopes to be able to announce the membership within the next two weeks. □

Focus on astronomy

by John Gribbin and Ian Ridpath

A RECENT flurry of activity related to observational astronomy has emphasised that the obituaries for British optical astronomy may have been written too soon. Certainly the prospects for optical astronomy in Britain are dim; but the stirrings of the supposed corpse are having repercussions around the world, emphasising the international nature of modern astronomy.

Anglo-Australian Telescope: The AAT is the furthest of these projects from Britain geographically, but perhaps the most important in scientific terms. In spite of the usual difficulties of marking out territory on international projects (which will be familiar to readers of Fred and Geoffrey Hoyle's book *The Inferno* (Heinemann, 1973)) the project is still more or less on schedule. The official timetable, which sees commissioning of the telescope in October with completion of alignment and testing of optics and instrumentation in December of this year, looks a trifle optimistic. But it does seem that the facility will be ready for use early in 1975.

Infrared telescope for Hawaii: The SRC recently announced the decision to go ahead with construction of a 3.8 m (152-inch) infrared flux collector on Mauna Kea in Hawaii. This ends long speculation about the future of British infrared astronomy, and will result in the world's biggest 'purpose built' infrared telescope being located at a site which is recognised by astronomers as one of the best in the world. The SRC will lease the site for the instrument from the University of Hawaii, which already operates an observatory on Mauna Kea, at a height of 4,200 m.

This project has suffered more than most from political intervention, with sites such as Tenerife being ruled out because of poor relations between the British and Spanish governments. But for once the astronomers may have come out well from the politicking, since it seems likely that the Hawaii site would have been felt too remote for administrative convenience if an alternative closer to home had remained viable. The site is also convenient from a practical point of view, because it is far enough south to provide a good view of the galactic centre, a region of great interest to infrared astronomers. The construction costs for this telescope are estimated as £1.25 million at January 1974 prices, and construction is expected to take three years.

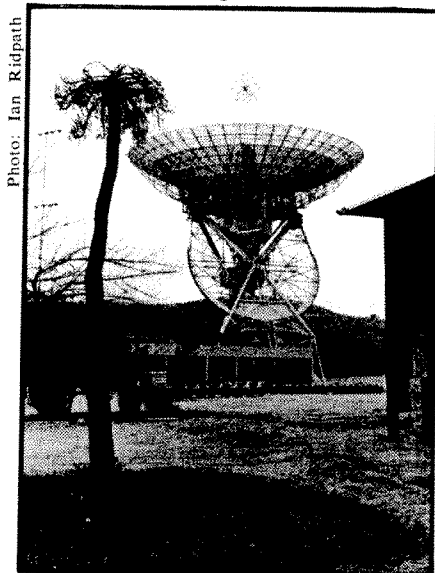
Mr Gordon Carpenter, the project manager, said last week that tenders

are now out and are expected back in early October. The site allocated is on the same mountain ridge as the University of Hawaii 88-inch optical telescope, and so most of the access roads and so on are already there. It should be possible to pour the first concrete in 1975, he said.

All this must make hot favourite as the location for the Northern Hemisphere Observatory, about which a decision is thought to be imminent.

South Africa: Amidst all this lightening of the astronomical gloom which has been so prevalent in recent years, a few storm clouds remain as a reminder that astronomers have not entirely abandoned their centuries-old tradition of internecine squabbling.

Since the opening of the new South



Hartebeesthoek tracking station

African Astronomical Observatory, with its observing station at Sutherland in the Karoo and its headquarters at the Cape Observatory, the former Republic Observatory in Johannesburg has been effectively abandoned. The observatory's buildings in Gill Street stand silent and empty, with the 26½-inch refractor, responsible for the observatory's world-renowned work on double stars and classic colour photography of Mars, now under mothballs in its dome.

Only one astronomer remains at the observatory, examining plates taken for minor planets with the 10-inch Franklin Adams telescope at Hartebeestpoort, 50 miles outside Johannesburg.

The decision to cannibalise the Republic Observatory and concentrate efforts at the Sutherland was received with mixed feelings by astronomers a few years ago, and Dr William Finsen, formerly Director of what was once South Africa's national observatory, was among the critics who spoke out against what he saw as a wrong decision by the SRC and the South African

Council for Scientific and Industrial Research (see *Nature*, 229, 355; 1974). Today, Finsen is in retirement and virtually an outcast from the South African astronomical establishment. For good or bad, however, the reorganisation of observational astronomy in South Africa is now essentially complete, and under the forceful direction of the former Astronomer Royal Sir Richard Woolley, it is hardly likely to become a backwater.

South African radio astronomers, meanwhile, like their infrared colleagues in Britain, seem to have benefited from a partially politically oriented decision. The 26-m (85-foot) radio dish of the former NASA deep-space tracking station at Hartebeesthoek, near Pretoria, ceased operation at the end of June this year, officially as part of "an overall consolidation activity" to prune the NASA tracking network. Privately, however, astronomers say that pressure from black politicians in the United States is believed to have influenced the decision. The associated tracking station for Earth-orbiting satellites is to close next year.

The deep-space station, opened in 1960, has been used to track all NASA's unmanned probes to the Moon and planets; it has now been taken over by South Africa's Council for Scientific and Industrial Research, and is being modified to work as a radio astronomy observatory. Initially the aerial will work at a wavelength of 13 cm, and eventually wavelengths as short as 4 cm will be available. The South African radio astronomers hope to have the telescope operational by the end of the year, and perhaps as soon as September. Surprisingly, however, no firm programme has yet been decided; but the interest of the observatory's director, George Nicholson, in quasars and extragalactic work is certain to make itself felt.

Leaving aside this somewhat fortuitous windfall to the radio astronomers, the revival of British and associated optical astronomy can in retrospect be traced back over several years. Certainly five years ago there was cause for concern; but with hindsight it is becoming clear that the problems have been recognised and to a large extent tackled. Projects such as the Schmidt at Siding Spring, the AAT and the new infrared telescope are now becoming physical realities, and allowing for the inevitable delay in the filtering of the resulting benefits through the system, the patient may not only be able to take up his bed and walk but may even be running around in the first team in another ten years or so.

correspondence

Megalithic alignments

SIR,—In your editorial "Science beyond the fringe" (April 12) you speak of archaeology as "plagued by a series of ideas which have achieved a following particularly among the young". The extreme example you give of this is "people busily poring over Ordnance Survey maps of Britain plotting mythical alignments between ancient monuments and erecting fanciful hypotheses about prehistoric technological civilisations." To this, as you say, Professor Glyn Daniel has objected in his *Antiquity* editorials.

Please allow me, as an author on the subject ridiculed in your editorial, the privilege of a brief comment.

You declare that alignments between ancient monuments are "mythical" and hypotheses about advanced prehistoric civilisations "fanciful", although as editor of a scientific journal you are of course aware that your statement of opinion does not necessarily make them so. It is ironical that the first man to detect significant alignments between megalithic sites was your predecessor, Sir Norman Lockyer, editor of *Nature* for the first 50 years of its existence. In fact, despite the studied neglect with which Lockyer has been treated by archaeologists throughout this century, his main conclusions, that megalithic sites were inter-related and orientated by astronomical considerations, are now generally accepted, even by the editor of *Antiquity*, as the result of recent work by Professor Thom and other astro-archaeologists. The quality of criticism brought against Lockyer and his school is typified by the comment with which O. G. S. Crawford, a former editor of *Antiquity*, dismissed the evidence of A. Watkins relating to the deliberate alignment of ancient sites: that it was "based on a misconception of primitive society." This comment perfectly illustrates the determination of modern archaeologists to prefer the basic historical paradigms they have set up above any evidence that might contradict them.

Personal experience shows that archaeologists, Professor Daniel included, simply refuse to review facts and evidence tending against current orthodoxy. In a book published by Garnstone Press early this year, *The Old Stones of Land's End*, I demonstrated, in a way that must convince any reasonable person who cares to

check the facts, the existence of planned megalithic alignments in West Cornwall. The book attracted a good number of local reviews but not one public comment by any archaeologist. It is therefore rather galling to read your account of archaeologists being "plagued" by theories of the inter-relationship of megalithic sites. Would not their best remedy be to attempt a serious refutation of the evidence offered them?

Your faithfully,

JOHN MICHELL

11, Miles Buildings,
Bath, Somerset, UK

Call for biohazard legislation

SIR—Although Brian Ford (*Nature* August 2) refers to various Acts of Parliament, he makes no mention of the Safety and Health at Work etc. Act which received the Royal Assent on July 31. Clauses 2(2)(b) and 3(1) of the Act place a general duty on employers to protect both their workers and other persons from risks to health in connection with the use, handling, storage and transport of articles and substances; and under Clauses 15 and 16, the Secretary of State may make Regulations, or the Health and Safety Commission may prepare and issue Codes of Practice (or approve Codes of Practice prepared by others) for any of the general purposes of the Act. I would therefore suggest that no further legislation is necessary and that the way is clear for agreed Codes of Practice to be promulgated to deal with the different groups of organisms, as Brian Ford suggests.

Yours faithfully,

J. A. TANNAHILL

Employment Medical Advisory Service,
Department of Employment,
London, UK

Population policy study

SIR,—I should like to bring to the attention of readers of *Nature* a survey of the field of reproductive biology and contraceptive development sponsored by the Ford Foundation. The Foundation has asked for an inventory of where we stand in terms of (1) knowledge concerning the reproductive process and fertility control and (2) the human and financial resources that are being brought to bear on the matter of bringing population growth

within tolerable limits. For the conduct of this study, a headquarters has been established in Boston with a worldwide network of collaborators and consultants and an international advisory committee drawn from public and private donor agencies, universities and the pharmaceutical industry.

The force that motivated this major study is the accumulating evidence that an inadequate global effort is being waged in this field. The illusion is that the pressure of more immediate bio-social problems supersedes the need to control human fertility. The facts are otherwise.

The survey was initiated in July 1973 and is expected to be completed in 1975. The findings will be published in full on completion of the survey and as interim reports on special topics.

The comments, views and opinions by readers of *Nature* concerning any or all aspects of this survey will be welcome and helpful in drafting recommendations and guidelines for building on present progress in the understanding of reproductive phenomena and stabilisation of the global populace on a level commensurate with a high quality of life and human dignity.

Yours faithfully,

ROY O. GREEP

Laboratory of Human Reproduction
and Reproductive Biology,
45 Shattuck Street,
Boston, Massachusetts 02115

Astronomical independence

SIR,—I write to correct John Gribbir who, in discussing (*Nature* 250, 538 1974) the thousandth issue of *The Observatory*, wrongly describes it as the house journal of the Royal Astronomical Society. His mistake is understandable since *The Observatory* does by long tradition, report the proceedings of the Society's meetings and is circulated to all Fellows of the Society. It is, however, published by 'The Editors of "The Observatory"', as is clearly stated in each issue, and has no official connection with the Society. The Editors, indeed, fiercely maintain their independence.

Our house journal is *The Quarterly Journal of the Royal Astronomical Society*.

Yours faithfully,

JOHN SHAKESHAFT

Royal Astronomical Society,
London

news and views

Giant extragalactic radio sources

MANY of the powerful extragalactic radio sources have dimensions much larger than those of the active galaxies which have supplied the vast energies required. In the case of the brightest radio source for example, Cygnus A, most of the radio emission originates, not in the galaxy associated with the source, but in two symmetrically disposed regions, one on either side of it. The peaks of maximum radio brightness are located about 100 kiloparsecs (equivalent to 3×10^5 light years) from the galaxy, the radius of which is only about 10 kpc. Such objects are the most common type of powerful extragalactic radio source found in source surveys although, as compared with the space density of ordinary galaxies, they are very rare indeed.

Detailed radio studies of the structures of these objects have been made possible by the development of the large aperture synthesis telescopes, first at Cambridge and more recently in The Netherlands (the Westerbork Synthesis Radio Telescope, WSRT). Several hundred of the brighter ones have been mapped with an angular resolution of $23''$ and a smaller number with higher angular resolution, that of the Cambridge 5 km telescope being $2''$ at a wavelength of 6 cm. There is no detailed source model which commands universal acceptance since the radio maps have revealed difficulties with each of the models proposed. All models, however, postulate the presence of magnetic fields within the components to produce the synchrotron radio emission, and of cold intergalactic gas in the vicinity of the sources to account for the complex radio structures observed.

Until now the maximum overall dimensions of these radio sources have been thought to be about 1 Mpc, which is the characteristic scale of clusters of galaxies rather than of galaxies themselves. Many radio sources are known to lie close to the centre of clusters of galaxies and for a while it has looked as if there might be a relationship between these two scales particularly since there is good evidence, from X-ray observations, for the presence of hot diffuse gas in clusters. If this relationship were true, however, it would be disappointing from the cosmological point of view because one might have hoped to use the radio components of the largest double radio sources as probes of the diffuse intercluster gas where most of the mass of the Universe may well reside.

On page 625 of this issue of *Nature*, Willis, Strom and Wilson report very interesting observations made with the WSRT and show that radio sources with physical sizes greater than 1 Mpc do indeed exist: the radio sources 3C236 and DA240 are extended, with total physical sizes, projected on to the celestial sphere, of 5.7 and 2.0 Mpc respectively. Credit for drawing attention to these objects goes to workers at the National Radio Astronomy Observatory (Bridle, Davis, Fomalont and Lequeux, *Astr. J.*, **77**, 405; 1972) who, in a definitive survey of bright radio sources at 1,420 MHz, found them to lie in "confused" regions and were unable to decide whether the radio sources in close proximity on the sky were physically related or simply chance associations of unrelated objects. In the first two cases studied by Willis, Strom and Wilson, the maps

show unambiguously that these sources are in fact associated and form components of extended double radio sources on a scale much larger than has been known before.

These observations are important for cosmologists, cosmic ray physicists and for the student of the physics of extragalactic radio sources. For the cosmologist there is the prospect of probing the intercluster gas and setting direct limits to the pressure of such gas. For the cosmic ray physicist, there is now the direct evidence that clouds of cosmic ray electrons can be ejected beyond the confines of a cluster of galaxies in times much shorter than cosmological time scales.

For the radio source theorist, these objects stimulate new speculations. First, nobody has ever been clear about the ultimate fate of a powerful double radio source. The present observations suggest that in some cases the components expand, preserving the relativistic particles within their lobes, until very late stages in their evolution. Second, the observation of polarised radio emission from the components of DA240 indicates surprisingly low densities of cold matter inside the components. Third, and perhaps most interesting, there is the observation of a compact double radio source associated with the parent galaxy in the case of 3C236. It is usually assumed that these components are moving away from the nucleus of the galaxy in opposite directions. The axis of this compact double is almost perfectly aligned with the axis of the giant double source components. On any theory of the extensive components, the latter must be able to 'remember' for hundreds of millions of years the direction in which to supply energy. Similar phenomena have been known before—in the radio galaxies Centaurus A and 3C66 for example—but 3C236 is probably the clearest case yet. Observations of Cygnus A forced Hargrave and Ryle to conclude that a continuous supply of energy over tens of millions of years is essential if one is to account for the structure of the components, but present observations are evidence that a continuous supply of energy may occur for even longer, in the oldest sources. These new observations, and others in progress at Westerbork, will therefore be seized upon by cosmologists and cosmic ray physicists, and will provide yet another link in the chain of our understanding of the physical evolution of extragalactic radio sources.

M. S. LONGAIR

Organisation of sequences of rRNA

THAT the sequences of both 28S rRNA of the large ribosome subunit and 18S rRNA of the small subunit are contained in a single precursor has been known for some time; for this constitutes one of the rare cases where a synthetic system was defined first with eukaryotic and only later with prokaryotic cells. The size of the precursor varies; in HeLa cells it sediments at 45S, so that only 56% is conserved in the form of mature rRNA sequences, but in *Xenopus* it is smaller (2.7×10^6 daltons instead of the mammalian 4.1×10^6 daltons) and almost 80% is conserved. The processing

pathway is best defined in HeLa cells. By utilising an electrophoretic analysis of nucleolar RNAs, Weinberg and Penman (*J. molec. Biol.*, **47**, 169–178; 1970) found that the 45S primary transcript is cleaved to a 41S molecule, which in turn is split into a 32S and a 20S RNA; the 32S molecule then matures into 28S rRNA and the 20S molecule matures into 18S rRNA. By analysing the oligonucleotides into which these species are broken by ribonuclease, Maden, Salim and Summers (*Nature new Biol.*, **237**, 5–9; 1972) confirmed that the 41S precursor contains both 28S and 18S sequences and that the 32S and 20S precursors respectively contain sequences of only the 28S and 18S rRNA molecules. Because oligonucleotides were identified by their content of labelled methyl groups, these experiments showed also that all the methyl groups added to 45S RNA are located in regions of the molecule destined to become mature rRNAs.

The identification by Seeber and Busch (*J. biol. Chem.*, **246**, 7151–7158; 1971) of common 5' terminal sequences in 45S, 32S and 28S RNAs of Novikoff hepatoma cells showed that the 28S rRNA sequences is located at this end of the precursors which contain it. The elegant analysis of RNA secondary structures reported by Wellauer and Dawid (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2827–2831; 1973) achieved a more precise location of ribosomal RNA sequences in the precursors. The 45S precursor contains a 5' terminal structure corresponding to 28S rRNA, with the 18S rRNA located within the remaining sequences; the 41S RNA structure is similar but lacks all the material on the 3' side of the 18S sequence. The cleavage that generates 32S and 20S precursors must take place within the central non-rRNA region, since 32S RNA has 5' terminal 28S sequence and 3' terminal non-rRNA sequence; and 20S RNA has a 5' non-

rRNA sequence with 18S rRNA located 3' terminally.

Each ribosome contains one 5S rRNA molecule in addition to the major RNAs and this seems to be coded by genes dispersed in the genome whose transcription is independent of that of the main precursor. The presence of another small RNA was first revealed by Pene, Knight and Darnell (*J. molec. Biol.*, **33**, 609–623; 1968), when they found that denaturing treatments release a small fragment from 28S rRNA. Originally characterised as 7S RNA, this molecule must be covalently linked to 28S rRNA, and more accurate characterisation has led to its recent description as 5.8S RNA. Synthesis of 5.8S RNA seems to be linked to that of the large rRNAs—kinetic experiments suggest its derivation by cleavage from the 32S precursor in HeLa cells. The sequence of the HeLa 5.8S RNA has now been examined by Maden and Robertson (*J. molec. Biol.*, **87**, 227–236; 1974), who have compared the oligonucleotides released by T1 ribonuclease with those cleaved from the rRNA precursors. When 28S rRNA is prepared by cold phenol extraction, it retains the 5.8S fragment; but heat treatment separates 28S rRNA from its small attached molecule. Correspondingly, upon T1 ribonuclease analysis, these two preparations of 28S RNA differ in the oligonucleotides characteristic of 5.8S RNA. One equivalent of the 5.8S RNA sequences is present for every equivalent of 28S RNA.

Fingerprints of the 32S RNA precursor show that it possesses the oligonucleotides constituting 5.8S RNA, again in molar yields which suggest the presence of one 5.8S RNA sequence in each precursor molecule. Some evidence for the presence of these fragments in 45S RNA is also presented. Although not yet precisely placed within the precursors, the 155 nucleotide long 5.8S RNA presumably lies between

Multifunctional gene in a eukaryote

ON page 630 of this issue of *Nature* Bollon presents a genetical analysis of a "multifunctional" eukaryotic gene, threonine deaminase, concerned with both structure and regulation.

Working with the yeast, *Saccharomyces cerevisiae*, Bollon found that the *ilv1* gene not only encodes for a gene product that is catalytically active (that is, L-threonine deaminase; EC4.2.1.16) but also that the gene product is involved in multivalent repression of the other isoleucine-valine enzymes. It has thus been possible, by careful genetical analysis, to discriminate between the nucleotide sequences that encode for that structural (=catalytic) and regulatory (=multivalent repression) functions of the *ilv1* gene. The advantage of using the yeast system, for studying regulatory phenomena, is that yeasts lend themselves well for intragenic complementation. Using many different *ilv1* mutants—impaired in threonine deaminase activity—intragenic complementation and fine structure analyses have been carried out.

Goldberger (*Science*, **183**, 810–816; 1974); Levinthal *et al.* (*Nature new Biol.*, **246**, 65–68; 1973) and Kasai (*Nature*, **249**, 523–527; 1974) have provided excellent background information about gene regulation. The first suggestion of an enzyme participating in its own regulation—by repression—was made by Vogel (in *The Chemical Basis of Heredity*, edit. by McElroy and Glass, Johns Hopkins Press, Baltimore 1957, page 276); it is also pertinent to note that the yeast system developed by Bollon and Magee (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2169–2173; 1971; *J. Bact.*, **113**, 1333–1344; 1973) offered the first evidence *in vivo* for the regulatory role of threonine deaminase, or for some form of the

ilv1 gene as Bollon shows in this issue. Of course, Umbarger and his many colleagues, as well as Hatfield's group, have provided a vivid picture of the role of *ilvA* (which specifies threonine deaminase) in multivalent repression in bacteria.

Although the *ilv1* gene product in yeast seems to be involved in multivalent repression, other elements are involved too. As already mentioned, Bollon and Magee noted that the involvement of leucine in multivalent repression may be unique (compare Levinthal *et al.*, 1973) and they suggest that leucine may function in repression by way of leucyl-tRNA; thus the *ilv1* gene product may be only one component of the multivalent machinery. One vital feature of the yeast which may have been overlooked is that the genes specifying its various isoleucine-valine enzymes are found on different chromosomes (compare *ilvADE*, *ilvB* and *ilvC* in *Escherichia coli*).

The principal points of Bollon's work can be summarised as follows:

- A multifunctional gene (*ilv1*) has been analysed in detail in a eukaryotic organism for the first time.
- The *ilv1* product, threonine deaminase, catalyses the conversion of L-threonine → α -ketobutyrate in yeast.
- The regulatory role of the *ilv1* gene product is considered a positive effector for the derepression of the isoleucine-valine enzymes.
- Thus *ilv1* is truly 'multifunctional' with catalytic and regulatory parts being played by threonine deaminase.
- Intracistronic discrimination denoting the catalytic and regulatory functions of *ilv1* is presented.

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the 28S and the 18S sequences, and the 5' side of the point where cleavage takes place later to generate 32S RNA. The single methyl group present in 5.8S RNA is also present in 32S and 45S precursor RNAs; this is consistent with the idea that methylation is confined to rRNA sequences in the precursors. Furthermore, two other sites of modification contain pseudouridine; it is not yet confirmed that the modification is introduced into the precursor, although this seems likely. Modification at one of these sites may be incomplete. Future determination of whether the 5.8S sequence is contiguous with the 28S sequence in the precursor, or whether there is a space between them corresponding to a non-rRNA sequence, may help to decide upon models for processing and the possible implication of secondary structures or particular nucleotide sequences. Whether the 5.8S RNA sequence is noncovalently bonded to the 28S sequence in the precursor or takes up this association after maturation is another relevant question.

Another approach to defining the organisation and synthesis of RNA sequences is to describe their location in the genome. By hybridising *Xenopus* DNA with 5.8S RNA, Speirs and Birnsteil (*J. molec. Biol.*, **87**, 237-256; 1974) found that the relationship of 5.8S to 28S and 18S sequences seems to be similar to that in HeLa cells. When *Xenopus* DNA is fractionated, the rDNA containing the sequences coding for 28S and 18S rRNAs can be separated from the bulk of the genome by its distinct content of G-C base pairs, a technique used some time ago by Brown and Weber (*J. molec. Biol.*, **34**, 681-698; 1968) and Birnsteil *et al.* (*Nature*, **219**, 454-463; 1968) to show that 28S and 18S sequences alternate and each pair is separated from the next by a spacer. Speirs and Birnsteil find that the fraction of a density gradient hybridising with 5.8S forms a satellite with the density characteristic of rDNA. The conclusion that 5.8S coding sequences are interspersed with 28S and 18S sequences is suggested also by their observation that the nucleolate mutant of *Xenopus* (which lacks both the 28S and 18S genes) does not synthesise 5.8S RNA; nor does 5.8S RNA hybridise with DNA extracted from mutant cells.

Because DNA-RNA hybrids have a buoyant density different from that of denatured DNA, the extent of the density shift when DNA fragments are annealed with rRNA reveals the proportion of the fragment complementary to RNA. And in linkage experiments, the DNA hybridising with one rRNA can be tested for its ability to hybridise with the other. By using DNA fragments of different lengths, it is possible to follow the organisation

of sequences in DNA that code for these RNAs. Extending the previous experiments of this nature with 28S and 18S rRNA to 5.8S RNA, Speirs and Birnsteil observed a small density shift when DNA fragments of 8 and 3 times its length are hybridised with 5.8S RNA—the low density shift implies that 5.8S coding sequences are interspersed with other sequences rather than clustered together. Linkage experiments show that prehybridisation with 28S or 18S rRNA induces a large shift in the density of the fragments hybridising with 5.8S RNA; 18S rRNA causes a smaller shift than 28S rRNA, so that the 5.8S sequence must be closely linked to the 28S sequence and less closely related to the 18S sequence. Taken together with the analysis of HeLa rRNA precursors, these results suggest that the 5.8S sequence lies between the 28S and 18S sequences in the genome. The model which Speirs and Birnsteil present for the rRNA sequences in the genome postulates a small separation between the 3' end of the 28S sequence and the 5' end of the 5.8S sequence, with another separation between the 5.8S and 18S sequences.

The role of the 'transcribed spacer' regions which separate the sequences constituting the mature rRNAs is not clear; it is tempting to speculate, however, that it is concerned with folding of the rRNA regions or their interactions with proteins during maturation. Perhaps against this model is the observation that the mature RNA sequences are very similar in length in, for example, *Xenopus* and HeLa cell, but the nontranscribed spacer lengths are very different. And in *Escherichia coli*, although the bacterial precursors are only slightly larger than the mature RNA molecules, ribosome assembly seems to take place perfectly well with isolated 23S and 16S rRNAs. A second type of spacer, the 'nontranscribed spacer', separates the precursor-coding sequences in nucleolar DNA and its function also is obscure, although recent experiments encourage the speculation that these sequences may in some way be concerned with the maintenance of identity in all the repeated rRNA sequences in the genome.

BENJAMIN LEWIN

New 30S ribosomal assembly map

from a Correspondent

THE updated version of Nomura's *Escherichia coli* 30S ribosomal "assembly map" (Held, Ballou, Mizushima and Nomura, *J. biol. Chem.*, **249**, 3103; 1974) raises two interesting points. First, of course, the additions to the map are themselves important, particu-

larly the inclusion of protein S12, which was absent from the original map (Mizushima and Nomura, *Nature*, **226**, 1214; 1970) and which is now receiving more and more attention (see for review Pongs, Nierhaus, Erdmann and Wittmann, *FEBS Lett.*, **40** Suppl., 28; 1974). The role of S12 in the streptomycin effect has been widely investigated, and although mutations in S12 confer streptomycin resistance (see, for example, Ozaki, Mizushima and Nomura, *Nature*, **222**, 333; 1969), it seems that proteins S3 and S5 are the ones which actually bind the antibiotic (Schreiner and Nierhaus, *J. molec. Biol.*, **81**, 71; 1973). Furthermore, revertant mutants from streptomycin dependence to independence show alterations in proteins S4 and S5 (Hasenbank, Guthrie, Stöffler, Wittmann, Rosen and Apirion, *Molec. gen. Genet.*, **127**, 1; 1973). This suggests a strong relationship between proteins S5 and S12, and it is therefore gratifying to find an interaction between these two proteins in the new assembly map.

Second, there is the question of which proteins are RNA-binding. The evidence presented by Held *et al.* that S17 (which now incidentally has become a separate entity from S16, the two proteins being treated as one in the original assembly map) can specifically bind to 16S RNA under reconstitution conditions is convincing. But in view of the controversy (discussed in detail by Held *et al.*) which has occurred over this protein and also S13, it is perhaps worthwhile to consider precisely what the term 'RNA-binding protein' means. The term normally defines proteins which can bind singly to ribosomal RNA under reconstitution conditions. The reconstitution buffer is necessary to obtain a specific interaction, but it must be remembered that this is a high salt buffer which reduces electrostatic interactions. This has the effect not only of preventing non-specific interactions, but also of weakening the specific RNA-protein interaction. Held *et al.* say that "it is conceivable that proteins other than the initial binding proteins also interact with 16S RNA in the finished ribosome structure". Perhaps it would be more appropriate to say that (in a particle which is two-thirds RNA, and whose proteins are almost all basic) it is almost inconceivable that other proteins do not interact with the RNA in the final structure.

This has of course been suggested already (see, for example, Lutter, Bode, Kurland and Stöffler, *Molec. gen. Genet.*, **129**, 167; 1974), although few people would go as far as Cox and Bonanou (*Biochem. J.*, **114**, 769; 1969) in arranging all the proteins in neat rows with identical RNA environments. It seems likely that the conclusion will be that in physiological salt conditions

the proteins have a widely varied but positive interaction with the RNA, with no sharp distinction between "binding" and "non-binding" proteins; the very existence of the controversy over the specificity of S17 and S13 binding supports this view.

Organic pollutants in the sea

from a Correspondent

THE requirements of industry and of agricultural practice have in recent years greatly enlarged the search for and use of a variety of classes of organic compounds which in large quantities eventually reach the sea. The nature, origin, dispersal persistence and fate of some of the more commonly occurring pollutants including halogenated hydrocarbons, organic heavy metal compounds and crude oils were reviewed at a discussion meeting organised by the Royal Society on July 4-5.

N. S. Thom and A. R. Agg (Water Research Centre, Stevenage) cited more than 200 substances believed to be significant pollutants of water in the United Kingdom, and C. R. Pearson and G. D. McConnell (ICI Ltd, Brixham and Runcorn) reviewed a range of industrial hydrocarbons in widespread use. It seemed from the papers of E. D. Goldberg (Scripps Institution, La Jolla) and J. E. Portmann (Fisheries Laboratory, Burnham-on-Crouch) that although the input of both DDT and dieldrin into the oceans of the northern hemisphere may have passed its peak, the output is increasing in the equatorial regions and southern hemisphere and world production of organochlorine pesticides is increasing. Although, as Pearson and McConnell pointed out, the many kinds of chlorinated hydrocarbons derived from C_1 and C_2 hydrocarbons used as intermediates in further manufacture or as solvents and carriers are not unexpectedly dispersed to the sea substantially through the atmosphere, heavier synthetic halogenated hydrocarbons such as PCBs and DDT residues may similarly be transported as gas molecules or adsorbed on airborne dust. Goldberg and Portmann each suggested a 25% transference to the oceans by these means.

Within the sea persistence of organic pollutants varies greatly according to their chemical stability, susceptibility to biodegradation and the routes and terminal situations of their passage. Half lives varying from a few hours (for example, CCl_4) to days or weeks (EDC tars deriving from vinyl chloride production) to years (for example, PCBs, DDT, aromatic compounds of high carbon number) were cited by S. Jen-

sen (Wallenberg Laboratory, Stockholm), R. Lange (Odense University) and E. D. S. Corner (Marine Biological Association, Plymouth) among other speakers as examples of varying persistence.

G. Eglinton (University of Bristol) showed a film demonstrating a newly developed computerised gas chromatography-mass spectrometry system used for the determination and assay of pollutants in coastal muds, a system which may prove to be useful also in determining the natural occurrence of organic compounds in muds deposited in pre-industrial times.

The uptake of organic pollutants by marine organisms and their levels of occurrence and sites of accumulation were discussed by several speakers. Their differential absorption by lipids was stressed and Portmann made the point that although many measurements had been made of the concentrations of the more persistent compounds in a variety of organisms of the higher trophic levels, little was known of transfers at lower trophic levels, for example in the grazing of phytoplankton by zooplanktons. Corner gave an account of work in progress on the utilisation, transformation and breakdown of fossil fuel hydrocarbons by marine crustaceans, a subject of particular interest in studies of the effects of oil spills. D. E. Hughes and P. McKenzie (University College, Cardiff) reported laboratory and field experiments indicating that about 40-90% of oil may be degraded by microbial attack; alkanes and other saturated compounds are more readily degraded than aromatic and heterocyclic compounds. A. J. Van Benekom (Netherlands Institute for Sea Research, Texel) reported on some consequences of discharges from the Rhine on the chemistry and plankton production of coastal water of the Southern Bight of the North Sea. Diatom growth was at times inhibited and the general level of production was unusually low and subject to irregular peaks. Blooms of *Phaeocystis* and possibly other toxic flagellates seemed to be favoured by these conditions.

New model of lunar interior

from Peter J. Smith

ACCORDING to Toksöz *et al.* (*Science*, **176**, 1012; 1972), the Moon has a 50-60 km thick crust in the eastern part of Oceanus Procellarum; and Nakamura *et al.* (*Science*, **181**, 49; 1973) concluded that the deep lunar interior below a depth of about 1,000 km is probably partially molten. But what is the state of the Moon between these

two extremes, in the mantle which accounts for the major part of the lunar volume? The complete answer to this question is not yet available, although Nakamura *et al.* (*Geophys. Res. Lett.*, **1**, 137; 1974) have now moved some way towards it in reporting new seismic data from the four Apollo seismic stations still in operation.

Nakamura and his colleagues have attempted to determine both P and S wave velocities throughout the lunar mantle from large meteoroid impacts, high frequency teleseismic events and deep moonquakes. The observed P wave velocity shows a systematic variation with epicentral distance, indicating that the subcrustal region cannot have a constant velocity. For example, P wave arrival times at distances greater than 70° are delayed by 10 s with respect to those expected from data at shorter ranges—an offset which could arise from a negative velocity gradient in the upper few hundred kilometres of the mantle, from a small stepwise decrease in velocity at a depth of the few hundred kilometres, or from a combination of the two.

For a negative velocity gradient, the travel-time curve would be continuous between 50° and 70°; and on this assumption, Nakamura *et al.* calculate that the P wave velocity would decrease from about 8.1 km s⁻¹ at the top of the mantle to about 7.8 km s⁻¹ at a depth of about 500 km, followed by a slight increase. For a stepwise decrease, the travel-time curve would be discontinuous with a shadow zone somewhere between 50° and 70°; and calculations put the minimum depth of the step at 200-300 km and its velocity contrast at 0.3 km s⁻¹. For a combination situation, the velocity gradient and velocity contrast would be smaller than those I have given. Unfortunately, the available data are too uncertain to enable a decision to be made about which of the three cases actually obtains, although Nakamura and his colleagues seem to favour the combination.

More certain (though based on a single data point) is a very large delay in P wave arrival at 168° which, if subsequently confirmed, would suggest that the Moon has a low velocity core. The present data put a limit of 170-360 km on the radius of such a core and a limit of 3.7-5.1 km s⁻¹ on its P wave velocity. Also conspicuous is the near linearity of the S wave travel-time curve beyond distances of 90°, indicating a large, and apparently increasing, delay of S wave arrivals relative to P wave arrivals at greater distances. Possible explanations involve one or more of the following effects: refraction of waves by a large negative velocity gradient at about 300 km depth, diffraction of waves around a discontinuity at this depth, or penetration of

waves into a lower velocity zone below. In fact, calculations suggest that the S wave velocity decreases from about 4.7 km s^{-1} at the top of the mantle to about 4.4 km s^{-1} at 300 km, followed by a more rapid decrease to a value of $3.8 \pm 0.2 \text{ km s}^{-1}$. Below 800 km, S waves are highly attenuated.

From these and other results, the most up-to-date picture of the lunar interior is then as follows:

- Zone 1: crust. This uppermost zone is 50–60 km thick in the region of the Apollo 12 and 14 stations and has seismic velocities consistent with plagioclase-rich material. The top few hundred metres is highly pulverised.
- Zone 2: upper mantle. This zone is about 250 km thick and has a P wave velocity of about 8.1 km s^{-1} which probably decreases slightly with depth. The seismic data are consistent with mineral assemblages comprising olivines (mostly) and pyroxenes (partly). The temperature gradient at the top of this zone is calculated to be $2\text{--}5^\circ \text{C km}^{-1}$.
- Zone 3: middle mantle. This is a zone with reduced S wave velocity and extends from 300 km to 800 km. Deep moonquakes occur at the base of it.
- Zone 4: lower mantle. This zone below 800 km is marked by high S wave attenuation and is possibly partially molten (as the terrestrial asthenosphere).
- Zone 5: core. This zone has a radius of 170–360 km and is marked by a greatly reduced P wave velocity. Molten?

Microclimate of open shade habitat

from Peter D. Moore

EXTREME habitats and the morphological and physiological adaptations necessary for life within them have always been more popular topics for study than have moderate conditions. It is becoming apparent, however, that immediate habitats, or ecotones, often have interesting attributes which make them worthy of special attention. An example of such a situation is provided by the study of open and shaded habitats.

For many years it has been conventional to classify plants into sun-demanding (heliophytes) and shade-requiring (sciophytes), and a great deal of research has been carried out both upon the physiological adaptation of these plants (for example, Björkman and Holmgren, *Physiologia Pl.*, **16**, 889; 1963; Björkman, *ibid.*, **19**, 854; 1966) and upon the light climates of shade habitats (for example, Anderson, in *Plant Photosynthetic Production*, edit. by Sestak, Catsky and Jarvis, Junk, The Hague, 1971). Little atten-

tion, however, has been given to intermediate situations, which can be termed 'open shade'.

A study of the open shade habitat from the microclimatic point of view has now been made by Stoutjesdijk (*Acta bot. neerl.*, **23**, 125; 1974). Surface temperatures on the north side of a patch of hawthorn (*Crataegus monogyna*) scrub in a Dutch sand-dune system were found to be as much as 10°C below those of ambient air. Although surfaces temperatures above ambient are commonplace, to find surfaces considerably below ambient at midday is extremely unusual and can only be explained in terms of the overall energy balance in these situations. At 11.30 h on a bright day in September, Stoutjesdijk found the surface temperature to be 9.1°C whereas ambient air was 15°C . Incoming radiant energy was in the form of diffuse solar radiation ($0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$) and long wave radiation from the sky ($0.395 \text{ cal cm}^{-2} \text{ min}^{-1}$). Radiant energy loss was accounted for by reflection ($0.01 \text{ cal cm}^{-2} \text{ min}^{-1}$) and by long wave radiation ($0.523 \text{ cal cm}^{-2} \text{ min}^{-1}$). The net outcome of this situation is negative energy balance of $-0.038 \text{ cal cm}^{-2} \text{ min}^{-1}$, this being largely the result of the high long-wave radiation losses. The negative radiation budget is rather weak, however, and cannot fully account for the low surface temperature. The true energy budget is more strongly negative because of latent heat losses from the surface during the evaporation of dew; this explains the low surface temperatures.

This type of energy balance situation can occur only in open shade, for only here are long-wave losses large in comparison with gains and evaporation can take place at a rate sufficient to maintain high latent heat losses. Surface temperatures were found to be below ambient in daytime throughout the year, but differences were least in June and July (shading effects diminished) and greatest in late summer (strong shading and considerable dew formation).

Some work has been carried out on the influences of leaf canopies upon the spectral quality of light, but most of this has been concentrated on fairly dense canopies (see, for example, Federer and Tanner, *Ecology*, **47**, 555; 1966; Daynard, *Can. J. Bot.*, **47**, 1989; 1969; *Acta bot. neerl.*, **21**, 185; 1972). These studies have shown that the rate of visible to far-red radiation decreases considerably as shading increases. Measurements by Stoutjesdijk in his open shade habitat showed that there was a far better penetration of photosynthetically usable light ($<700 \text{ nm}$) than in the deep shade beneath hawthorn scrub.

Open shade habitats, then, provide

an interesting combination of physical conditions—low daytime temperatures (with consequent high relative humidities) and moderate photosynthetically-effective light levels. Undoubtedly some plant species characteristic of open shade habitats, such as, for example, *Mnium undulatum*, *Rhytidiadelphus triquetrus*, find such conditions to their liking. Some anomalous plant distributions may also be explicable in terms of this unusual combination of microclimatic factors. For example, it may be that the steep, north facing scarp slopes of the South Downs provide a similar microclimatic habitat to Stoutjesdijk's 'open shade'. In which case it might explain the persistence in such situations of montane bryophyte species, such as *Racomitrium lanuginosum*, which are known to require high humidities, fairly low temperatures and moderately high light intensities for their survival.

Nature of Hoag's object

by John Gribbin

It is now nearly a quarter of a century since Hoag discovered a remarkable astronomical object which appeared to be a "perfect halo" surrounding a diffuse nucleus. But only now is Hoag's object, as it has come to be known, receiving the attention which its peculiarity merits. According to O'Connell, Scargle and Sargent (*Astrophys. J.*, **191**, 61–62; 1974) the object is certainly worth investigating and "one of the more exotic possible interpretations" is that the ring is the image of a background galaxy produced by a gravitational lens effect.

Hoag's object is clearly visible on *Sky Survey* prints (at $\alpha = 15 \text{ h } 15.0 \text{ min}$; $\delta = +21^\circ 46'$) but it has not previously been studied in detail. The core of the object is a fuzzy circle $6''$ in diameter, and the surrounding blue ring or annulus has inner diameter $28''$ and outer diameter $45''$ with an axial ratio of 0.93 ± 0.05 . The ring is structureless, and there is no sign of connecting material bridging the gap between core and annulus.

In the study now reported by O'Connell *et al.* new information has been obtained from spectrograms taken with the 200-inch telescope and the Lick 120-inch. The observed redshift of $12,740 \text{ km s}^{-1}$ and an assumed Hubble constant of $75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ imply a distance of 170 Mpc, corresponding to a linear diameter of 5 kpc for the core and inner and outer radii of 23 kpc and 37 kpc for the ring.

Almost all of the properties of the core seem to be in line with those expected for compact galaxies, but there seems little doubt that the ring is "an

independent structural component . . . and not simply the unobscured rim of a normal SO disk". Several other similar systems are known, and O'Connell *et al.* cite the examples of NGC6028, NGC2859 and IC5285. "There is no reason to suppose that radiation from these rings is anything other than direct starlight", and in the case of Hoag's object the radiation certainly cannot be reflected light from the core, since the ring is "at least" as luminous as the core.

The interpretation which seems to be favoured by O'Connell *et al.* involves gravitational encounters between galaxies which could initiate star formation in "trapped" rings; they point out that a pair of galaxies near Hoag's object on the sky could, if they are at the same distance as the object, have passed by it 10^8 to 10^9 yr ago, just right to account for the apparent age of the stars in the ring.

Merokeratins

from Peter Speakman

WOOL is a mixture of about 100 different proteins, but Haylett's group in South Africa have been able to purify several proteins with a high cysteine content and to show homologies in their amino acid sequences. Argument is now over whether these proteins have evolved from a repeating penta- or deca-peptide (Swart and Parris, *Nature*, **249**, 580; 1974). Lindley and Cranston have pointed out that because of the heterogeneity of proteins a new experimental approach is needed to supplement sequence studies in order to study the pattern of disulphide cross-links between proteins as they are arranged together in the fibre (*Biochem. J.*, **139**, 515; 1974).

Protein fragments which are very similar in molecular weight, dimensions and α -helix content have been prepared from mammalian epidermal keratin and from reduced wool by partial proteolysis, the method which was used to prepare the meromyosins and more recently, myosin subfragments 1 and 2. A sharp low-angle X-ray diffraction pattern from oriented films of the wool fragment showed that it could aggregate in a specific way into long, thin assemblies. These fragments from epidermis and reduced wool may be in the native conformation; and so studies of their internal structure and dimensions in solution and investigations of their specific aggregates, are beginning to give information about the conformation of the protein chains within the keratin macromolecular components, and about the mutual positions of the macromolecules in the fibre or tissue. Analogous studies of dissolved muscle proteins and their modes of aggrega-

tion, and of tropocollagen and its polymorphic aggregates have contributed to a very detailed understanding of muscle and connective tissue structure.

The small-angle X-ray diffraction pattern published from the CSIRO Division of Protein Chemistry in Melbourne shows that a wool keratin fragment can be made to form specific aggregates which are about 200 Å in diameter and greater than 2,000 Å long, with a longitudinal repeat of 160 Å (Suzuki *et al.*, *J. molec. Biol.*, **73**, 275; 1973). The method of preparing the fragment is interesting and relevant. Wool proteins are dissolved by reduction in a solution of a denaturing agent (8M urea). The thiol groups are blocked by reaction with iodoacetate ions, and the urea is removed by dialysis. The proteins (SCMKA) with a smaller proportion of cysteine residues and a higher α -helix content than the original wool are separated by fractional precipitation and purified by chromatography.

It is clear that some refolding of the SCMKA proteins, into conformations which are not completely flexible, takes place when the urea is removed, because a fairly large protein fragment (as well as small peptides) is produced when the SCMKA proteins are treated briefly with chymotrypsin. The fragment has a molecular weight of 41,000, a length of 170–200 Å measured in solution or 160 Å in the electron microscope, and a diameter of 20 Å. It has a higher α -helix content (80%) than the original SCMKA proteins (50%) and it is believed to consist of three chains (Crewther and Harrap, *J. biol. Chem.*, **242**, 4310; 1967; Dobb *et al.*, *J. Textile Inst.*, **64**, 374; 1973). A similar wool fragment has been prepared without using a denaturing agent (Hilburn *et al.*, *Biochim. biophys. Acta*, **214**, 245; 1970; Dilley *et al.*, Abstracts, British Biophysical Society Meeting, Leeds, April 1971). If wool is reduced at pH 10 (which itself may cause some denaturation) and treated with trypsin, a rod-shaped fragment, 200 Å long and 20 Å wide, molecular weight 55,000, with a higher α -helix content (64%) and a lower cysteine content than the original wool, can be separated from the digest.

An analogous fragment prepared by partial proteolysis of epidermal keratin protein is reported from Matoltz's laboratory (Skerrow *et al.*, *J. biol. Chem.*, **248**, 4820; 1973). It is not necessary to reduce disulphide crosslinks in order to extract protein from mammalian epidermis but a high or low pH is needed (in this case pH 2.6), which may again, cause some denaturation. The solution of epidermal protein in acid buffer is added dropwise to a trypsin solution maintained at pH 8.8, and a protein fragment can be separated from the digest, length 200 Å, molecular weight 46,000, which is 83% α -helical.

Polyacrylamide gel electrophoresis in denaturing conditions showed that the fragment consisted of three chains with approximately similar molecular weights.

The protein chains of SCMKA were denatured during the preparation, and therefore information about the structure of wool can only be inferred from the structure of the SCMKA proteolytic fragment, and from the mutual arrangement of the fragments in the specific aggregate, if partial or exact renaturation occurred when the denaturant was removed. Similarly, the other wool fragment and the epidermal protein fragment have been exposed to pH values which might cause denaturation. On the other hand, the similarity between the three fragments strongly suggests that undenatured or accurately renatured parts of the keratin structure have been prepared in these experiments. Their compositions and α -helix contents imply that they originate in the wool protofibril and the epidermal protofilament, but the low yield in each preparation (for example, less than 10% in the case of the wool fragment prepared without a denaturing agent) means that they may not represent the whole of these structures.

This work will encourage attempts to dissolve native proteins from epidermis or reduced wool without the use of proteolytic enzymes or denaturing agents, and the study of their structure in solution. Fairly extreme conditions of pH or temperature dissolve large quantities of keratin proteins but they may cause denaturation. Milder conditions are less likely to cause denaturation, but smaller amounts of protein dissolve and it may not be clear whether they are minor components or partly soluble major components. In both cases, even if the protein has a definite conformation in solution this will not necessarily be identical to the conformation adopted by the same protein in the fibre or tissue. A similar problem arose in collagen research in the 1950s. Acid or neutral buffer solutions will only dissolve small amounts of tropocollagen from calf skin, for example, but it was possible to show that the conformation of the protein chains in dissolved tropocollagen is the same as in intact connective tissue. The length of the tropocollagen molecule measured in solution was shown to be consistent with the dimensions of one of its specific aggregates (SLS) in the electron microscope; and the mutual arrangement of the tropocollagen molecules in another specific aggregate (quarter-stagger) was deciphered, and shown to be very similar to the arrangement in connective tissue. Experiments with the native fibrous protein in solution, and research with intact tissue dramatically confirmed each other.

3C236, DA240; the largest radio sources known

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A high resolution study has been made of faint and very extensive radio components associated with the galaxies 3C236 and DA240. Their respective linear dimensions of 5.7 Mpc and 2 Mpc are without precedent. Their properties are compared with those of other radio galaxies and the consequences for present models of the evolution of these objects are briefly discussed.

It is important for several reasons to determine the maximum size to which a radio source may evolve. First, the relativistic electrons in the radio components will suffer unavoidable energy losses by various processes as they move away from the associated optical object^{1,2}. These losses are likely to be severe for very old or large sources and it may be that the study of such objects will allow a choice to be made between the various modes of energy replenishment that have been suggested². Second, these sources might serve as probes of the intergalactic medium, for their structure is likely to be strongly influenced by it. Finally, radio components of large angular size can be mapped in detail to obtain much needed clues as to the physical processes occurring in them.

So we felt it essential to ascertain whether the apparent upper cutoff in radio source sizes at about 1 Mpc (refs 3, 4) is a real effect or largely a consequence of observational selection. We have begun a programme of observation at a wavelength of 49 cm with the Westerbork Synthesis Radio Telescope (WSRT) to search for very large sources

which had not been recognised as such in earlier observations. Such a search requires a telescope with good sensitivity, as well as a resolving power sufficient to both delineate the extent of large objects and reliably separate them from unrelated confusing sources. A telescope is most sensitive to extended emission at long wavelengths because of its greater beam area and the non-thermal spectra of the radio sources. The effective sensitivity of existing single dishes can be increased only marginally by observing at longer wavelengths because of the severe limitations imposed by confusion.

With an aperture synthesis instrument like the WSRT, however, we can take full advantage of the improvement expected from a lowering of the frequency. A change in observing wavelength from 21 cm to 49 cm renders the instrument ten times more sensitive to typical radio emission distributed over several synthesised beams. Confusion remains tolerable, with an average of one detectable source in about 200 beam areas, while the twenty baselines simultaneously obtained ensure a low level of 'grating response confusion'.

Source selection and first results

We have selected for study a number of confused regions listed in the catalogue of Bridle *et al.*³. Observing with 10' resolution at 21 cm, they were unable to decide whether the objects were composed of physically associated components, or unrelated sources. Here we report our first results; the sources 3C236 (1003+35) and DA240 (0744+55) are huge radio galaxies having overall sizes

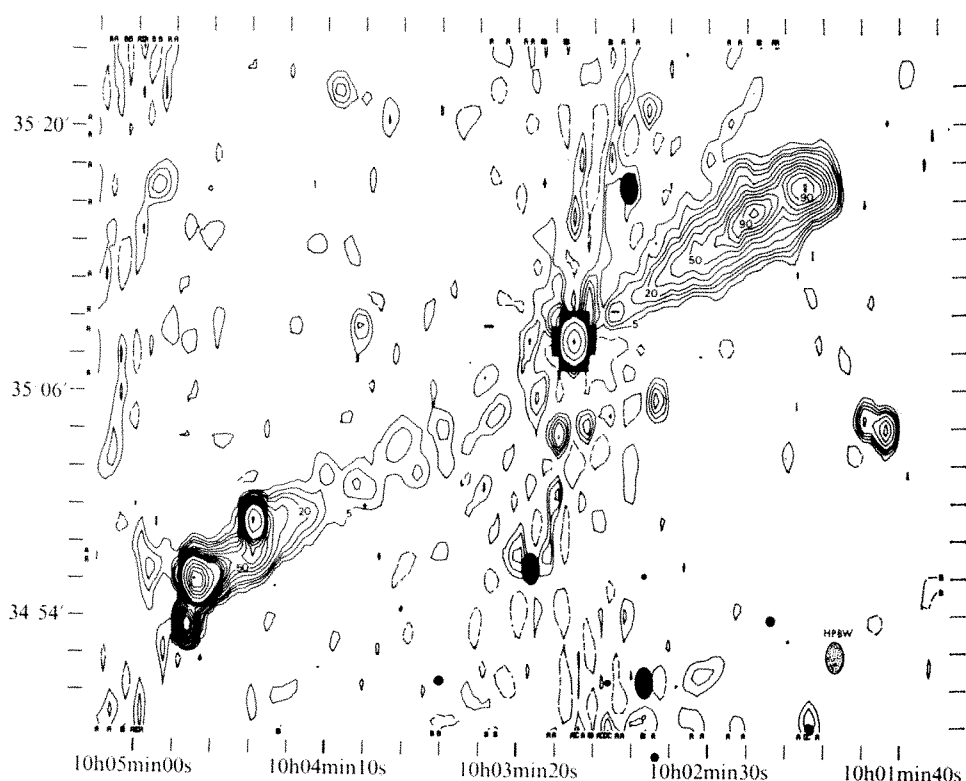


Fig. 1 Contour map of 3C236 at 49 cm. The field centre, at RA 10 h 04 min, Dec. 35° 00', is marked by a cross. The dashed contour is at a level of -7.5 mJy and continuous contours are plotted at intervals of 5 mJy per synthesised beam area from 5 mJy to 20 mJy, 10 mJy from 20 mJy to 100 mJy, and 50 mJy from 100 mJy to 350 mJy. Continuous contours at the 500, 1,500, 3,000 and 4,500 mJy level are also plotted. Stripes extending approximately north-south from the central intense source are due to instrumental effects as discussed in the text. The three black ellipses represent intense background sources, the effects of which have been removed from the map.

Table 1 Observations of 3C236 and DA240

	3C236	DA240
Observation length (half days)	1	2
No. of spacings, increment (m)	20, 72	40, 36
Synthesised half-power beamwidth ($\alpha \times \delta$)	57'' \times 99''	57'' \times 69''
Grating ring radius ($\alpha \times \delta$)	24' \times 41'	47' \times 57'
R.m.s. noise (mJy)	~ 1.5	~ 1.0
Primary beam pattern half-power width	82'	

of 5.7 and 2.0 Mpc respectively (assuming Hubble's constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and an Einstein-de Sitter cosmology).

Early interferometric observations⁶ indicated that most of the emission from 3C236 was contained within a very compact source $< 3''$ in extent. Wilkinson⁷ resolved this source with a baseline of $112,300 \lambda$ at 1,423 MHz and found that two unresolved components separated by $1''$ in position angle 119° gave the best model fit to his data. Wyndham⁸ identified the source with a 16 mag elliptical galaxy. Examination of the Palomar Sky Survey (PSS) prints yields no evidence that the galaxy is in a cluster. Only a few 19 mag objects are visible nearby, and another 16 mag elliptical is present some $4'$ to the north-east. Sandage⁹ measured a redshift $z = 0.0988$ for 3C236 based on a single emission line identified with $[\text{O II}] \lambda 3727$. His accurate photometric data¹⁰ showed that 3C236 lies very close to the line of best fit in the Hubble diagram for radio galaxies. Bridle *et al.*⁵ found two radio components separated by $34'$ straddling the compact source. They pointed out that if all the radio emission were associated, the overall size could be as great as 8 Mpc.

The source DA240 was resolved by Bridle *et al.* into a double whose components were extended and separated by $17'$. Caswell and Wills¹¹ identified 4C56.16, the stronger eastern component, with a 15 mag galaxy, number 9-13-66 in the catalogue of Vorontsov-Velyaminov and Arhipova¹² (hereafter referred to as V-V). This galaxy shows weak emission lines and has a redshift $z = 0.0356$ (ref. 13). Our observations show that instead the galaxy to be identified with DA240 lies midway between the two components found by Bridle *et al.*

Observations and reduction

3C236 and DA240 were observed at 49 cm with the WSRT, a detailed description of which is given elsewhere¹⁴. It consists of 12 equatorially mounted 25-m parabolic dishes situated along an east-west line. Telescope parameters and information relevant to the present observations are summarised in Table 1. Phase stability of the 49-cm system during a twelve hour observing period is about $\pm 1^\circ$, and the gain stability is certainly better than $\pm 1\%$. In our experience, the detection of weak emission having a brightness 0.003 of the most intense peak on the map is feasible with the present system; in the 3C236 observation, which is particularly well calibrated, this value has been extended to almost 0.001.

Two major problems had to be overcome in analyzing the data. First, it was found that both sources contain extremely intense components more than one hundred times as bright as the surrounding emission. Since the inner sidelobes of the synthesised antenna pattern are about 4% of the main response, sidelobes from the intense structure initially masked the low-level radiation. Second, in the case of 3C236, grating rings from some parts of the source distorted the emission from other parts. We have therefore used the CLEAN technique¹⁵, in which structure in the map is iteratively decomposed into a set of point sources, the effects of which are then removed from the map using the antenna pattern with its sidelobes and grating rings. Subsequent convolution of the point sources with a 'clean' antenna pattern leads to a map free from the effects of the subsidiary responses.

The reception pattern of the individual paraboloids causes emission from regions far from the field centre to be attenuated. The maximum effect occurs at the outer edge of the western component of 3C236, whose brightness is reduced by 30%. Flux densities and other tabulated data have been corrected for this effect, but the contour maps have not.

Maps and basic data

A contour map of 3C236 is shown in Fig. 1. The source consists of two extended components on opposite sides of the intense compact source, with outer edges at distances

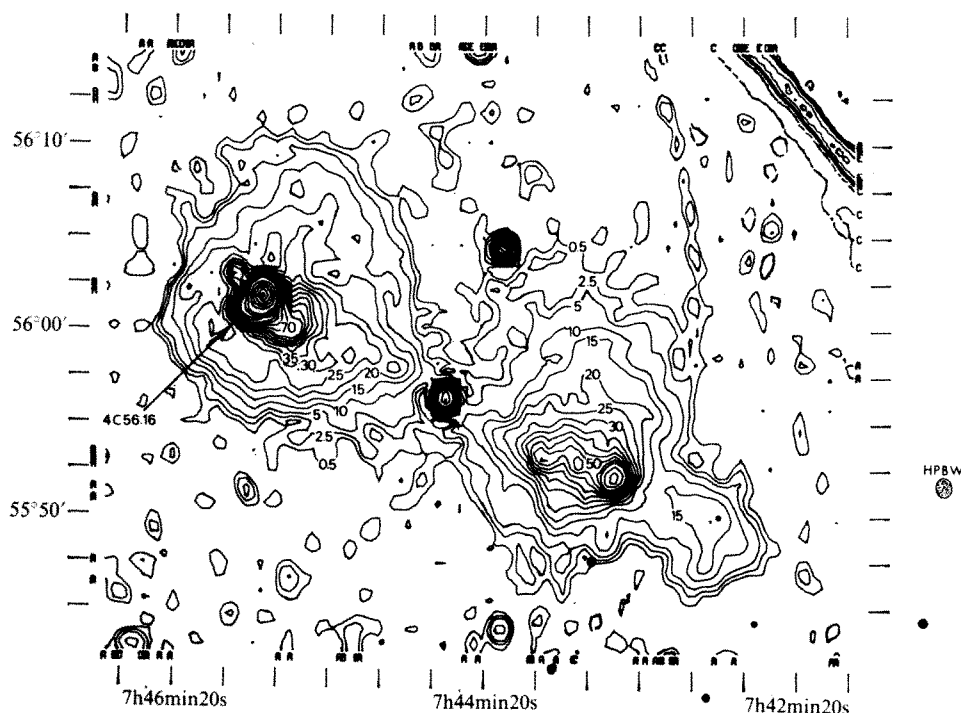


Fig. 2 A contour map of DA240 at 49 cm. The field centre, at RA 07 h 44 min 30 s, Dec. $55^\circ 55'$, is marked by a cross. The dashed contour is plotted at a level of -5.5 mJy and continuous contours are at levels of 0.5 mJy and 2.5 mJy per synthesised beam area, and then in steps of 5 mJy from 5 mJy to 60 mJy, 10 mJy from 60 mJy to 100 mJy, 25 mJy from 100 to 150 mJy, 50 mJy from 150 to 250 mJy and 250 mJy from 250 mJy to 1,500 mJy. The greatest contour is at a level of 1,600 mJy.

Table 2 Some basic data on 3C236 and DA240

	3C236	DA240
Position of central source (1950.0)	10 h 03 min 05.5 s \pm 0.05 s 35° 08' 49.0" \pm 0.6"	07 h 44 min 34.82 s \pm 0.07 s 55° 56' 28.3" \pm 0.6"
Redshift	0.0988	0.0356
Visual magnitude (uncorrected for absorption)	15.97 (ref. 10)	\sim 15.2 (ref. 16)
Angular size along major axis (minutes of arc)	39	34
Size along major axis* (Mpc)	5.7	2.0
Size transverse to major axis (Mpc)	\sim 0.5	\sim 0.7
Size of central source (kpc)	2.44	$<$ 2.9
Flux density (mJy) (49 cm)		
Central source	5.08 \pm 0.05	0.267 \pm 0.007
East component	1.8 \pm 0.2	5.4 \pm 0.3
West component	2.1 \pm 0.2	3.3 \pm 0.3
Total	9.0 \pm 0.3	9.0 \pm 0.5
Mean surface brightness of the outer components (mJy per synthesised beam) (49 cm)	37.4 \pm 3.3	20.8 \pm 1.2
Spectral index α ‡Integrated	-0.6 (ref. 18)	-0.77 (ref. 18)
Central source	-0.62 \pm 0.03 (ref. 17)	-0.40 \pm 0.06
49-cm integrated polarisation (%)	$<$ 1.3	8.3 \pm 1.3
at position angle (°)	—	118 \pm 2

*Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and an Einstein-de Sitter cosmology.

†The flux densities are derived assuming 3C147 has a 49-cm flux density of 37.78 Jy, from extrapolation of the data of Kellermann *et al.*¹⁷.

‡ α defined in the sense flux density \propto frequency α .

of 24' and 15' from it. The extension of the outer components along the line joining them and the precision of the collimation (lines from the two prominent outer peaks meet the central one at an angle of 179.5°) indicate the association suggested by Bridle *et al.*⁵ to be real. Basic data relevant to 3C236 (and DA240) are summarised in Table 2.

The contour map shows a number of spurious, predominantly radial features emanating from the central component, the most prominent of which are nearly vertical. These stripes are instrumental effects produced in the vicinity of intense point sources by small gain and phase fluctuations during the observation, and determine the dynamic range discussed earlier. The peaks beyond the south-east edge of the following component result from the same effect. Their narrowness and radial elongation are usually sufficient to distinguish such features from real structure.

The south-following component has three strong peaks near its south-east end. The brightest of these, with a maximum intensity of 364 mJy per beam area, is centrally located at the eastern end of the low brightness plateau. This component is resolved, except on its outer edge. We think it likely that the two other peaks are unrelated background sources for the following reasons. Both are unresolved and the southernmost one lies adjacent to, rather than within, the low brightness emission. Our polarisation data show the following component, including the main peak, to have significant linear polarisation; the two unresolved sources, however, are unpolarised. From the number of background sources stronger than 6 mJy in a typical WSRT field at 49 cm, we expect 3C236 to coincide, on average, with about 0.5 unrelated objects, which is not significantly different from the two suggested here. For the remainder of our discussion, the main peak will be assumed to define the eastern end of 3C236.

We find the position angle of the source axis to be 122.5°, very close to the 119° which Wilkinson⁷ found for his model of the intense central double source. Since Wilkinson's value has an error of about $\pm 2^\circ$, the alignment may well be exact. This alignment shows that the process responsible for the double structure, which spans a linear scale ratio $\sim 2,000$, has maintained its orientation for at least 10⁷ yr. It is also interesting that the position angle of the minor axis of the optical galaxy seems roughly coincident with the radio source axis.

The distribution of brightness in DA240 is shown as a contour map (Fig. 2) and as an intensity modulated radio photograph (Fig. 3), the latter being particularly suitable for the display of faint, extended regions. Like 3C236,

DA240 is dominated by a very intense source (4C56.16), so the map is slightly affected by limitations of dynamic range. Thus several faint stripes appear to radiate from 4C56.16, and there is a weak residual grating ring near RA07 h 43 min.

DA240 consists of two nearly circular diffuse components, each about 12' in diameter. These regions are more sharply bounded in the south than the north, suggestive of a gradient in the external gas density across the source. Each component has a dominant maximum, and these peaks are almost equidistant from an unresolved central component. These three peaks have widely differing intensities, the easternmost one (4C56.16) having a maximum surface brightness of 1.74 Jy per beam area. From its visibility curve at 49 cm, we find that 4C56.16 emits 1.4 Jy from a region smaller than 15". The westernmost peak is twenty times less intense than the 4C source and is fully resolved, even along its leading edge. Preceding the entire western component and clearly associated with it, is a faint elongated feature some 6' in length and 4' wide. The centre component has a flux density of 267 mJy at 49 cm and, according to our preliminary data, at 6 cm has an angular size less than 3" and a flux density of 115 \pm 10 mJy.



Fig. 3 An intensity modulated radiophotograph of DA240, produced on a rotating drum photo plot system designed by W. J. Jaffe. The declination scale is compressed by about 12% relative to the right ascension scale.

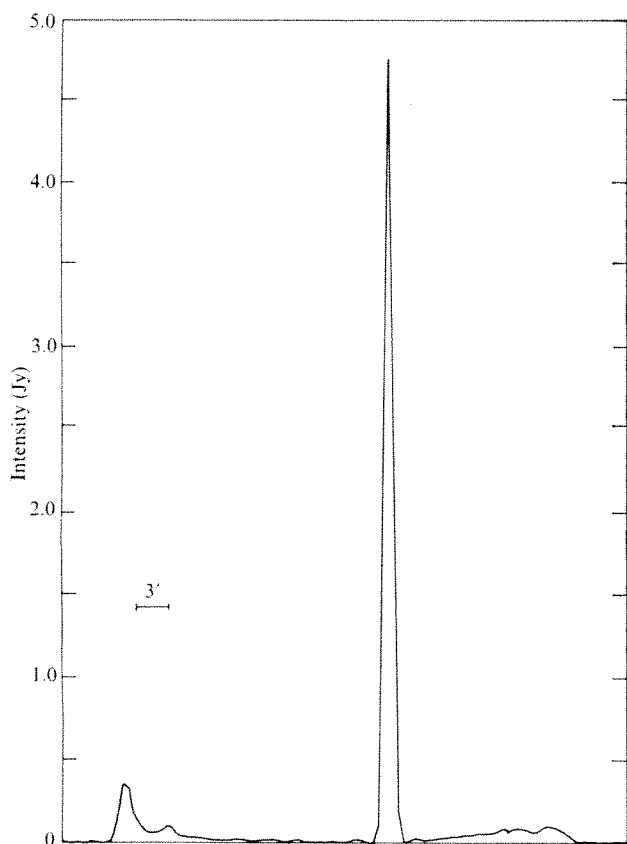


Fig. 4 A crosscut in position angle 122.5° along the major axis of 3C236.

We have identified the compact central source with an elliptical galaxy having a diffuse outer envelope (number 9-13-57 in the V-V catalogue). V-V gives the dimensions of the inner core as $30'' \times 24''$. Schmidt (private communication) has measured a redshift $z=0.0356$ for this galaxy.

On the north-east side of the intense component 4C56.16 there is an unresolved subpeak with an intensity of about 38 mJy which is coincident with the galaxy V-V 9-13-66, the object thought by Caswell and Wills¹¹ to be associated with 4C56.16 itself. The redshift ($z=0.0356$) given by Burbidge¹³ is exactly the same as that measured by Schmidt for V-V 9-13-57. V-V 9-13-66 is classified as "a flat galaxy whose arms have an indefinite direction of winding". The flux density implies a radio power of $1.5 \times 10^{22} \text{ W Hz}^{-1} \text{ sr}^{-1}$ at 49 cm, much stronger than that of normal spirals¹⁹. The tantalising question of whether V-V 9-13-66 is actually immersed in the eastern component of DA240 cannot be conclusively answered with the present data.

A ridge of emission extends from each outer component of DA240 towards the central one. The position angle of a line joining the end of each ridge near the central component is almost the same as the position angle of the major axis of the optical galaxy, giving the ridge system the shape of an open 'S'.

From the available data^{5,18} we have derived an integrated 178 MHz flux density of 22 Jy for DA240 which is well above the nominal completeness limit of the 3C survey. As shown by Bennett²⁰, however, the 3C observations are increasingly insensitive to sources whose angular size exceeds about $5'$. It is essential for workers deriving statistical properties of radio source samples to appreciate the inherent limitations of many source surveys in this respect.

Our observations also show that both 3C236 and DA240 have strong linear polarisation at 49 cm. Although the integrated polarisation of 3C236 is negligible, individual regions show degrees of polarisation up to 20%. The eastern peak is polarised by 10% but as we have already noted, the other two intense sources near it are unpolarised. Polarisations of up to 40% and possibly higher occur in the extensive low brightness regions of DA240. The intense unresolved structure in 4C56.16 is 22% polarised, and in its immediate surroundings similar values are found. DA240 has an integrated percentage polarisation at 49 cm of 8.3%, higher than previously found for any radio galaxy at this wavelength²¹. The polarisation of the central components, coincident with the optical galaxies are $< 0.4\%$ and $< 2\%$ for 3C236 and DA240 respectively.

Discussion

A remarkable feature of the 3C236 and DA240 maps, and the main reason that much of the structure has previously escaped detection, is the huge range of surface brightness. In 3C236 a ratio of more than 1,000:1 is found between the intense central source and the faintest emission in the south following component. A profile of intensity along the major axis of the source (Fig. 4) indicates these differences. Although DA240 does not display such an extreme disparity the peak intensity of 4C56.16 exceeds the average brightness of DA240 by more than a factor of 80. It is not yet possible to say whether these large variations in surface brightness, and the prominence of the central component at 49 cm, qualify 3C236 and DA240 as rare source types.

The ratio of the flux densities of the outer components and their relative distances from the optical galaxy are not unusual²² in these sources. It is, however, interesting to note that both outer components of 3C236 are well resolved transverse to the source axis, unlike the majority of 3C double sources when observed with a similar resolution²².

Table 3 Comparison of 3C 236, DA240, Cen A and Cyg A

Source	3C236	DA240	Cen A	Cyg A
Distance* (Mpc)	554	206	10	320
Linear size (Mpc)	5.70	1.99	1.57	0.16
$P_{\lambda_{49}}^\dagger$ ($\text{W Hz}^{-1} \text{ sr}^{-1}$)	2.6×10^{25}	3.6×10^{24}	2.1×10^{24}	2.7×10^{27}
Radio luminosity (\sim)	2.4×10^{36}	3.4×10^{35}	1.9×10^{35}	3.0×10^{38}
Component properties	east	west	east	west
Volume (10^{72} cm^3)	35	43	8.0	7.9
Minimum Energy† (10^{58} erg)	54	63	29	22
Energy density ($10^{-14} \text{ erg cm}^{-3}$)	1.6	1.5	3.6	2.8
Equipartition magnetic field† (10^{-7} G)	6.3	6.1	9.5	8.4
			north	south
			2.9	2.9
			9.1	9.3
			3.1	3.2
			8.8	9.0
			1800	2000
			2.4×10^{-6}	1.9×10^{-6}
			3.0	3.0
			1.3×10^9	1.6×10^9

*Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and an Einstein-de Sitter cosmology.

†Minimum energy estimates have been made assuming the energy of cosmic ray protons to be negligible. If the energy in protons exceeds that in electrons by a factor of 100, the minimum energies and the equipartition magnetic field strengths must be multiplied by 13.9 and 3.7, respectively.

Table 4 Minimum energies (U_{\min}), equipartition magnetic fields (B_{eq}), thermal electron densities (n_e) and masses (M) of selected regions

Source	Region	U_{\min}^* (erg)	B_{eq}^* (gauss)	n_e (cm^{-3})	M (M_{\odot})
DA240	Intense eastern compact component	1.3×10^{58}	2.4×10^{-6}	$< 6 \times 10^{-5}$	$< 8 \times 10^8$
DA240	Extended eastern low brightness area	1.6×10^{59}	5×10^{-7}	$< 2.4 \times 10^{-5}$	$< 1 \times 10^{11}$
3C236	Eastern head	3.3×10^{58}	2.2×10^{-6}	$< 7 \times 10^{-5}$	$< 1.7 \times 10^9$

*Minimum energy estimates have been made assuming the energy of cosmic-ray protons to be negligible. If the energy in protons exceeds that in electrons by a factor of 100, the minimum energies and the equipartition magnetic field strengths must be multiplied by 13.9 and 3.7, respectively.

DA240, also, has unusual features. In many respects—the relaxed nature of the large outer components, the 'S'-shaped ridge linking the brighter peaks—the source is reminiscent of Centaurus A (ref. 23). It may be significant that Centaurus A is also a large source exceeding 1 Mpc in length. Like 3C236, Centaurus A has a compact double source well within the large outer components.

In Table 3 we present a number of derived properties for 3C236, DA240, Cen A and the powerful radio galaxy Cyg A (ref. 24). We have assumed that the components are prolate spheroids whose depths are equal to their width in the plane of the sky along the minor axis and that the energy in cosmic ray protons is negligible. Examination of Table 3 shows that DA240 and Centaurus A do, indeed, have many similar properties. Cyg A, a much smaller, very intense source, has a higher luminosity, energy density and magnetic field strength than the other three.

In columns 1 and 2 of Table 4 we show the minimum energies U_{\min} in the form of relativistic electrons and magnetic field, and the corresponding equipartition magnetic fields B_{eq} for two selected regions of DA240 and one of 3C236.

As we have already noted, both sources are strongly polarised at 49 cm. Stringent upper limits may therefore be placed on the thermal gas density in the sources, for high gas densities would cause depolarisation by differential Faraday rotation. Assuming the line of sight depths through the components to be equal to their size in the plane of the sky, and the magnetic field to take its equipartition value, we find the upper limits to the thermal density, n_e , given in column 3, while the corresponding masses are presented in column 4. The gas densities found are at most an order of magnitude greater than the cosmological density of 3×10^{-6} hydrogen atoms cm^{-3} , and could be very much less. Such low densities, and the large overall sizes of the sources have interesting implications for certain models of the evolution of extragalactic radio sources which we now discuss in turn.

De Young and Axford²⁵ proposed a dynamical containment mechanism in which the ram pressure of the intergalactic gas confines the radio emitting components. In the context of their model it has been suggested²⁶ that the extensive 'tails' of low brightness often seen to extend back towards the parent galaxy result from electrons which have diffused out of the compact outer components. Such a scheme does not seem applicable to DA240, for here the extended low brightness emission completely envelopes the compact regions. As the motion of the component is envisaged to be supersonic it is not clear how electrons could diffuse ahead of it. Of course, if the components were more or less stationary (and contained by some process other than ram pressure), the observed morphology could result. The ram pressure model may also be criticised on the grounds that it supposes the components to leave the galaxy with sizes not much smaller than their present ones. For 3C236, there is evidence from the double structure of the compact central source that the original component sizes are not bigger than 1 kpc. In the subsequent expansion to the observed diameter of ~ 150 kpc for the compact eastern component, adiabatic losses would decrease the flux by a factor of more than 10^9 . It is, therefore, necessary that energy in the form of magnetic field and relativistic particles

be distributed over volumes much larger than a galactic nuclear region in a loss-less way, probably to be supplied to the components throughout most of their lifetimes.

A useable feature of the model is that the components are continually decelerated and eventually brought to a halt after which the source expands rapidly. Christiansen²⁷ has shown that a plasmon can travel a distance

$$D = M / (\pi \times 1.67 \times 10^{-24} n_s r^2) \quad (1)$$

before dispersing. Here M is the mass of the plasmon, r its radius and n_s the number density of the gas surrounding the component. Although equation 1 was derived assuming ram pressure containment, it yields an estimate of the distance travelled by the plasmon before it is strongly decelerated even if it is contained by other processes. This is because the plasmon must, in any case, experience a retarding force of (ram pressure) \times (cross-sectional area); r should then be interpreted as the component radius, suitably averaged over the source lifetime. In spite of uncertainty in this radius, it is interesting to apply equation 1 to the eastern component of 3C236 and so derive an upper limit to n_s by taking D and r as their present observed values and the upper limit to M from Table 4. We find $n_s < 10^{-6} \text{ cm}^{-3}$. If the source axis is not in the plane of the sky, the limit becomes correspondingly lower. In spite of this low density, the fact that the component leading edge is sharper than the trailing one suggests an interaction with an external gas. If the eastern component in 3C236 is, in fact, confined by ram pressure, $n_s > 3.6 \times 10^{-8} \text{ cm}^{-3}$, assuming the component velocity $v < 0.08c$ (ref. 28).

Gull and Northover²⁹ have proposed an elegant mechanism for the propulsion and confinement of radio components by a circumgalactic medium. The radially decreasing density of the medium, maintained by the galactic gravitational field, causes the components to 'float' away from the centre. The model seems, however, to be inapplicable to 3C236 and DA240 for several reasons. The most telling of these are the long component travel times ($\sim 10^{10}$ yr) and the difficulty of maintaining sufficient density gradients over the distances involved. Similar difficulties have been noted for Cyg A (ref. 24); for these giant sources they are even more severe.

Further observations of 3C236 and DA240 with the WSRT at 6 cm and 21 cm have either been planned or are already in progress. A presentation of these results and a full discussion of their implications will follow. We have also begun observations of other candidate objects, for it seems most improbable that these two are the only radio sources of moderate strength whose large scale structure has been overlooked. Before long we may know if 3C236 is atypical, or whether it must relinquish its distinction of being the largest known object.

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- ¹ Van der Laan, H., and Perola, G. C., *Astr. Astrophys.*, **3**, 468 (1969).
- ² Longair, M. S., Ryle, M., and Scheuer, P. A. G., *Mon. Not. R. astr. Soc.*, **164**, 243 (1973).
- ³ Fomalont, E. B., *Astrophys. J.*, **157**, 1027 (1969).
- ⁴ Fanaroff, B. L., and Riley, J. M., *Mon. Not. R. astr. Soc.*, **167**, 31P (1974).
- ⁵ Bridle, A. H., Davis, M. M., Fomalont, E. B., and Lequeux, J., *Astr. J.*, **77**, 405 (1972).
- ⁶ Clark, B. G., and Hogg, D. E., *Astrophys. J.*, **145**, 21 (1966).
- ⁷ Wilkinson, P. N., *Mon. Not. R. astr. Soc.*, **160**, 305 (1972).
- ⁸ Wyndham, J. D., *Astrophys. J.*, **144**, 459 (1966).
- ⁹ Sandage, A., *Astrophys. J. Lett.*, **150**, L145 (1967).
- ¹⁰ Sandage, A., *Astrophys. J.*, **178**, 25 (1972).
- ¹¹ Caswell, J. L., and Wills, D., *Mon. Not. R. astr. Soc.*, **135**, 231 (1966).
- ¹² Vorontsov-Velyaminov, B. A., and Arhipova, V. P., *Morphological Catalogues of Galaxies*, 4 vols (Univ. Moscow, 1964).
- ¹³ Burbidge, G. R., *A. Rev. Astr. Astrophys.*, **8**, 369 (1970).
- ¹⁴ Baars, J. W. M., van der Brugge, J. F., Casse, J. L., Hamaker, J. P., Sondaar, L. H., Visser, J. J., and Wellington, K. J., *Proc. IEEE*, **61**, 1258 (1973).
- ¹⁵ Högbom, J., *Astr. Astrophys. Suppl.*, **15**, 417 (1974).
- ¹⁶ Zwicky, F., and Herzog, E., *Catalogue of Galaxies and of Clusters of Galaxies*, **III** (Cal. Tech., Pasadena, 1966).
- ¹⁷ Kellermann, K. I., Pauliny-Toth, I. I. K., and Williams, P. J. S., *Astrophys. J.*, **157**, 1 (1969).
- ¹⁸ Roger, R. S., Bridle, A. H., and Costain, C. H., *Astron. J.*, **78**, 1030 (1973).
- ¹⁹ Van der Kruit, P. C., *Astr. Astrophys.*, **29**, 263 (1973).
- ²⁰ Bennett, A. S., *Mem. R. astr. Soc.*, **68**, 163 (1962).
- ²¹ Strom, R. G., *Astr. Astrophys.*, **25**, 303 (1973).
- ²² Mackay, C. D., *Mon. Not. R. astr. Soc.*, **154**, 209 (1971).
- ²³ Cooper, B. F. C., Price, R. M., and Cole, D. J., *Aust. J. Phys.*, **18**, 589 (1965).
- ²⁴ Hargrave, P. J., and Ryle, M., *Mon. Not. R. astr. Soc.*, **166**, 305 (1974).
- ²⁵ de Young, D. S., and Axford, W. I., *Nature*, **216**, 129 (1967).
- ²⁶ Mills, D. M., and Sturrock, P. A., *Astrophys. Lett.*, **5**, 105 (1970).
- ²⁷ Christiansen, W., *Mon. Not. R. astr. Soc.*, **145**, 327 (1969).
- ²⁸ Mackay, C. D., *Mon. Not. R. astr. Soc.*, **162**, 1 (1973).
- ²⁹ Gull, S. F., and Northover, K. J. E., *Nature*, **244**, 80 (1973).

Fine structure analysis of a eukaryotic multifunctional gene

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The ilv 1 gene of Saccharomyces cerevisiae contains functions associated with both structural and regulatory genes and therefore is termed a multifunctional gene. The ilv 1 gene product catalyses the first step in isoleucine biosynthesis and is involved in repression of the isoleucine-valine pathway. The intracistronic discrimination of the two functions is presented.

IN spite of considerable progress in the understanding of the underlying molecular mechanisms involved in the regulation of gene expression in prokaryotes¹, such mechanisms remain obscure in eukaryotes. Although several approaches for studying eukaryotic control mechanisms have involved animal systems², much information about eukaryotic regulatory phenomena has been obtained from studies with fungi. Work with *Neurospora* has revealed several complex forms of regulation, for example, enzyme aggregation^{3,4} and compartmentation⁵, and *Saccharomyces* has been the subject of several elegant studies⁶⁻⁹. Nevertheless, a regulatory macromolecule like a repressor protein involved in the control of gene expression in eukaryotic organisms remains to be identified and characterised.

There is evidence from *in vivo* studies that threonine deaminase or some form of the *ilv 1* gene product, is involved in multivalent repression of the isoleucine-valine enzymes, probably functioning as a positive effector. Altered repression of the isoleucine-valine biosynthetic enzymes was observed in *Saccharomyces cerevisiae* MAR-33 (ref. 10), with a mutation in or closely linked to the *ilv 1* gene which renders threonine deaminase 100 times less sensitive than the wild type to isoleucine inhibition^{11,12}. The isoleucine-valine biosynthetic enzymes from MAR-33 are fully derepressed when the strain is grown on minimal medium while normally only partial derepression is attained under these conditions^{11,12}. Altered regulation of the isoleucine-valine enzymes was observed also in strain D106-la, which contains a nonsense mutation in the middle of the *ilv 1*

gene^{11,12}. In D106-la, the isoleucine-valine biosynthetic enzymes fail to derepress when cells are grown in minimal medium or in a medium limited for isoleucine, a condition that normally leads to complete derepression. Magee and I have shown that the altered regulatory effects on the isoleucine-valine enzymes cosegregate with the desensitising threonine deaminase mutation in MAR-33 and the *ilv 1* nonsense mutation^{11,12}.

In vivo evidence for threonine deaminase or some form of the *ilv 1* gene product in multivalent repression of the isoleucine-valine pathway has been obtained also in bacteria in Umberger's laboratory^{13,14} as well as in that of Hatfield¹⁵, who suggested

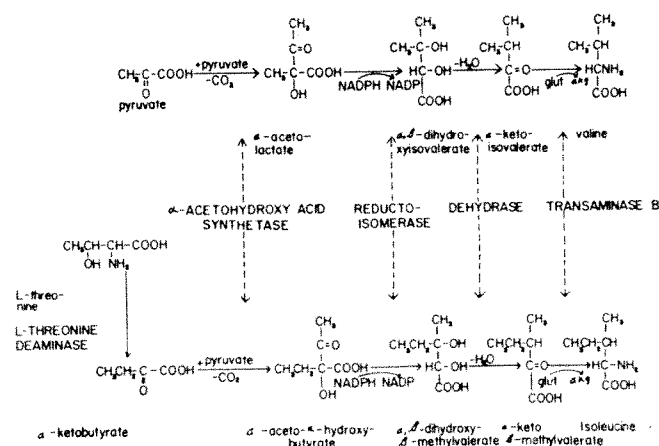


Fig. 1 Biosynthetic pathway for isoleucine and valine in *Saccharomyces cerevisiae*. Threonine deaminase (EC 42116) is the first enzyme specific for the biosynthesis of isoleucine. The other four enzymes are involved in the biosynthesis of both valine and isoleucine. Repression of AHAS, reductoisomerase, dehydrase and transaminase B requires isoleucine, valine and leucine each at 5 mM. In *Saccharomyces cerevisiae*, the genes which code for the five different isoleucine-valine enzymes are on different chromosomes.

Table 1 List of strains containing *ilv 1* mutations

Genotype	Phenotype	Reference
<i>ilv 1-1</i>	Nonsense mutation	11
<i>ilv 1-8</i>	Nonsense mutation	11
<i>ilv 1-15</i>	Nonsuppressible	20
<i>ilv 1-41</i>	Nonsuppressible	20
<i>ilv 1-48</i>	Nonsuppressible	20
<i>ilv 1-51</i>	Nonsuppressible	20
<i>ilv 1-67</i>	Nonsense mutation	20
<i>ilv 1-74</i>	Nonsuppressible	20
<i>ilv 1-82</i>	Nonsuppressible	20
<i>ilv 1-83</i>	Nonsuppressible	20
<i>ilv 1-101</i>	Nonsense mutation	20
<i>ilv 1-113</i>	Nonsense mutation	20
<i>ilv 1-1 SUP, ilv 3-</i>	Contains suppressor for <i>ilv 1-1</i>	11
<i>ilv 1-101 SUP, ilv 3-</i>	Contains suppressor for <i>ilv 1-101</i>	This study
M21	Nonsuppressible <i>ilv 1</i>	11
MD11	Wild type for <i>ilv 1</i>	24

some role for threonine deaminase in repression¹⁶. In addition to the isoleucine-valine pathway, the involvement of a biosynthetic enzyme in repression of subsequent enzymes of the histidine pathway has been shown by Goldberger *et al.*^{17,18}.

The diversity of protein function became clearer with reports of proteins with several activities^{7,11,17}. I report here the analysis of the catalytic and regulatory functions of the *ilv 1* gene. I present evidence confirming the multifunctional nature of the *ilv 1* gene where by genetic and biochemical analyses, the intracistronic discrimination of some of the *ilv 1* regions associated with each function are assigned.

Catalytic function of *ilv 1*

Figure 1 shows one activity of the *ilv 1* gene product is the conversion of L-threonine to α -ketobutyrate, the first biosynthetic step leading to the synthesis of L-isoleucine. This catalytic activity is performed by threonine deaminase whose activity is 50% inhibited by 0.5 mM L-isoleucine and stimulated by 5 mM L-valine¹⁰. The threonine deaminase from *S. cerevisiae* contains two identical subunits of approximately 100,000 molecular weight^{10,19}.

Several strains of *S. cerevisiae* with mutations in the *ilv 1* gene, rendering threonine deaminase catalytically inactive²⁰, have been tested (Table 1). Each *ilv 1* allele represented in the table has been subjected to genetic fine structure analysis and the map is given in Fig. 2 (ref. 20). As Table 1 shows the *ilv 1* mutations 1, 101, 67, 117, 113 and 8 are adjudged to be nonsense mutations by suppression studies²⁰, whereas suppressibility of the other *ilv 1* mutations has not been observed²⁰.

As Zimmerman²⁰ indicated, the genetic length of the *ilv 1* gene using the *ilv 1-41* and *ilv 1-8* alleles as its extremities is about 16 map units of induced mitotic recombination. Based on previous data^{21,22} one unit of recombination represents approximately 130 nucleotides. By these calculations the *ilv 1* gene product involved in catalytic function would have a molecular weight of approximately 100,000 which is consistent with a dimeric protein of approximately 200,000 molecular weight, as indicated above.

Threonine deaminase activity was found in extracts of several diploid strains each containing different pairs of *ilv 1* alleles. Intragenic complementation between various *ilv 1* alleles has been found by Zimmermann *et al.*²³. The diploid strains constructed by mating different haploid strains each containing various *ilv 1* mutations, as well as a diploid strain containing two wild type *ilv 1* genes, are given in Table 2.

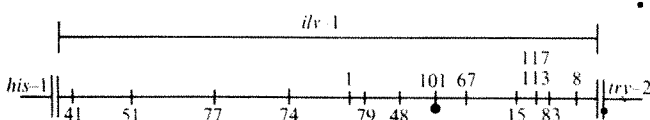


Fig. 2 Fine structure map of the *ilv 1* gene. The alleles above the line are of the nonsense type.

Extracts were prepared from the various diploid strains and the specific activity of threonine deaminase was determined as before^{10,12}. In addition, the sensitivity of the diploid threonine deaminase to inhibition by 1.0 mM and 100 mM L-isoleucine was determined. Table 2 shows threonine deaminase with a specific activity of 3.21 (μ mol of product formed per 20 min per mg of protein) was found in extracts from the diploid containing two wild type *ilv 1* genes. An unexpectedly high threonine deaminase specific activity of 1.23 was found for the diploid containing the *ilv 1-51* and *ilv 1-79* alleles, which is 40% of the specific activity found in the wild type diploid. Table 2 shows the threonine deaminase obtained from the diploid containing the *ilv 1-51* and *ilv 1-79* alleles was not inhibited by 1.0–100 mM L-isoleucine which inhibits *ilv 1*⁺ \times *ilv 1*⁺ wild type threonine deaminase. As previously reported, 100 mM L-isoleucine inhibits the threonine deaminase obtained from strain MAR-33 harbouring a mutation in *ilv 1* (or closely linked to it) which renders the enzyme 100 times less sensitive to isoleucine inhibition. Table 2 shows of the other diploids tested for threonine deaminase activity, that containing the *ilv 1-48* and *ilv 1-77* alleles had a threonine deaminase specific activity of 0.31 which seemed to be sensitive to inhibition by 1.0 mM isoleucine. Neither the diploid containing the *ilv 1-48* and *ilv 1-82* alleles nor that containing the *ilv 1-51* and *ilv 1-82* alleles had detectable threonine deaminase activity.

Therefore, in addition to showing several strains containing *ilv 1* mutations rendering threonine deaminase either catalytically inactive or less sensitive to isoleucine inhibition, the use of diploids containing different *ilv 1* alleles has facilitated, by intragenic complementation, the *in vivo* construction of threonine deaminase molecules with altered properties for isoleucine inhibition.

Regulatory function of *ilv 1*

As previously indicated, in addition to catalytic activity, some form of the *ilv 1* gene product must be involved in multivalent repression of the isoleucine-valine biosynthetic enzymes,

Table 2 Threonine deaminase specific activity in diploid strains

Diploid genotype	L-isoleucine added (mM)		
	0	1.0	100
<i>ilv 1</i> ⁺ \times <i>ilv 1</i> ⁺	3.21	0	0
<i>ilv 1-51</i> \times <i>ilv 1-79</i>	1.23	1.14	1.29
<i>ilv 1-48</i> \times <i>ilv 1-77</i>	0.31	0	0
<i>ilv 1-48</i> \times <i>ilv 1-82</i>	0		
<i>ilv 1-51</i> \times <i>ilv 1-83</i>	0		

The threonine deaminase was assayed as previously described¹⁰ in the absence of L-isoleucine and in the presence of 1.0 mM and 100 mM L-isoleucine. Specific activity is expressed as μ mol of α -ketobutyrate formed per 20 min per mg of protein. Diploid strains were grown in minimal medium and extracts were obtained for the threonine deaminase assay, as previously described^{10,12}.

probably acting as a positive effector^{11,12}. This function is designated the '*ilv 1* regulatory function'. Evidence for altered *ilv 1* regulatory function was sought in various strains containing different *ilv 1* mutations as well as in the diploid strains constructed by mating the various haploid *ilv 1* auxotrophs.

I analysed the *ilv 1* regulation function by two approaches. First, determinations were made of the acetohydroxy acid synthase (AHAS) specific activity obtained from the various *ilv 1* auxotrophs and diploid strains grown in minimal, repressing and isoleucine limiting medium (Table 3). Second, the AHAS differential rate of synthesis was determined in cultures which had been transferred from repressing medium to isoleucine limiting medium (that is kinetics of escape from repression).

Two major conclusions can be drawn from Table 3. First, the *ilv 1* regulatory function is altered in strains containing the *ilv 1* nonsense alleles *ilv 1-1* and *ilv 1-101*. Second, altered

Table 3 Effect of different *ilv 1* mutations on *ilv 1* regulatory function

<i>ilv-1</i> Mutation	Specific activity of AHAS			ILM* RM ratio
	Repressing medium	Minimal medium	Isoleucine limiting medium	
MD11	0.049 ± 0.013	0.147 ± 0.019		
M21	0.057 ± 0.014	0.139 ± 0.019	0.364 ± 0.029	6.40
<i>ilv 1-1</i> nonsense	0.069 ± 0.012	0.063 ± 0.015	0.067 ± 0.014	0.97
<i>ilv 1-8</i> nonsense	0.064 ± 0.014	0.133 ± 0.019	0.364 ± 0.032	5.70
<i>ilv 1-15</i>	0.061	0.131	0.351	5.75
<i>ilv 1-41</i>	0.041	0.110	0.282	6.88
<i>ilv 1-48</i>	0.064	0.131	0.315	4.93
<i>ilv 1-51</i>	0.059 ± 0.010	0.127 ± 0.020	0.341 ± 0.030	5.78
<i>ilv 1-67</i> nonsense	0.069 ± 0.015	0.140 ± 0.018	0.371 ± 0.035	5.38
<i>ilv 1-74</i>	0.056	0.132	0.322	5.75
<i>ilv 1-79</i>	0.063	0.125	0.341	5.41
<i>ilv 1-82</i>	0.056	0.124	0.311	5.55
<i>ilv 1-83</i>	0.073	0.141	0.348	4.77
<i>ilv 1-101</i> nonsense	0.062 ± 0.011	0.071 ± 0.013	0.069 ± 0.014	1.11
<i>ilv 1-113</i> nonsense	0.063 ± 0.013	0.128 ± 0.020	0.324 ± 0.030	5.14
<i>ilv 1-1 SUP, ilv 3-</i>	0.067 ± 0.013	0.134 ± 0.021	0.343 ± 0.029	5.11
<i>ilv 1-101 SUP, ilv 3-</i>	0.059 ± 0.014	0.121 ± 0.018	0.335 ± 0.033	5.67
<i>ilv 1+ × ilv 1+</i>	0.053 ± 0.011	0.135 ± 0.016		
<i>ilv 1-51 × ilv 1-79</i>	0.056 ± 0.013	0.281 ± 0.024		
<i>ilv 1-48 × ilv 1-77</i>	0.041 ± 0.010	0.143 ± 0.021		
<i>ilv 1-48 × ilv 1-82</i>	0.061	0.141		
<i>ilv 1-51 × ilv 1-83</i>	0.053	0.119		

The repressing medium used is minimal salts medium plus L-isoleucine, L-valine and L-leucine, each at 5 mM^{12,24}. Minimal medium consisted of minimal salts medium as previously described^{10,22}. Where isoleucine was necessary for growth, a nonrepressing concentration of 2 mM L-isoleucine was added^{12,24}. Isoleucine limiting medium consisted of minimal salts medium plus 2.5 mM L-isoleucine and 20 mM L-valine which competes for isoleucine utilisation^{12,24}. AHAS was assayed in toluenised cells as previously described^{12,24}. Its specific activity is expressed as μmol of product produced per 20 min per mg of protein. MD11 is a strain wild type for the *ilv 1* gene. M21 contains a nonsuppressible *ilv 1* mutation which renders threonine deaminase catalytically inactive. In the cases where standard deviations are represented, four determinations were done.

* Ratio of AHAS activity obtained from cells grown in isoleucine limiting medium (ILM) compared with cells grown in repressing medium (RM).

ilv 1 regulatory function is not observed in strains containing the *ilv 1* nonsense alleles, *ilv 1-67*, *ilv 1-113*, *ilv 1-117*, and *ilv 1-8*.

As Table 3 shows, AHAS was derepressed approximately 2.5-fold when the normally regulated strains MD11 and M21^{11,12} were grown on minimal medium compared with repressing medium: the ratio of AHAS specific activity of M21 cells grown in isoleucine limiting medium compared with repressing medium was 6.4. Similar results were obtained for all strains containing the various *ilv 1* alleles including the *ilv 1-67*, *ilv 1-113*, *ilv 1-117* and *ilv 1-8* nonsense alleles, with the exception of strains containing the *ilv 1-1* and *ilv 1-101* nonsense alleles where no AHAS derepression was observed when cells were grown in minimal or isoleucine limiting medium. The ratio of AHAS specific activity when strains containing either the *ilv 1-1* or *ilv 1-101* alleles were grown on isoleucine limiting medium compared with repressing medium was close to unity. Since the AHAS specific activity obtained from strains containing the *ilv 1-1* or *ilv 1-101* alleles grown in repressing medium was similar to that obtained for the normally regulated strains MD11 and M21, unity represents a failure to derepress

under minimal and isoleucine limiting conditions.

Since only the *ilv 1-1* and *ilv 1-101* nonsense mutations and not the *ilv 1-8*, *ilv 1-67*, *ilv 1-117* and *ilv 1-113* nonsense mutations affect the *ilv 1* regulatory function, one might conclude that the *ilv 1* region excluded by the *ilv 1-1* and *ilv 1-101* nonsense mutations are needed for regulatory function whereas the regions excluded by the other *ilv 1* nonsense mutations are not.

These results were substantiated by examining the differential rate of synthesis of AHAS by the second method in cultures transferred from repressing medium to isoleucine limiting medium^{11,12}. Figure 3 gives the differential rate of AHAS synthesis in M21, which is normally regulated for the isoleucine-valine enzymes—it was 0.19. The AHAS differential rate in the strain containing *ilv 1-1* was 0.017 and in the strain containing *ilv 1-101*, 0.021. As Table 4 shows AHAS differential rate of 0.02 was obtained when M21 was grown in repressing medium. The low AHAS differential rate obtained from the strains containing the *ilv 1-1* and *ilv 1-101* alleles grown on isoleucine limiting medium therefore, reflects lack of derepression. Also Table 4 shows the strains containing the other nonsense alleles, *ilv 1-113*, *ilv 1-8* and *ilv 1-67*, strains containing suppressors for the *ilv 1-1* and *ilv 1-101*, *ilv 1-1 SUP* and *ilv 1-101 SUP* and strains containing the nonsuppressible *ilv-1* alleles 51 and 79 gave AHAS differential rates of synthesis comparable with the normal value obtained with M21.

Figure 3 also shows that the increase in the differential rate of synthesis of AHAS was inhibited by 50 $\mu\text{g ml}^{-1}$ of cycloheximide, which inhibits protein synthesis. Thus the increase in activity required protein synthesis and probably represented synthesis of new enzyme. In addition, since the level of AHAS after inhibition of protein synthesis remained constant for 4 h, no substantial degradation of AHAS occurred within the 4 h period.

The altered *ilv 1* regulatory function and the *ilv 1-1* and *ilv 1-101* mutations cosegregate in four tetrads tested. As Table 3 shows, both catalytic and regulatory function was restored when suppressors for the *ilv 1-1* and *ilv 1-101* nonsense mutations were present. In addition, the altered *ilv 1* regulatory

Table 4 Differential rate of AHAS synthesis in *ilv 1* auxotrophs

<i>ilv</i> Mutation	Differential rate of synthesis of AHAS*
M21	0.190
<i>ilv 1-1</i>	0.017
<i>ilv 1-101</i>	0.021
<i>ilv 1-113</i>	0.160
<i>ilv 1-8</i>	0.205
<i>ilv 1-67</i>	0.170
<i>ilv 1-51</i>	0.168
<i>ilv 1-79</i>	0.210
<i>ilv 1-1 SUP ilv 3</i>	0.180
<i>ilv 1-101 SUP ilv 3</i>	0.230
(M21-grown in repressing medium)†	0.020

* μmol of product produced per 20 min per 10^6 cells.

† Differential rate of synthesis for AHAS when strain M21 is grown on repressing medium.

Strains were transferred from repressing medium to isoleucine limiting medium as described for Fig. 3.

function resulting from the *ilv 1-1* and *ilv 1-101* mutations was recessive to wild type (Table 5). When a strain containing either the *ilv 1-1* or *ilv 1-101* mutations was mated with a strain which was *ilv 1⁺; ilv2⁻*, and the resultant diploid strain was grown on minimal medium, the AHAS specific activity was derepressed 2.5 times more than the normal repressed level. These results suggest that the *ilv 1* gene product is necessary for derepression of the isoleucine-valine enzymes.

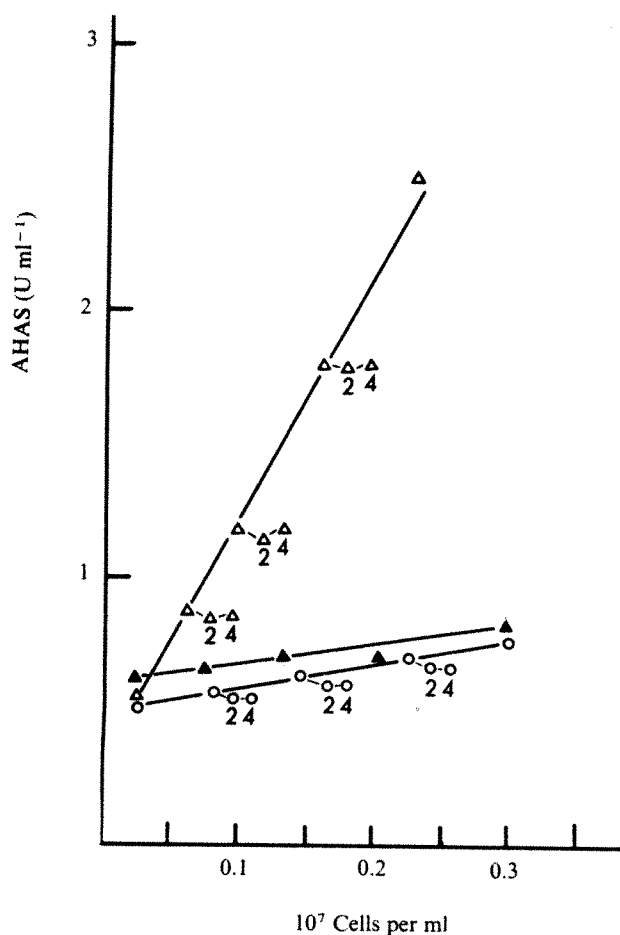


Fig. 3 Cells were grown in 50 ml of repressing medium to about 5×10^7 cells per ml, centrifuged, washed and diluted five times into 250 ml of minimal medium plus 20 mM valine and 2.5 mM isoleucine (isoleucine limiting medium). Samples of 50 ml were taken after 0, 2, 4, 6 and 8 h. Cells were made permeable by toluene and the AHAS was assayed as previously reported^{12,24}. The numbers 2 and 4 shown after each point represent AHAS activity 2 and 4 h after the addition of 50 μ g of cycloheximide. Open triangles represent strain M21, closed triangles represent the strain containing the *ilv 1-1* allele and the open circles represent the strain containing the *ilv 1-101* allele.

There is evidence^{11,12} that the isoleucine inhibition property of threonine deaminase is related to the *ilv 1* regulatory function. In a strain MAR-33, which contains a mutation in (or closely linked to *ilv 1*), the threonine deaminase is 100 times less sensitive to isoleucine inhibition¹⁰. When MAR-33 was grown on minimal medium, the AHAS as well as the other isoleucine-valine enzymes were derepressed approximately six-fold, attaining a specific activity usually obtained only under isoleucine limiting conditions. If 5 mM L-isoleucine was added to the medium, the isoleucine-valine enzymes were derepressed only 2.5-fold which is usually attained when a wild type strain is grown on minimal medium^{11,12}. In another strain TIR-9 which also contains a mutation in *ilv 1* (or closely linked to it), the threonine deaminase is only ten times less

sensitive to isoleucine inhibition¹⁰. The levels of the isoleucine-valine enzymes when TIR-9 was grown under minimal conditions were like wild type. Therefore, it seems that a 100-fold but not a ten-fold isoleucine desensitisation of the threonine deaminase affects the *ilv 1* regulatory function.

Table 5 Recessivity of altered *ilv 1* regulatory function

Medium	Diploid genotype	Specific activity of AHAS
Minimal	<i>ilv 1-1 ilv 2⁺ × ilv 1⁺ ilv 2⁻</i>	0.123 ± 0.017
	<i>ilv 1-101 ilv 2⁺ × ilv 1⁺ ilv 2⁻</i>	0.112 ± 0.019
Repressing	<i>ilv 1-1 ilv 2⁺ × ilv 1⁺ ilv 2⁻</i>	0.041 ± 0.011
	<i>ilv 1-101 ilv 2⁺ × ilv 1⁺ ilv 2⁻</i>	0.047 ± 0.010

AHAS was assayed as previously described^{12,24}, and specific activities are expressed as μ mol of product produced per 20 min per mg of protein.

The *ilv 1* regulatory function was examined in the various diploid strains previously described, especially that which contains the *ilv 1-51* and *ilv 1-79* alleles which result in threonine deaminase desensitised to isoleucine inhibition (Table 3). In all diploids tested except one, the AHAS-specific activity was approximately 2.5 times greater when cells were grown in minimal medium compared with repressing medium. In the diploids strain containing the *ilv 1-51* and *ilv 1-79* alleles, the AHAS specific activity was five times greater when cells were grown in minimal media compared with repressing media. This increased derepression of the AHAS when the diploid strain containing *ilv 1-51* and *ilv 1-79* alleles was grown on minimal media further indicates the interrelationship of the isoleucine inhibition site of the *ilv 1* product, and the *ilv 1* regulatory function.

Multifunctional map of *ilv 1*

A summary of the effects of the various *ilv 1* mutations on *ilv 1* catalytic and regulatory function is represented in Fig. 4. The results are represented as a multifunctional map which consist of three representations of the *ilv 1* gene each corresponding to a different property of the *ilv-1* gene product and each containing the *ilv-1* alleles causing defects in the corresponding *ilv 1* function.

As Fig. 4 shows, the *ilv 1-1* and *ilv 1-101* nonsense alleles concordantly affect both catalytic and regulatory function, whereas both functions are discordantly affected by the *ilv 1-113*, *ilv 1-117*, *ilv 1-67*, and *ilv 1-8* nonsense alleles where only the catalytic function is affected. In addition, the other nonsuppressible *ilv 1* alleles did not affect *ilv 1* regulatory function.

These results suggest that the *ilv-1* regions affected by the *ilv 1-117*, *ilv 1-113*, *ilv 1-67* and *ilv 1-8* nonsense mutations are not needed for regulatory function but are necessary for catalytic function. On the contrary, *ilv 1-1* and *ilv 1-101* nonsense mutations affect *ilv 1* regions needed for both regulatory and catalytic functions. In addition, these results represent the strongest indication that the regulatory function actually is within the *ilv 1* gene and not in an adjacent regulatory region.

In addition, as seen in Fig. 4, alleles *ilv 1-51* and *ilv 1-79* do not affect *ilv 1* regulatory function in haploid strains. Nevertheless, regulatory function is altered in a diploid strain containing both *ilv 1-51* and *ilv 1-79* alleles, where intragenic complementation results in restoration of threonine deaminase activity that is insensitive to isoleucine inhibition. These results are consistent with the report of a concordant affect on isoleucine inhibition and regulatory function in strain MAR-33 (refs 11, 12). Preliminary data indicate that MAR-33 has a

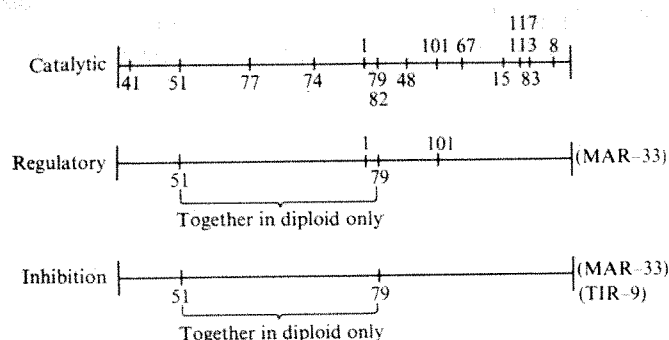


Fig. 4 Multifunctional map of the *ilv I* gene. Each representation of the *ilv I* gene corresponds to one of the *ilv I* functions which are represented at the left of the corresponding cistron. Only the alleles which result in altered function are represented. The alleles above the line are of the nonsense type.

mutation near the middle of the *ilv I* gene and that it is not suppressible (my unpublished data).

In conclusion, the results presented here strongly indicate that the *ilv I* gene is multifunctional, coding for a product which functions in the catalysis of L-threonine to α -keto-butyrate, as well as being a positive effector in the derepression of the isoleucine-valine enzymes. I have found correlation between the isoleucine inhibition property of threonine deaminase and *ilv I* regulatory function. Furthermore, there is intracistronic discrimination of the *ilv I* catalytic and regulatory functions. Apparently the regulatory function of the multifunctional *ilv I* gene represents an example of a eukaryotic regulatory gene which has been identified and partially characterised both genetically and biochemically.

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- ¹ Vogel, H. J. (ed.) *Metabolic regulation*, 5, (Academic Press, New York, 1971).
- ² Wolstenholme, G. E. W., and Knight, J. (ed.) *Control processes in multicellular organisms* (Churchill, London, 1970).
- ³ Giles, N. H., Case, M. E., Partridge, C. W. H., and Ahmad, S. I., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1453-1460 (1967).
- ⁴ Arroyo-Begovich, A., and DeMoss, J. A., *J. biol. Chem.*, **248**, 1262-1267 (1973).
- ⁵ Weiss, R. L., and Davis, R. H., *J. biol. Chem.*, **248**, 5403-5408 (1973).
- ⁶ Shaffer, B., Rytka, J., and Fink, G. R., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 1198-1205 (1969).
- ⁷ Wiame, J. M., in *Current topics in cellular regulation* (edit. by Horecker, B. L., and Stadtman, E. R.) **4**, 1-20 (Academic Press, New York, 1972).
- ⁸ Kellems, R. E., and Butow, R. A., *J. biol. Chem.*, **247**, 8043-8050 (1971).
- ⁹ Hartwell, L. H., Culotti, J., Pringle, J. R., and Reid, B. J., *Science*, **183**, 46-51 (1974).
- ¹⁰ Betz, J. L., Hereford, L. M., and Magee, P. T., *Biochemistry*, **10**, 1818-1824 (1971).
- ¹¹ Bollon, A. P., and Magee, P. T., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2169-2173 (1971).
- ¹² Bollon, A. P., and Magee, P. T., *J. Bact.*, **113**, 1333-1344 (1973).
- ¹³ Pledger, W. J., and Umbarger, H. E., *J. Bact.*, **114**, 195-207 (1973).
- ¹⁴ Levinthal, M., Williams, L. S., Levinthal, M., and Umbarger, H. E., *Nature new Biol.*, **246**, 65-68 (1973).
- ¹⁵ Calhoun, D. H., and Hatfield, G. W., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2757-2761 (1973).
- ¹⁶ Hatfield, G. W., and Burns, R. O., *Proc. natn. Acad. Sci. U.S.A.*, **66**, 1027-1035 (1970).
- ¹⁷ Goldberger, R. F., and Kovach, J. S., in *Current topics in cellular regulation* (edit. by Horecker, B. L., and Stadtman, E. R.), **5**, 295-308 (Academic Press, New York, 1972).
- ¹⁸ Blasi, F., Bruni, C. B., Avitable, A., Deeley, R. G., Goldberger, R. F., and Meyers, M. M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2692-2696 (1973).
- ¹⁹ Katsunuma, T., Elsasser, R., and Holzer, H., *Eur. J. Biochem.*, **24**, 83-87 (1971).
- ²⁰ Thuriaux, P., Minet, M., ten Beige, A. M. A., and Zimmerman, F. K., *Molec. gen. Genet.*, **112**, 60-72 (1971).
- ²¹ Manney, T. R., and Mortimer, R. K., *Science*, **143**, 581-583 (1964).
- ²² Parker, J. H., and Sherman, F., *Genetics*, **62**, 9-22 (1969).
- ²³ Zimmerman, F. K., Schmidt, I., and ten Beige, A. M. A., *Molec. gen. Genet.*, **104**, 321-330 (1969).
- ²⁴ Magee, P. T., and Hereford, L. M., *J. Bact.*, **98**, 857-862 (1969).

letters to nature

Cosmogony of the asteroidal belt

WE have to derive the state of partial corotation in a way which, by being very simple, stresses its character as a basic state in cosmic plasma dynamics. We also substitute the mass density (m, a) for the number density (n, a) in the plot of asteroids, as a function of their semimajor axis a . This gives a better definition to the limits of the main belt, and demonstrates more clearly that the asteroids—like the Saturnian rings—have condensed from a partially corotating plasma.

If a rotating body has an axisymmetric dipole field and is surrounded by a plasma, and if the electrical conductivity of the body and of the plasma is infinite, the plasma will rotate with the same angular velocity Ω as the body; the magnetic field lines are 'frozen-in'. This idealised model is, however, not applicable to cosmic problems because celestial bodies have gravitation, and the plasma is subject both to that and to the centrifugal force produced by the rotation¹⁻⁴. Moreover, the 'frozen-in' picture is seldom applicable in astrophysics⁴⁻⁷. The assumption of infinite conductivity is unrealistic, because electric fields parallel to the magnetic field play an important role^{8,9}. It is now evident that the magnetosphere is to a high degree uncoupled from the ionosphere¹⁰.

This means that instead of a plasma corotating with an angular velocity $\omega = \Omega$, we have typically a 'partial corotation' with $\omega < \Omega$ (refs 3 and 4). The rotational velocity is given by the condition that the centrifugal force, f_c , the gravitation, f_g , and the electromagnetic force, f_B , acting on a plasma element should balance each other, so that

$$f_c + f_g + f_B = 0 \quad (1)$$

(We assume that the plasma temperature is so low that pressure and diamagnetic effects can be neglected—see Fig. 1.) If M_c is the mass of the central body, κ the constant of gravitation, B the magnetic field deriving from a dipole with moment a, m the mass of a plasma element, I the current through it, and \mathbf{x}_0 a unit vector perpendicular to the axis, there is in a coordinate system (r, λ) in the meridian plane of the dipole:

$$f_c = \omega^2 m r \cos \lambda \mathbf{x}_0 \quad (2)$$

$$f_g = (\eta M_c m / r^3)(-\mathbf{r}) \quad (3)$$

$$f_B = I \times B \quad (4)$$

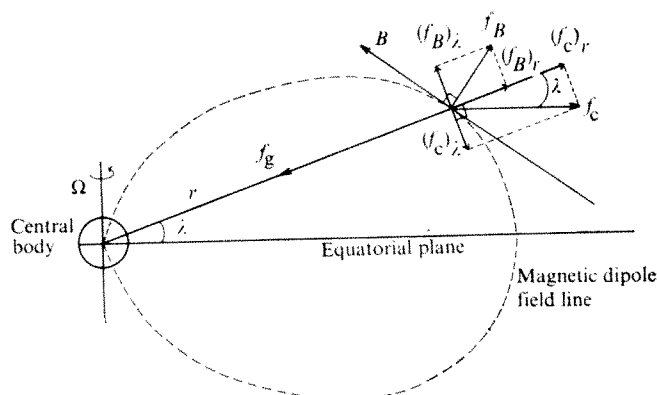


Fig. 1 Configuration of partial corotation. Equilibrium between gravitation f_g , centrifugal force f_c , and electromagnetic force $f_B = \mathbf{I} \times \mathbf{B}$, means that $f_g + f_c + f_B = 0$. Because $(f_c)_\lambda + (f_B)_\lambda = 0$, the geometry of the dipole field requires that $(f_c)_r = 2(f_B)_r = 2/3(-f_g)$. a , Central body; b , magnetic dipole field line.

In a dipole field $B_r = -(2a/r^3) \sin \lambda$, $B_\lambda = (a/r^3) \cos \lambda$, and so from equations (1)–(4) (for $\lambda \neq 0$)

$$f_c = 2aI/r^3$$

and

$$f_g = [f_c + (Ia/r^3)] \cos \lambda$$

showing that: $(f_c)_r = 2(f_B)_r = (2/3) |f_g|$. Thus the gravitational force is compensated to 2/3 by the centrifugal force, and to 1/3 by the electromagnetic force. The factor 2/3 is given by the geometry of a dipole field.

If condensation of grains (planetesimals) takes place from a partially corotating plasma, and the resulting grains are so large that the electromagnetic forces become small compared to f_c and f_g , then they will move in Kepler orbits. As the centrifugal force does not fully compensate the gravitation, the condensed grains will move in ellipses with eccentricity, $e = 1/3$ (refs 1–4). If a large number of grains have condensed in the neighbourhood of the central body and they collide with one another, the eccentricities of their orbits will diminish, so that eventually they will all move in more circular orbits, with a semimajor axis with a length which is 2/3 the distance to the point where the condensation occurred.

There are reasons to believe that condensation from a partially corotating plasma was a basic process in the formation of the Solar System. In fact, the structure of the Saturnian ring, and of the asteroid belt can be understood in this way^{1–3,11}. The factor 2/3 occurs in two places in the Saturnian ring system, and in two places in the asteroid belt. In the latter case the usual (n, a) diagram of asteroids has been used, giving the number density, n , of asteroids as a function of their semimajor axes, a .

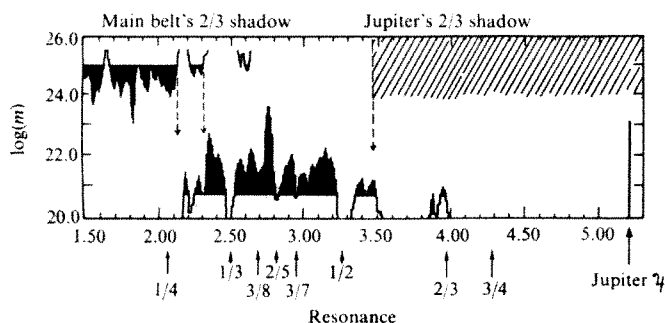


Fig. 2 The smoothed (m, a) diagram. The curve has been smoothed with the weight function 1:3:5:3:1. Mass distribution in unit of gram within a radial distance interval of $\Delta a = 0.01$ AU. In order to emphasise the log scale, high density regions are darkened. The same diagram diminished by a factor 2/3 and turned upside down is shown above, in order to demonstrate the 'shadow' effect which produces the inner cutoff of the asteroidal belt. Similarly Jupiter's 'shadow', which generates the outer cutoff, is shown. Kirkwood gaps from Jupiter resonances are arrowed.

The diagram (n, a) only gives an approximate picture of the mass distribution in the asteroid belt. We have now, however, calculated an (m, a) diagram, giving the mass distribution. We have assumed that all asteroids have the same albedo and density, so that we can put their mass proportional to $(\text{brightness})^{3/2}$ (Fig. 2).

The (m, a) diagram shows the Kirkwood gaps, especially at 1/2 and 1/3, somewhat more clearly than the usual (n, a) diagram.

The limits of the main belt at $a = 2.15$ and 3.49 are also sharper (especially because a large number of asteroids below $a = 2.15$ have a negligible mass).

In order to study the effect of the fall-down ratio, 2/3, the (m, a) diagram, diminished by a factor 2/3, is shown reversed in the upper part of Fig. 2.

The diagram shows that the whole main belt is located below 2/3 of Jupiter's orbit, which means that the main belt asteroids may have derived from condensation which took place inside Jupiter's orbit down to $a = 3.49$. At this limit plasma would have condensed directly on already existing asteroids (or asteroidal grains), which therefore produced a 'shadow' at 2/3 this distance. As the diagram shows, there is a marked drop in mass density at $2/3 \times 3.49 = 2.32$. Above the 1:2 Kirkwood gap however, the mass density does not seem to be large enough to make the plasma density drop to zero. That does not occur until the 'shadow' of the bulk of asteroids below 3.20 appears at 2.13 (that is, $2/3 \times 3.20$).

There is no other known way to explain the upper and lower limits of the main belt.

The only asteroids outside the main belt which are massive enough to appear in the diagram are the Hildas, at $a = 3.95$ AU. As 5.9 AU ($3/2 \times 3.95$) exceeds Jupiter's distance from the Sun ($= 5.2$) they must have condensed outside Jupiter's orbit. When falling down in ellipses with $e = 1/3$ they avoided capture (or dispersal) by Jupiter because they were commensurable with Jupiter's orbit period to a degree of 2/3 that kept them from coming close to Jupiter. (Compare the Neptune–Pluto resonance and similar cases¹²).

The 2/3 ratio is found in three places in the asteroid (m, a) plot (Fig. 2). In fact, the observed ratios agree with the theoretical value to within 1%. Including the two occurrences in the Saturnian ring system there are five cases which confirm that the condensation from a plasma in partial corotation was an important process in the formation of the Solar System.

The mass distribution of the asteroids is not altogether explained by the resonances and the 2/3 fall-down. For example, there is a very strong peak at 2.75 which includes Ceres and Pallas, and a secondary peak at 2.36 which includes Vesta. These may result from a later evolution which concentrates mass through a process which eventually leads to the formation of planets.

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¹ Alfén, H., *Stockh. Obs. Annl.*, **14**, No. 2 (1942); **14**, No. 5 (1943); **14**, No. 9 (1944).

² Alfén, H., *On the Origin of the Solar System* (Oxford University Press, Oxford, 1954).

³ Alfén, H., *Icarus*, **7**, 387 (1967).

⁴ Alfén, H., and Arrhenius, G., *Astrophys. Space Sci.*, **21**, 117 (1973).

⁵ Alfén, H., and Fälthammar, C. G., *Cosmical Electrodynamics Fundamental Principles* (Oxford University Press, Oxford, 1963).

- ⁶ Alfven, H., *Annls Géophys.*, **24**, 1 (1968).
⁷ Alfven, H., in *Relations between Cosmic and Laboratory Plasma Physics: Cosmic Plasma Physics* (edit. by Schindler, K.), 1 (Plenum, New York, 1972).
⁸ Fälthammar, C.-G., *Space Sci. Revs.*, **15**, 803 (1974).
⁹ Block, L. P., *Cosmic Electrodynamics*, **3**, 349 (1972).
¹⁰ Bostrom, R., in *Magnetospheric Physics* (edit. by McCormac, B. M.), (Reidel, Dordrecht, in the press).
¹¹ Alfven, H., *Icarus*, **8**, 75 (1967).
¹² Alfven, H., and Arrhenius, G., *Astrophys. Space Sci.*, **8**, 338 (1970).

A Lagrangian community?

It seems that it may be economically feasible, within the limits of the technology of this decade, to establish at one of the Lagrange libration points of the Earth-Moon system (called here L_5) a habitat capable of supporting and maintaining some 10,000 people. Projections indicate that this habitat, using free solar energy and the rich mineral resources of the lunar surface, could construct a still larger habitat, in a progression leading, possibly within 30 yr from now, to communities of from 10^6 to 10^7 people. These communities could be as comfortable as the most desirable parts of the Earth, with natural sunshine, controlled weather, normal air, apparent gravity and complete freedom from pollution. Replication of these communities could lead to the exponential growth of new land area, with a growth rate more rapid than that of the total human population.

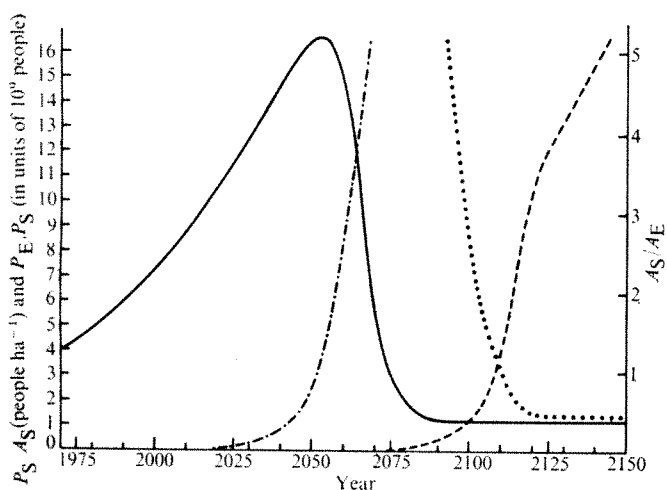


Fig. 1 A graph, technically realisable in my opinion, showing that most industry could be removed from the biosphere of the Earth within a century. The graph is based on a "worst-case" assumption: no reduction of the population growth rate either on earth or in the space communities. P_E is the population on earth, P_S the population in space, A_S/A_E the ratio of land area in space (all usable) to the total land area of Earth. Changes within wide limits in the assumed input numbers do not affect reaching a solution stable in P_E and P_S/A_S . The final stable value of P_E (1.2×10^9 people) is equivalent to the 1910 value. —, P_E ; ---, P_S ; ·····, P_S/A_S ; ---, A_S/A_E .

A technically possible time-development is shown in Fig. 1, not as a prediction but as an illustration of the power of the technique.

Economic feasibility is defined here as the achievement of an overall cost less than or equal to that of the Apollo project. A reduction in the design size of the first habitat, with a corresponding reduction in the size of its population, would result in some further reduction in the estimates.

The solutions to several problems were necessary in order to achieve this feasibility:

- (a) Geometry: A physical arrangement has been found which would allow the use of natural sunlight for industry, farming and the maintenance of an attractive, earthlike environment with normal day/night and seasonal cycles.
 (b) Transport from the earth to L_5 : A reconfiguration of hardware already under development for the space-shuttle (to be operational by 1980) seems adequate to transport the necessary minimum of materials from earth.
 (c) Water: The combination, at L_5 , of hydrogen from the earth with oxygen from the abundant oxides of the lunar surface effects an important saving of a factor 9 in the mass of material needed from earth per unit mass of water at L_5 .
 (d) Materials: Obtaining nearly all the mass of the habitat from the moon appears essential for economy. Two alternative designs have been studied for the acceleration of lunar materials to the 2.4 km s^{-1} escape velocity of the moon. Both designs depend on the vacuum environment of the moon. Neither is conventional, but the technology of the present decade would suffice for either.

The ultimate benefits of this new possibility depend on the production of successively larger communities by a workforce which is housed, fed and maintained within the communities rather than on earth, and so to the eventual achievement by the communities of the ability to sustain their own growth by the construction of new lands and production facilities from lunar and asteroidal material.

These studies have been discussed in lectures at a number of universities during the past 18 months. Knowledge of the work has therefore spread, additional people have joined the effort, and recent progress has been rapid. On May 10, 1974, the first public meeting on this topic was held at Princeton University.

Detailed information on these studies will be found in forthcoming publications in *Physics Today* and in *Icarus*.

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Intensity of the near infrared OH airglow

THE OH airglow was studied from the balloon-borne telescope Thisbe on three flights launched in 1971 and 1972 from the NCAR balloon flight station, Palestine, Texas (32°N , 95°W). The principal goal of these flights has been the measurement of the zodiacal light in the near infrared¹ with the airglow as a troublesome foreground contribution which had to be determined carefully.

The measurements have been performed from a float altitude of 32 km at wavelengths $\lambda = 0.82$, 2.1, and $2.4 \mu\text{m}$ with half power bandwidths $\Delta\lambda = 0.023$, 0.1 and $0.1 \mu\text{m}$ respectively. For the shorter wavelengths a straightforward photometer with a multiplier as detector has been used. The larger wavelengths were measured by a dry ice cooled PbS-photometer. The field of view of both photometers was 2° in diameter. The $0.82 \mu\text{m}$ photometer was calibrated by observations of 33 stars. The PbS-photometer was calibrated before the flight by a 240 K blackbody and during the flight by the star $\alpha\text{-Ori}$

$$\begin{aligned} (I_{2.1 \mu\text{m}} &= 1.9 \times 10^{-12} \text{ W cm}^{-2} \mu\text{m}^{-1}; \\ I_{2.4 \mu\text{m}} &= 1.3 \times 10^{-12} \text{ W cm}^{-2} \mu\text{m}^{-1}). \end{aligned}$$

The airglow intensity was determined from several elevation scans. As an example Fig. 1 shows the result of an elevation scan at $\lambda = 2.1 \mu\text{m}$.

A van Rhijn curve for an emission altitude of 92 km was fitted to the data. The resulting zenith intensities at all measured wavelengths are listed in Table 1.

Table 1 Zenith intensities of the OH airglow ($\text{W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$)

Date	Time (CST)	λ (μm)	$\Delta\lambda$ (μm)	Intensity
May 13, 1972	23:00	0.82	0.023	$(2.5 \pm 0.4) \times 10^{-10}$
November 1, 1971	2:40	2.1	0.1	$(1.6 \pm 0.5) \times 10^{-9}$
October 24, 1972	2:00	2.1	0.1	$(1.6 \pm 0.3) \times 10^{-9}$
October 24, 1972	5:00	2.4	0.1	$< 6 \times 10^{-11}$

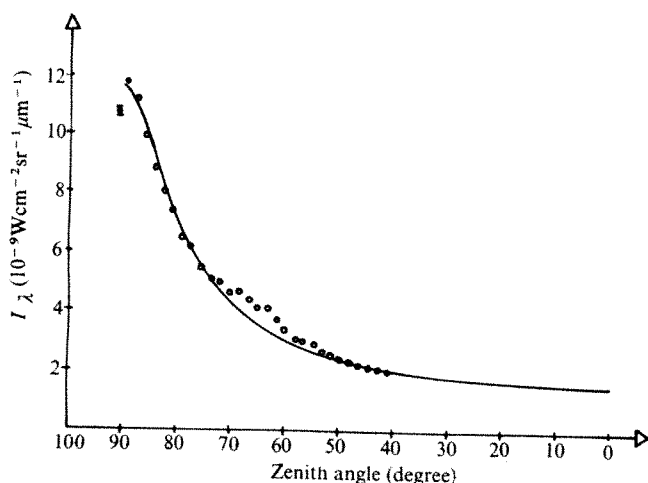


Fig. 1 OH airglow intensity at $2.1 \mu\text{m}$ determined from an elevation scan. A van Rhijn curve for an emission altitude of 92 km is fitted to the data. Notice the patchy structure at 65° zenith angle. $\lambda = 2.1 \mu\text{m}$; $\Delta\lambda = 0.1 \mu\text{m}$.

For comparison the results of other investigators are given. Sternberg and Ingham² found an average intensity of $2.9 \times 10^{-10} \text{W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$ at $\lambda = 0.82 \mu\text{m}$. At $\lambda = 2.1 \mu\text{m}$ Noxon *et al.*³ determined an intensity of 1×10^{-8} and Moros⁴ an intensity of $5 \times 10^{-10} \text{W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$. Peterson and Kieffaber⁵ determined at $\lambda = 2.2 \mu\text{m}$ ($\Delta\lambda = 0.54 \mu\text{m}$) an airglow intensity of $3.2 \times 10^{-9} \text{W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$ from a broadband measurement. The differences of the results may be

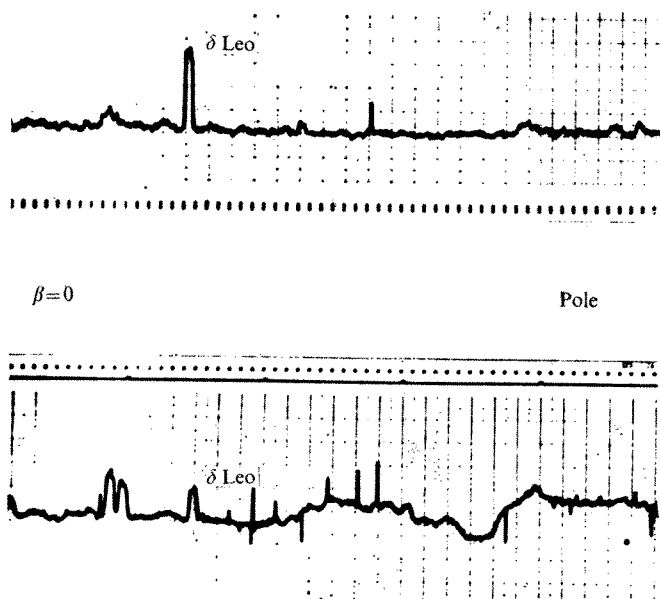


Fig. 2 Simultaneous scans from the ecliptic plane ($\beta = 0$) to the north celestial pole (5,000 Å above, 8,200 Å below). Visual magnitude of the star δ Leo (A 4 V) is 2.56 mag. The signal registered at 8,200 Å is dominated by strong variations of the OH-airglow, at 5,000 Å the airglow contribution is much smaller.

explained by the local and temporal variations of the airglow intensity. At $\lambda = 2.4 \mu\text{m}$ Moros⁴ found an upper limit of $< 2 \times 10^{-10}$ and Noxon and Vallance Jones⁶ an upper limit $< 10^{-9} \text{W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$. Our measurements indicate that at $2.4 \mu\text{m}$ the airglow intensity is at least three times smaller than previously known.

The patchy structure of the airglow was detected at 0.82 and $2.1 \mu\text{m}$ (see Figs 2 and 1). The patches have diameters of about 10° to 20° corresponding to 20 to 40 km in linear size. They differ from the average intensity up to 20%. Patches were also found at $0.8 \mu\text{m}$ by Peterson⁷ and at $2.2 \mu\text{m}$ by Peterson and Kieffaber⁵ using ground based measurements.

Our results have confirmed the existence of a theoretically predicted gap in the OH airglow emission at $\lambda = 2.4 \mu\text{m}$, which is also indicated in spectroscopic work^{8,9}. So surface photometry of extended celestial sources as the zodiacal light and the Milky Way is possible within this $\sim 0.3 \mu\text{m}$ wide gap from balloon altitudes. Because of the large intensity and irregularities of the OH airglow at shorter wavelengths serious problems arise in subtracting the airglow foreground from the night sky radiation.

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- Hofmann, W., Lemke, D., Thum, C., and Fahrbach, U., *Nature phys. Sci.*, **243**, 140 (1973).
- Sternberg, J. R., and Ingham, M. F., *Mon. Not. R. astr. Soc.*, **159**, 1 (1972).
- Noxon, J. F., Harrison, A. W., Vallance Jones, A., *Can. J. Phys.*, **36**, 246 (1958).
- Peterson, A. W., and Kieffaber, L. M., *J. atmos. ter. Phys.*, **34**, 1357 (1972).
- Moros, W. I., *Dokl. Akad. Nauk S.S.S.R.*, **126**, 983 (1959).
- Noxon, J. F., and Vallance Jones, A., *Can. J. Phys.*, **39**, 1120 (1961).
- Peterson, A. W., *Nature*, **242**, 321 (1973).
- Chamberlain, J. W., and Smith, C. A., *J. geophys. Res.*, **64**, 611 (1959).
- Lowe, R. P., and Lytle, E. A., *Appl. Opt.*, **12**, 579 (1973).

Positron annihilation in EAS and ball lightning

CRAWFORD¹ has suggested that the bursts of γ -ray energy that correspond to positron annihilation at rest (0.511 MeV), which have been observed by Ashby and Whitehead², may be caused by extensive air showers (EAS) of primary energy $E_0 \approx 10^{16}$ eV. We present two major objections to that hypothesis.

First, we shall discuss the duration of the four γ -ray bursts which were detected. Ashby and Whitehead² report an upper limit of about 10 s for the duration of three events, and the fourth lasted about 3 s. Those two values corresponded to two different time constants of the recording device: 25 s and 1 s, respectively. The lower limit for the duration can be derived from the time resolution of the NaI(Tl) γ -ray detector which was used, about 0.25 μs . A lower limit of $3.7 \times 10^3 \times 2.5 \times 10^{-7}$, ≈ 9 ms, can be given for the duration of the four events, as the event with the lower flux corresponds to 3.7×10^3 distinct counts. On the other hand, the delays in the distribution of the various EAS components must be considered. The electronic component, at distances relatively close to the shower axis, shows time-spreads of less than a few tens of ns (ref. 3), where-

as the delays observed for muons are of the order of several hundreds of ns. Slow neutrons which are delayed by no more than several μ s are the most delayed particles detected⁴. Finally, considering that positron annihilation times which correspond to the slower annihilation modes are of the order $\approx 10^{-7}$ s (ref. 5), it is hard to understand a γ -ray emission of 0.511 MeV from an EAS of such a long duration (≈ 1 ms.)

The number of photons which it is claimed were created seems rather high. Consider the energy deposited by the shower particles in the first radiation length in the ground, inside a disk of radius $R = 2$ m. The energy dissipated by an EAS is represented by the track length integral (ref. 6). In the lower atmosphere, after the maximum longitudinal development, the integral is represented by

$$E_{\text{diss}} = \epsilon_0 N_{s,l} \int_{t_{\text{max}}}^{\infty} \exp [0.18 (t_{s,l} - t)] dt$$

where ϵ_0 is the 'critical energy' and its actual value in air is approximately 84 MeV. For a shower generated by a particle of energy $E_0 = 10^{16}$ eV, we assume $t_{\text{max}} = 18.6$, $t_{s,l} = 28.4$, and $t_c = 74.4$, considering t_c when the EAS 'age parameter', s , is 2. The integral over the portion of the curve of the longitudinal development which is extrapolated below sea level (s.l.) to t_c gives $E_{\text{diss}}/N_{s,l} = 465$ MeV, and the value of the same quantity for the first radiation length in the ground turns out to be 71 MeV. The quantity $N_{s,l}$ represents the size, at sea level, of a shower initiated by a particle of energy $E_0 = 10^{16}$ eV. On average it is 3×10^6 (ref. 7). Furthermore, we have to consider the lateral distribution of these particles to estimate the energy dissipated within the 2 m disk. Near the shower axis the density of particles, D , is well represented by the expression

$$D = N_{s,l}/500r$$

so that the number of particles inside the disk turns out to be approximately 2.5% of the total number. Thus, the energy dissipated in the first radiation length in the ground and in the region with $r = 2$ m will be

$$E_{\text{diss}} (r \leq 2 \text{ m}) = 2.5 \times 10^{-2} \times 3 \times 10^6 \times 71 \times 10^6 = 5.3 \times 10^{12} \text{ eV}$$

Assuming¹ that 5.5% of E_{diss} will be converted into photons with an energy of 0.511 MeV, a total of $5.7 \times 10^8 \gamma$ will be emitted. The detector has an estimated geometric factor of 3.6×10^{-4} so that about 210 photons are finally seen. That is an order of magnitude smaller than the lower value of γ detected in the four observed events.

To increase this value, a higher primary energy, E_0 , of more than 10^{17} eV is required. In this case, however, the flux of cosmic rays, Φ , is $2 \times 10^{-10} \text{ m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (ref. 8). Thus, with a sensitive area, A of 10 m^2 , and a solid angle of acceptance, Ω of 3.1 sr, and assuming a running time, T , of 1.3×10^7 s, the number of events which can be expected is

$$n = \Phi A \Omega T = 0.08$$

In fact, four were observed under the same conditions.

Therefore, it seems to us that the hypothesis of γ -ray production from positrons of an EAS annihilating at rest has to be ruled out.

We thank Professor M. Galli and Dr G. Cavallo for discussions.

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¹ Crawford, J. F., *Nature*, **239**, 395 (1972).

² Ashby, D. E. T. F., and Whitehead, C., *Nature*, **230**, 160 (1971).

³ Bassi, P., Clark, G., and Rossi, B., *Phys. Rev.*, **92**, 441 (1953).

⁴ Cocconi, G., *Handbuch der Physik*, **46/1**, 215 (1961).

⁵ Segre, E., *Nuclei and Particles* (Benjamin, New York, 1965).

⁶ Greisen, K., *Progress in Cosmic Ray Physics*, **3** (North-Holland, Amsterdam, 1956).

⁷ Dixon, H. E., Earnshaw, J. C., Hook, J. R., Smith, G. J., and Turver, K. E., thirteenth Int. Conf. on Cosmic Ray, *Conference Papers*, **4**, 2473 (1973).

⁸ Hayakawa, S., *Cosmic Ray Physics* (Wiley Interscience, New York, 1969).

Observations from Skylab of mesoscale turbulence in ocean currents

PHOTOGRAPHS and visual observations made by astronauts aboard Skylab have revealed remarkable eddies embedded in warm-water currents flowing poleward from equatorial oceans. These visual displays of mesoscale turbulence provide us with (1) a concept of the size and shape of such features, (2) an opportunity to examine the places of origin, (3) a feel for the rate at which they coalesce, and grow downstream, and (4) the opportunity to ascertain their significance in the thermal energy regime of the oceans.

I identified the eddies from photographs taken aboard Skylab 2 (May-June 73) over the northwest Caribbean Sea. Lying in the current, on the left-hand side, the vortices varied in diameter from 3-20 nautical miles. The associated atmospheric manifestations indicated cool surface water temperatures in the eddies, and down-current boundaries with waters warmer than those of the open current. There were (1) a smoother sea surface within the eddies than out-

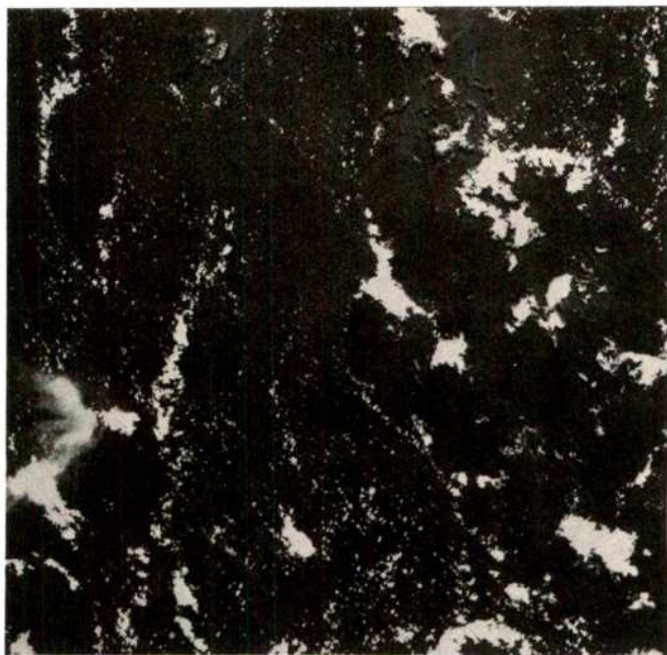


Fig. 1 Photograph (NASA No. SL2-10-072), taken vertically from the colour band of the Itek, 6-lens, 70 mm camera, with a 6 inch focal length lens, aboard the Skylab Space Station in June, 1973. The centre point of the area photographed is about $19^\circ \text{ N } 85^\circ \text{ W}$ and the ocean area covered is 60×60 nautical miles ($3,600 \text{ nm}^2$). Winds were from the southeast at 15-20 knots; the white, small "puff-ball" cumulus being aligned with the wind. The Yucatan Current flows north (top of picture) at speeds of 1-1.5 knots in this location, during this month of the year. The sea surface expression of the eddies are the oval, smoother, apparently indented features, and the atmospheric manifestations are the (1) disruption of the cloud streets, and (2) alignment of towering cumulus (to 35,000 feet) on the downstream edge of the eddies.

side, (2) a disruption of the wind-driven Langmuir circulation in both the sea surface and the overlying atmosphere, (3) towering crescent-shaped cumulus over the down current boundary, and (4) clear skies over the eddies (Fig. 1).

Although turbulent eddies of this nature and size have not been well documented, Spilhaus¹ noted eddies 30 miles in diameter during tests of the mechanical bathythermograph in the Gulf Stream. He pointed out that such scales

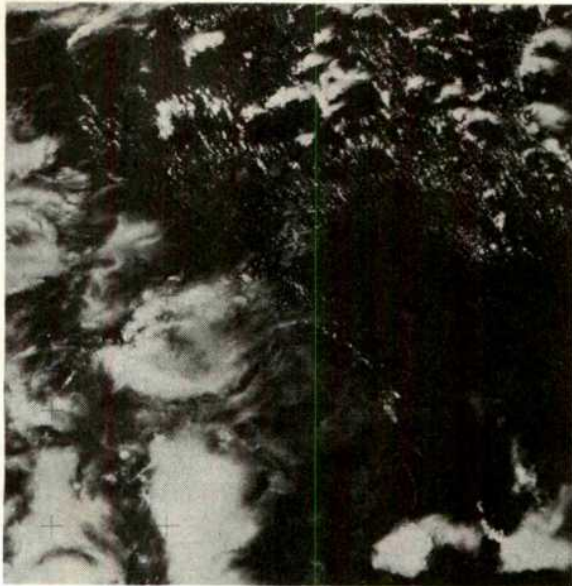


Fig. 2 Photograph (NASA No. SL4-137-3608) taken with a handheld Hasselblad, 70 mm camera, 100 mm focal length, from the Skylab Space Station, December, 1973, of the Southwest Atlantic Ocean near the coast of Argentina; Puerto Deseado was visible through the cumulus, middle left in the photograph. The centre point of the ocean covered by this photo is about $48^{\circ} 30' S$ $63^{\circ} W$ and the area is 210×210 nm ($44,400$ nm²). During this time of the year, upwelling takes place adjacent to the coast (beneath the huge, white cumulus caught in this photo) whereas about 100 miles offshore, a flow of warm water (50 – $55^{\circ} F$) moves south. Farther offshore, the cold (44 – $45^{\circ} F$) Falkland Current intrudes northward to the latitude of Montevideo ($35^{\circ} S$). On the day this photo was taken, the warm, nearshore counter-current was seen to be defined by the "puff-ball" cloud streets in the overlying marine atmosphere, into which intruded the clear skies overlying the cold eddies. The strong interaction between the countercurrent and the Falkland Current was manifested on this day by the strong blooms of phytoplankton; the light-coloured waters visible beneath the cumulus clouds.

implied a high rate of shear and were of the order of magnitude estimated by Rossby on the basis of a $10^{-3} s^{-1}$ shear. Further notations of "cold-water eddies"^{2,3} have been of eddies on current boundaries; formed from meanders, and are thus of a differing origin. That such turbulence has gone without much documentation is probably because of the (1) difficulty of detecting them by conventional oceanographic techniques, (2) a lack of knowledge of the sizes, persistence, and continuity of such features, and (3) the lack of photographs from orbital platforms which enhance the ocean/atmosphere manifestations of the eddies.

Because of their distinctive features, it was believed that the eddies could be examined visually by the three astronauts (Lt Col. Gerald Carr, Lt Col. William Pogue, and Dr Edward Gibson) aboard Skylab 4. Consequently, a request was made for visual observations by the astronauts early on the morning of 6 December 1973, as the spacecraft crossed the Caribbean Sea.

The characteristic clouds were indeed seen by all three astronauts, but the Sun angle was too low to enable examination of the ocean surface in the Sun's reflection. The next day, during a parallel orbital pass, but 180 miles to

the east, television on board scanned the Caribbean Sea, and the crescent-shaped clouds associated with the eddies were monitored to the coast of Columbia. Distinct eddies had also been caught by the camera during Skylab 3 (August–September), just south of those interpreted from Skylab 2. Similar cloud arrangements were also photographed south of Western Samoa, and the Gulf Stream east of Hatteras. The evidence was convincing, therefore, that turbulent vortices were constantly embedded within the main stream of warm ocean currents, and that they could be visually monitored from space.

Subsequently, observations from Skylab 4 confirmed that the turbulent features were in four other ocean areas (Coral Sea, North and South Equatorial Pacific, and western south Atlantic). Furthermore, eddies with similar surface manifestations, but not the towering cumulus, were observed east of New Zealand and in the Pacific north of Hawaii. These latter observations were confirmation that turbulent vortices of such magnitude are present wherever conditions are suitable, regardless of water temperature, and that eddies in warm currents are simply easier to see (and photograph) because of the atmospheric manifestations (Fig. 2).

We still had no definitive data, however, from the Caribbean Sea. Consequently, on January 24, 1974, personnel from the Navy's Weather Reconnaissance Squadron Four flew over the northwest Caribbean Sea, planting air expendable bathythermographs (AXBTs) along two lines, each about 200 miles long. An AXBT was dropped every ten nautical miles on both tracks. Heading south, the aircraft followed a flight line directly over waters on the left side of the core of the Caribbean Current. The second line was 60 miles to the east over the axis of the current.

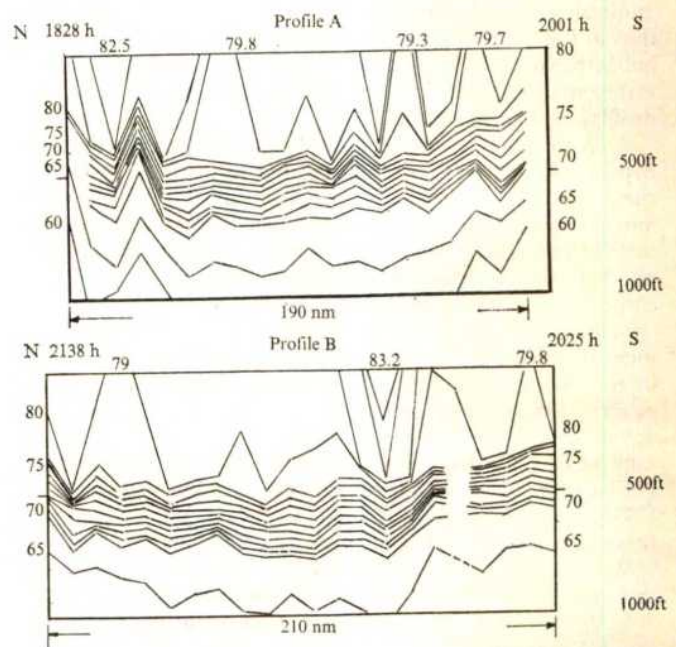


Fig. 3 North and south profiles of the vertical distribution of water temperature in the Yucatan Current on January 24, 1974. Air Expendable Bathythermographs (AXB) were dropped every 10 nm from a weather reconnaissance aircraft of the US Navy's VW-4 Squadron, Jacksonville, Florida. Profile A was flown first, beginning at 1828 GMT from $20^{\circ} 30' N$ $86^{\circ} 40' W$, the aircraft proceeding south-east for 190 nm. Skylab orbit overhead along the aircraft's track from 1831–1831:35 GMT. Profile B was started at 2138 GMT from $18^{\circ} N$ $83^{\circ} 40' W$, the aircraft proceeding north for 210 nm. The upward "doming" of cold water in the cyclonic eddies was encountered frequently along the first flight line, as expected in that the waters were well to the left of the current axis. (Note that the isotherm interval in the illustration is $1^{\circ} F$ to 70° ; then $5^{\circ} F$ to 60° . The vertical temperature gradient is nearly constant to depths of 1,000 feet.)

The temperature profiles (Fig. 3) indicated cyclonic eddies along the length of the flight line. The most dramatic modification of the ocean's thermal structure was in the vertical displacement of the deep-lying thermocline. Along the core of the current, the displacements of the isotherms were more subdued than to the west. It became clear from these data that the turbulent features were constantly forming and growing in the strongly flowing current.

The two most likely sources for the eddies are (1) seafloor irregularities projecting into the surface layers, and (2) shear stresses along discontinuities in the current.

There are reefs on the Honduras Shelf over which the Yucatan Current flows before reaching the waters in which the eddies were photographed first. That the eddies show no organised shedding, such as Von Kármán or Kelvin-Helmholtz vortices, could simply be the result of multiple points of origin, thus masking organised structure. But, similar eddies and their uniquely associated clouds were observed from the Skylab 4 spacecraft in the Brazil Current (Fig. 2), the equatorial Pacific, the Coral Sea, the southern Caribbean Sea, and the Gulf Stream. In none of these areas do seafloor features jut into the surface currents. Such an origin does not seem to account, therefore, for the observed eddies.

The density discontinuity of the thermocline is well formed in the Yucatan Current⁴. A shear along this horizontal surface would result in turbulence, probably in the form of elongated "rols", however. A rotation around a near-vertical axis would be precluded⁵.

Probably, shear stresses across the velocity profile initiate the turbulence (as noted by Spilhaus¹, and calculated by Rossby) rather than the other causes considered. Wherever the eddies have been observed, the current velocity diminishes sharply over distances of only a few miles from that in the current core. That the eddy shape is not circular indicates that the velocity shear takes place along a sloping rather than a vertical plane which is consistent with velocity profiles in ocean currents.

Such turbulence has been studied in the laboratory⁶, and Brown and Roshko⁷ have examined it in detail and theorised on the development and growth of eddies as they move downstream. Should the Yucatan-type eddies be of this origin, then we must expect them to decrease in number and increase in size in a down-current direction; conserving vorticity through the sequence.

Some details from the ocean may come from an experiment planned for the Coral Sea in March 1974, by scientists in the Royal Australian, New Zealand and United States navies. The earliest opportunity to check the concept of down-current eddy amalgamation, which in the ocean must take place over distances of 1,000 miles, will be during the joint orbital manned mission of the United States and the USSR, in July of 1975. By mid-1975, a better definition of these unique ocean features may be possible.

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¹ Spilhaus, A. F., *J. mar. Res.*, **3**, 51-75 (1940).

² Stommel, H., *The Gulf Stream*, 2nd edit. (University of California Press, Berkeley and Los Angeles, 1965).

³ Baranov, Ye. I., *Oceanology*, **7**, 61-65 (1967).

⁴ Wüst, G., *Stratification and Circulation in the Antillean-Caribbean Basins, 1, Spreading and Mixing of the Water Types with an Oceanographic Atlas*, 201 (Columbia University Press, New York, 1964).

⁵ Schubauer, G. B., and Tchen, C. M., *Turbulent Flow*, 123 (Princeton University Press, Princeton, 1961).

⁶ Liepman, Hans, in *Mechanics of Turbulence*, 211-227 (Gordon and Breach, 1961).

⁷ Brown, G. L., and Roshko, A., *J. Fluid Mech*s (in the press).

Lipid chemistry of eastern Mediterranean surface layers

It has been well established that organic surface films exist in most of the world's major oceans¹⁻⁹. They consist mainly of natural lipid material, with generally much smaller, though variable, amounts of petroleum hydrocarbons. Because the Mediterranean Sea is a restricted area the input of petroleum hydrocarbon is more noticeable than in most oceanic areas, and the level of surface pollution is probably increasing there because the major outflow occurs below the surface in the Straits of Gibraltar.

On a recent cruise of RRS Discovery in the Eastern Mediterranean, heavy oil slicks were encountered south of Cyprus and off the coasts of Libya and Egypt. Large coherent lumps of tar (tarballs) were often present with these heavy slicks, and in many places the associated surface films covered more than 60% of the surface area. In addition, when windspeeds were less than 10 knots, films not associated with oil slicks covered fairly large areas (30-40% at times). In certain coastal areas these surface films were accompanied by oil/water emulsions.

The surface films, emulsions, tarballs and surface living plankton were sampled at certain stations well away from heavy oil slicks in the eastern Mediterranean (Fig. 1). Their lipid composition and the extent to which petroleum hydrocarbons are entering the lipids of organisms which are exposed to high levels of oil pollution have been studied (Table 1).

A more detailed treatment of the results and sampling techniques will be published elsewhere¹⁰, but it should be emphasised that great care was taken to remove surface oil from organisms before analysis. The results can be summarised as follows:

Tarballs: in areas which were not grossly polluted by heavy slicks the distribution of tarballs was estimated to be 0.7-10 mg m⁻².

Emulsions: large scale distributions of oil/water emulsions were found off the Libyan and Egyptian coasts, possibly resulting from the practice of washing oil tanker's storage tanks down with high pressure water and discharging the washings before entering port. We made no estimate of the total organics present in the emulsion form, but a 54-mesh net collection gave a distribution of 0.1-0.5 mg m⁻² near the coast, with much lower concentrations in areas away from the coast. The emulsions were composed of both natural product organics (< 15% total extract) and a complex mixture of pollutant hydrocarbons (> 75% total extract).

Surface film: the surface film samples gave a distribution of 40-230 mg of organics per m², giving an estimated film thickness of 0.0005-0.003 cm. These values are similar to the estimate¹¹ that the oil slick observed six days after the stranding of the Torrey Canyon, which measured 10 by 40 miles, would have had an average thickness of 0.003 cm. The films were composed of both natural product lipids (< 5% total extract), which would be expected to form normal monomolecular films^{2,4}, and the complex mixture of pollutant hydrocarbons (> 85% total extract), which would give rise to polymolecular films^{9,12}.

Zooplankton: samples of near-surface crustaceans, fish and mixed plankton contained large amounts of hydrocarbons in their lipids (17-33% of total lipid; the lipid content of the animals was 2-3% of the total wet weight). These hydrocarbons

Table 1 Details of the samples

Sample number	Position	Type of sample	Estimated weight of tarballs (kg km ⁻²)	Estimated weight of emulsion (g km ⁻²)	Estimated weight of surface film (mg m ⁻²)	Hydrocarbon content of zooplankton (% of total lipid)
1*	35° 18' N 22° 5' E	Surface film	—	—	190	—
2	33° 8' N 22° 57' E	Tarballs	6.1	400	—	—
3	32° 59' N 25° 52' E	Emulsion	3.4	480	—	—
4	33° 11' N 27° 13' E	Tarballs	5.5	350	—	—
		Emulsion	—	—	—	—
		Crustaceans A	—	—	—	30
		Crustaceans B	—	—	—	19
		Fish	—	—	—	17
5	33° 3' N 29° 4' E	Tarballs	2.1	150	—	—
		Emulsion	—	—	—	—
6	32° 35' N 30° 31' E	Tarballs	0.7	100	—	—
		Emulsion	—	—	—	—
		Crustaceans	—	—	—	30
7	33° 27' N 31° 15' E	Tarballs	10.0	30	—	—
		Emulsion	—	—	—	—
8	35° 42' N 31° 41' E	Tarballs	5.4	65	—	—
		Emulsion	—	—	—	—
9	34° 37' N 31° 44' E	Tarballs	1.9	110	—	—
		Emulsion	—	—	—	—
		Mixed zooplankton	—	—	—	33
10a } †	34° 26' N	Surface film	—	—	135	—
b }	33° 11' E	Surface film	—	—	200	—
c }	(approximately)	Surface film	—	—	230	—
d }		Surface film	—	—	40	—

* Windspeed < 10 knots.

† Windspeed < 4 knots.

were composed mainly of the complex mixture found in the emulsion and surface film samples, quite unlike the much simpler, natural product hydrocarbon composition normally found in unpolluted marine organisms¹⁹⁻²¹. In addition, natural product hydrocarbons are usually only minor components of zooplankton lipids (< 5% total lipid)^{22,23}.

These results confirm that the surface layers of the eastern Mediterranean are polluted with petroleum hydrocarbons in the form of surface films, slicks, oil/water emulsions and tarballs;

light penetration must all be considered among the long term physical effects of this pollution. The presence of high levels of non-natural product hydrocarbons in the lipids of the zooplankton samples is also a cause for concern. We suggest that either the zooplankton species examined do not have an effective metabolic mechanism for dealing with non-natural product hydrocarbons, or this mechanism has been overloaded by high levels of pollutants. It may well be that these zooplankton species can tolerate high levels of pollutant hydro-

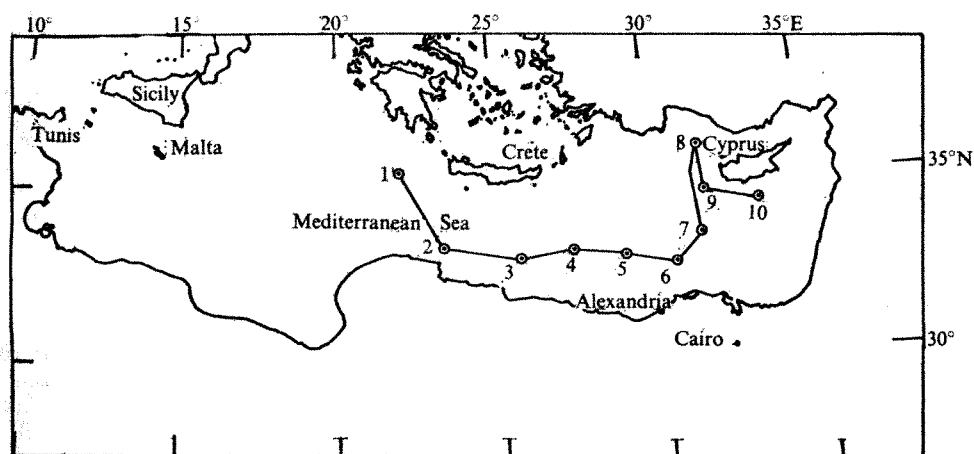


Fig. 1 Sample sites in the eastern Mediterranean. Numbers as in Table 1.

in many places the natural monomolecular surface films are swamped by polymolecular oil films. The combined natural and non-natural film material covers large areas of the surface of this region under calm conditions. The influence on interface properties such as evaporation, solid/liquid exchange, gas exchange between the atmosphere and sea, wave formation and

carbons in their lipids without being adversely affected but, nevertheless, the near-surface plankton do represent a stage at which these compounds can be introduced into the marine food web.

In view of the increase in oil tanker traffic in the Mediterranean which will result from the re-opening and proposed

enlarging of the Suez Canal, it seems likely that the situation can only become worse.

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Received June 17, 1974.

- ¹ Ewing, G., *J. mar. Res.*, **9**, 161 (1950).
- ² Garrett, W. D., *US Naval Research Laboratories Report 6201* (1964).
- ³ Garrett, W. D., *Limnol. Oceanogr.*, **10**, 602 (1965).
- ⁴ Garrett, W. D., *Deep Sea Res.*, **14**, 221 (1967).
- ⁵ Garrett, W. D., *J. mar. Res.*, **25**, 279 (1967).
- ⁶ Williams, P. M., *Deep Sea Res.*, **14**, 791 (1967).
- ⁷ Jarvis, N. L., *Limnol. Oceanogr.*, **12**, 213 (1967).
- ⁸ Garrett, W. D., in *Symposium on Organic Matter in Natural Waters* (edit. by Hood, D. W.), 469 (University of Alaska, Fairbanks, 1970).
- ⁹ Garrett, W. D., in *The Changing Chemistry of the Oceans* (edit. by Dyrssen, D., and Jagner, D.), 75 (Wiley Interscience, New York, 1972).
- ¹⁰ Morris, R. J., *Mar. Pollut. Bull.* (in the press).
- ¹¹ Smith, J. W., in *Torrey Canyon Disaster* (Warren Spring Laboratory, Stevenage, 1967).
- ¹² Garrett, W. D., *US Naval Research Laboratory Report 7372* (1972).
- ¹³ Blumer, M., Robertson, J. C., Gordon, J. E., and Sass, J., *Biochemistry*, **8**, 4067 (1969).
- ¹⁴ Blumer, M., Guillard, R. R. L., and Chase, T., *Mar. Biol.*, **8**, 183 (1971).
- ¹⁵ Han, J., and Calvin, M., *Proc. natn. Acad. Sci. USA*, **64**, 436 (1969).
- ¹⁶ Winters, K., Parker, P. L., and Van Baalen, C., *Science*, **158**, 467 (1969).
- ¹⁷ Albro, P. W., and Dittmer, J. C., *Lipids*, **5**, 320 (1970).
- ¹⁸ Ackman, R. G., *Lipids*, **6**, 520 (1971).
- ¹⁹ Lee, R. F., and Loeblich, A. R., *Phytochemistry*, **10**, 593 (1971).
- ²⁰ Youngblood, W. W., and Blumer, M., *Mar. Biol.*, **21**, 163 (1973).
- ²¹ Youngblood, W. W., Blumer, M., Guillard, R. R. L., and Fiore, F., *Mar. Biol.*, **8**, 190 (1971).
- ²² Morris, R. J., *J. exp. mar. Biol. Ecol.*, **13**, 55 (1973).
- ²³ Morris, R. J., and Sargent, J. R., *Mar. Biol.*, **22**, 77 (1973).

Archaeomagnetism and archaeoclimatic 'forecast'?

It has been suggested¹ that the semipermanent pattern of depressions of tropospheric pressure in the north polar regions may be associated with areas of high magnetic field intensity in Canada and Siberia. The physical reasons for the correlation are obscure, because contrary to earlier suggestions, field lines near the magnetic pole are not linked to the interior of the magneto tail². Nevertheless, the hypothesis, as an empirical principle, is of wide interest in that it would not only organise archaeoclimatic data but it would also form a basis for long term forecasting of general circulation changes.

I here test some aspects of the hypothesis by correlating archaeomagnetic and archaeoclimatic observations from various regions of the globe. Although there may be a tenuous correlation between them in some cases, the total pattern of archaeoclimatic regimes from central Europe to western America does not seem to follow the westward drift of the geomagnetic field pattern. I suggest that climatic regimes may be composed of a global component—related to variations of the solar constant—and of a possible westward drifting component.

First, if there is geomagnetic control of the pressure pattern in the troposphere^{1,3}, then a climatic response to long term variations of geomagnetic field intensity may be expected over the whole globe. Indeed, if the main dipole field weakens, as at present, there is reason to believe that cosmic ray particles would be more readily accessible to the atmosphere⁴. Because the geomagnetic dipole moment, M , declined to approximately

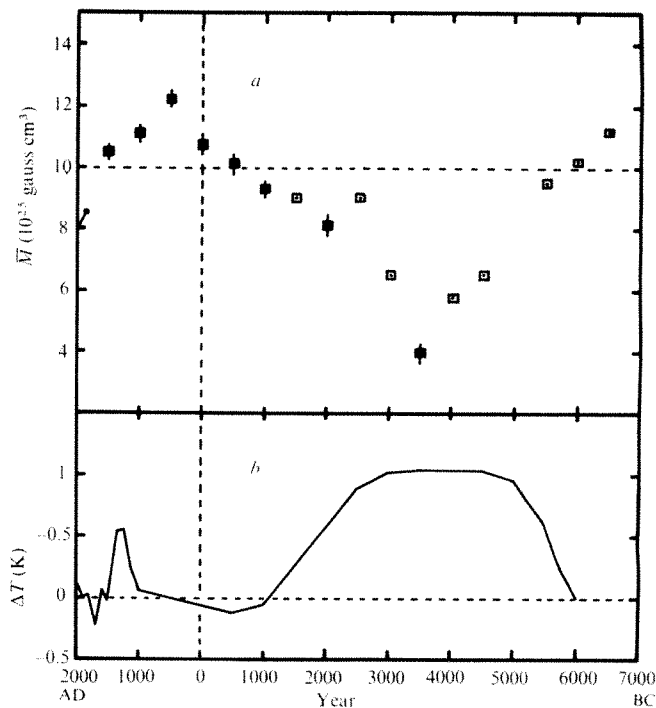


Fig. 1 *a*, World averaged dipole magnetic moment of the Earth for the past 9,000 yr (refs 5 and 6). Solid squares, averages with statistically significant number of samples. *b*, Damon's construction⁷ of deviations from the reference level (1890 AD) of long term averaged temperature. The construction is based on historical records and ocean cores.

half of its present value in the period 3000–5000 BC (Fig. 1*a*; refs 5 and 6), it may be expected that there was a major climatic response in the same period. Figure 1*b* shows a construction⁷ of deviations from the reference level (1890 AD) of long term averaged temperature according to Damon⁷. Although the actual value of ΔT is debatable⁷, it is important to note that the major climatic optimum of postglacial times—3000–5000 BC—seems to stand in anticorrelation with the intensity of the geomagnetic field. As the climate may be affected by many factors, including changes in the solar constant and in the oceanic circulation this single correlation cannot be construed as evidence in support of King's¹ hypothesis.

The hypothesis that the westward drift of the semipermanent depression of tropospheric pressure follows the westward drift of the geomagnetic field pattern, can be tested by considering local archaeomagnetic and archaeoclimatic data according to longitude (Fig. 2). Similar variations in the archaeomagnetic field intensity—in units of the present value, B_0 —in both central Europe and Arizona–Mexico (Figs 2*a* and *c*) can, according to Bucha⁸, be interpreted as resulting from the westward drift of the northern geomagnetic field pattern (there are two regions of stronger field, over both regions): that would account for the 500-yr phase difference between the two sets of data.

The qualitative climatic variations of Greenland—midway between Central Europe and Arizona–Mexico—can be represented in terms of $^{18}\text{O}/^{16}\text{O}$ isotopic ratio deviations obtained from ice cores (Fig. 2*b*; ref. 9). In order to trace the possible westward drift of clear-cut geomagnetic and climatic events, I have identified on Fig. 2*a* some climatic episodes in central Europe. The occurrence of a cold period in Poland and Czechoslovakia between 800 BC, and 200 BC, is well established^{10,11}, although a later warm period may have been interrupted by minor cold episodes. The empirical association of this pre-Christian cold episode with the peak in geomagnetic field intensity at 500 BC, is compatible with the hypothesis that a semipermanent low may have been located in the north of central Europe at that time. The consistency of this empirical association, as well as the westward drift hypothesis, can be

tested. A cold episode occurred in Greenland at about 100–400 BC, some 200–300 yr after that in central Europe (Fig. 2b). (Although there are difficulties in the chronology of ice-core analysis, ref. 9 shows that the data are consistent with historical records in the short term, and with varve and ocean core chronology in the long term). On the other hand there is no consistent association between climatic and magnetic episodes in post-Christian central Europe between their westward drift to Greenland (Figs 2a and b). Nevertheless, if the sequence of climatic and geomagnetic associations during the cold episode in the first millennium BC is taken seriously, King's hypothesis would lead to the conclusion that such an episode would have drifted to western North America by about 200 AD, in accordance with the geomagnetic field drift (Fig. 2). The climatic record of the White Mountains of California has been derived from tree rings of the bristlecone pine (Fig. 2d; ref. 12). It can be seen that a cold episode did occur in the

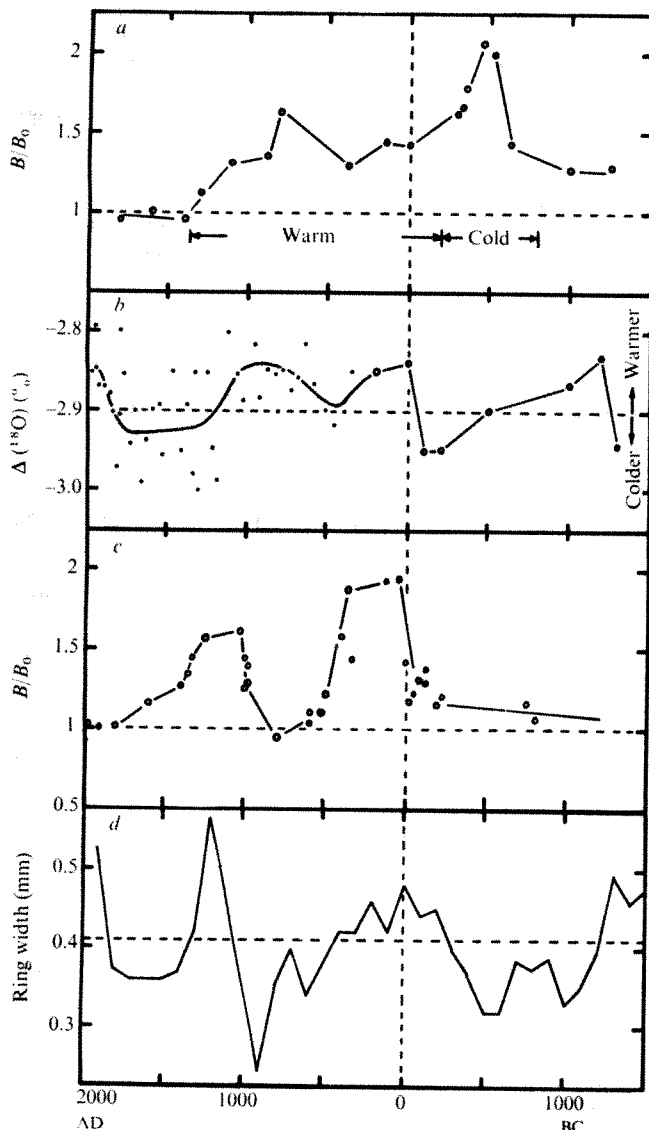


Fig. 2 a, Archaeomagnetic intensity, relative to the present value B_0 , for central Europe, 15° E (ref. 8). The indicated warm period, 200 BC–1400 AD may be interrupted by minor cold epochs. The Little Ice Age of circa 1600 AD is omitted. b, Archaeoclimatic data for Greenland (40° W) based on percentage deviation of $^{18}\text{O}/^{16}\text{O}$ isotopic ratio in ice cores⁹. Longer term averaged data before 200 AD taken from Fig. 4 of ref. 9. The continuous curve after 200 AD is drawn through the centroid of shorter term averaged data from Fig. 2 of ref. 9. c, Archaeomagnetic intensity, relative to the present value B_0 , for Arizona-Mexico, 105° W (ref. 8). d, Climatic record of the White Mountains of California (120° W) as indicated by the widths of bristlecone pine tree rings¹². In each case the horizontal dashed line is the mean.

first millenium AD; it was however, considerably later than 200 AD. Consequently, the archaeomagnetic and archaeoclimatic evidence available at present indicates only a tenuous correlation, at best.

In examining and interpreting the correlation of data discussed here, account must be taken of the preliminary nature of Bucha's⁸ interpretation of changes in field intensity observed for central Europe and western America. He suggested that they resulted from the westward drifting non-dipole field; even though the estimated rate of westward drift is in agreement with the better established nondipole drift of later epochs. As the data in Fig. 2 seem to be the only archaeoclimatic and archaeomagnetic data available at present for the same locations during the same epochs, I present them not only to indicate the present evidence for or against King's hypothesis, but also to emphasise the need for simultaneous and local data of both types. Evidence presented in support of the proposed hypothesis, including the original analysis¹, is based on isolated correlations; therefore, it seems that a systematic test, such as the determination of the westward drift of geomagnetic and climatic patterns, would be crucial in determining whether the hypothesis would be useful as an empirical principle for organising archaeoclimatic data. On the other hand, the organisation of climatic records according to longitude, such as in Figs 2a–d, does serve to distinguish global from local climatic episodes. Despite possible correlations with the geomagnetic field pattern, it is likely that the cold episodes at about 500 BC, and at about 1600 AD, are global in nature.

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- ¹ King, J. W., *Nature*, **245**, 443 (1973); *ibid.*, **247**, 131 (1974).
- ² Paulikas, G. A., *Rev. Geophys. Space Phys.*, **12**, 117 (1974).
- ³ Roberts, W. O., and Olson, R. H., *Rev. Geophys. Space Phys.*, **11**, 731 (1973).
- ⁴ Elsasser, W., Ney, E. P., and Winckler, J. R., *Nature*, **178**, 1226 (1956).
- ⁵ Cox, A., *Science*, **163**, 237 (1969).
- ⁶ Smith, P. J., *Geophys. J. R. astr. Soc.*, **12**, 321 (1967).
- ⁷ Damon, P. E., *Radiocarbon Variations and Absolute Chronology*, Nobel Symposium 12, 571 (edit. by Olsson, I. U., Wiley, New York, 1969).
- ⁸ Bucha, V., *ibid.*, 501.
- ⁹ Dansgaard, W., Johnsen, S. J., Moller, J., Langway, C. C., *Science*, **166**, 377 (1969).
- ¹⁰ Firbas, F., *Jena*, **1**, 480 (1949); **2**, 256 (1952).
- ¹¹ Starker, L., *Proc. int. Symp. World Climate 8,000 to 0 BC* (edit. by Swayer, J. S., 15, R. met. Soc., London, 1966).
- ¹² LaMarche, V. C., *Science*, **183**, 1043 (1974).

Interaction of coherent electromagnetic waves with relativistic electrons in a medium

A MECHANISM is proposed here for producing coherent photons by the interaction in a medium of relativistic electrons (moving with velocity greater than the electromagnetic phase velocity in the medium) with coherent electromagnetic waves incident in the opposite direction. A new effect of potential use is exhibited; it is a consequence of two distinct physical phenomena acting synergistically to result in double-Doppler-shifted coherent waves contained within a Mach cone. Its applications include production of narrow band radiation at higher frequencies and wave numbers than are at present available from

lasers, which has implications for holography and for improved pumping efficiency and production of narrow band submillimetre waves from microwaves, which has significance for communications.

When a material body moves in a medium with a speed greater than that of the waves it produces in the medium, the energy accumulated in these waves cannot get ahead of the moving body, resulting in a wave with a sharp discontinuity on the Mach cone. In the field of hydrodynamics, the basic phenomenon is a shock wave. For a charged particle moving at a constant speed in a medium with index of refraction greater than unity, and with particle speed exceeding the phase velocity of light in the medium, the radiated wavelets emitted by the medium under the action of the field of the particle at different points of its path interfere constructively at the Mach angle, resulting in Čerenkov radiation. The radiation vanishes outside the Mach cone and peaks sharply near the edge of the cone.

A similar discontinuity and attendant enhancement of radiation near the edge of the Mach cone ought to occur because of the effect, on suitable media and at appropriate particle velocities, of the scattered-radiation field of a charged particle accelerated by a coherent electromagnetic field, but at the double-Doppler-shifted frequency that characterises Thomson (Compton) backscattering of laser light from energetic electrons.

Conversion of optical photons into ones of higher frequency in a vacuum by Compton backscattering of laser photons from energetic electrons has been observed^{1,2} in the laboratory. This vacuum effect^{1,2} does not fully exploit the coherent-wave nature of the laser source, and the original discussions³⁻⁵ of this effect did not consider the coherence properties of the incident radiation.

We extend these considerations by taking into account the coherence of the field driving the charged particle and by examining the laser-stimulated wave emission under conditions where the velocity of the scattered-field source exceeds that of field propagation in the medium. The properties of the scattered wave in these conditions are essentially different from Compton-backscattered waves in vacuum. We also discuss possible constructive interference from many-electron effects. The calculation is classical since the basic effect does not depend essentially on the quantum nature of light. Quantum corrections are small for most parametric ranges of interest.

We consider a relativistic electron moving with speed u , along the positive z axis, in an infinite medium of unit magnetic permeability. The electric field of the untruncated plane wave incident in the opposite direction (head-on collision) is

$$E_0(\mathbf{x}, t) = E_0 \sin(\omega_0 t + k_0 z), \quad (1)$$

where $k_0 c = \omega_0 n$, c is the speed of light in vacuum and n is the index of refraction at the incident laser frequency. All quantities and results are in the rest frame of the dielectric. We consider first the case of a slightly dispersive medium, so that the dielectric constant ϵ at the scattered frequency can be reasonably approximated by a constant, though we include the possibility $\epsilon = n^2$, and take $\beta^2 \epsilon > 1$, where $\beta = u/c$. The scattered double-Doppler-shifted field (in cylindrical coordinates ρ, z) is, to order E_0 ,

$$E(\rho, z, t) = 2(r_e/\omega_0)(\gamma/\gamma_0)E_0 \frac{\partial}{\partial t} \left\{ (\tau) \Theta(\gamma u \tau - \theta) \right. \\ \left. \times \cos[\Omega(\gamma^2 \tau - z/u)] \frac{\cos[\alpha(\gamma^2 u^2 \tau^2 - \rho^2)^{1/2}]}{(\gamma^2 u^2 \tau^2 - \rho^2)^{1/2}} \right\} \quad (2)$$

where r_e is the classical electron radius, $\gamma_0 = (1 - \beta^2)^{-1/2}$, $\gamma = (\beta^2 \epsilon - 1)^{-1/2}$, $\theta(x) = 1$ for $x > 0$ and $\theta(x) = 0$ for $x < 0$, $\tau = t - (z/u)$, $\Omega = \omega_0 + k_0 u$, and $\alpha = \gamma \Omega \epsilon$.

The component K_z of the wave number for propagation of the scattered wave in the z direction is

$$K_z = (\Omega_z/u)(1 + \gamma^{-2}) \quad (3a)$$

where

$$\Omega_z = \gamma^2 \Omega = [\omega_0(1 + n\beta)/(\beta^2 \epsilon - 1)] \quad (3b)$$

is the corresponding frequency of propagation; that is, this component of the wave has a phase velocity v_z dictated in part

by the electron velocity,

$$v_z = u(1 + \gamma^{-2})^{-1} = c/(\beta \epsilon) \quad (4)$$

Note that the maximum enhancement of the frequency, at least for the simple model considered here, occurs just above threshold for the effect. As β increases beyond threshold, for a constant ϵ , Ω_z decreases. This differs qualitatively from the vacuum effect, where the scattered frequency increases monotonically with β . The effect can therefore be optimised for certain parametric ranges by suitable adjustment of ϵ for a given medium, for example, by varying the density of a gas. Scattered coherent waves of 10^2 to 10^3 times incident infrared frequencies should be realisable in low-density gases with $0 < \epsilon(\Omega_z) - 1 \ll 1$.

The scattered field vanishes outside the Mach cone of half angle $\phi = \sin^{-1}(v_p/u)$, where $v_p = (c/\epsilon)^{1/2}$ is the phase velocity of electromagnetic disturbances in the medium; $v_z = v_p \sin \phi$. The intensity increases sharply as the conical edge is approached. The singularity in Equation (2) for the field on the cone is in reality smoothed out by the absorptive properties of a dispersive medium, as in the Čerenkov case, and by energy losses of the charged particle, which serve to place a lower limit on the parameter $Q = \gamma^2 u^2 \tau^2 - \rho^2$ that results in the singularity for $Q = 0$ when dispersion and these losses are not taken into account.

To estimate the field energy of the scattered double-Doppler-shifted wave, we keep only the dominant contribution to the expression (2) in the frequency range 10^{14} to 10^{18} rad s⁻¹ for ω_0 and take the energy loss in the medium into account phenomenologically. The ratio η of the energy scattered from a single electron to that of the portion of length L of the laser beam which overlaps the electron beam in the dielectric is then $\eta = (2\pi r_e^2/A)(L/L_1)(\gamma/\gamma_0)^2(\Omega_z/\omega_0)^2(\beta^2 \epsilon)^2(\Omega_z \tau_a)^2 \ln(R/2L_1)$, (5)

where R is the range of the electrons, L_1 is the distance over which the Mach cone builds up (the interaction length), A is the cross section of the laser beam and τ_a is a representative value of τ during the time T of interaction of a single electron with the incident wave in the medium. We assume the total lengths of the laser and electron beams to be larger than the dielectric thickness, which is readily achievable and renders independent of these lengths the ratio η and, for fixed electron beam density, the quantities η_{coh} and η_{incoh} given below. By definition, we then have $L \simeq L_1$, and geometry of the cone dictates $\tau_a \simeq T/3$. From equations (5) and (3b), η is proportional to γ^{10} , so that a change of $\beta^2 \epsilon$ from 1.2 to 1.05, for example, increases η by three orders of magnitude. We have, however, made no systematic effort to optimise the various relevant parameters such as dielectric constant, electron energy, or pulse time and total energy of either the electron or laser beam.

Expressions (2) and (5) apply to scattering from a single electron. The number N of electrons in the beam that act at a fixed time to produce the desired effect is given in terms of the total number N_0 of beam electrons by $N = N_0(d/L_e)$, where we assume the electrons to be uniformly distributed in a beam of length L_e and d is the thickness of the dielectric. Two limiting cases will be considered.

For electrons bunched in space-time so that the resulting phases $(\Omega_z t - K_z z)$ of the waves scattered from different electrons are all within, say, the same quadrant, these waves will add constructively. Because the electron and wave velocities are nearly equal, waves scattered from electrons bunched within $1/4$ wavelength λ of the scattered radiation will also be within $1/4$ period of one another. Such constructive interference from randomly distributed electrons may be realisable by using a thin dielectric film of thickness $\gtrsim \lambda/4$. The number N_c of electrons in the beam that can act coherently is then given by $N_c = N_0(\pi c/2 \Omega_z L_e)$. Additional enhancement may be obtainable by using a suitable array of periodically spaced thin films, by modulating the electron beam and by time phasing of the laser pulse. In adding coherently the contributions from different electrons, one should in principle fold in a factor for the spread in electron velocities. In practice, the phenomeno-

logical treatment referred to above takes this partly into account. For an interaction region larger than a wavelength, the total intensity of radiation from randomly distributed electrons will be the incoherent sum of the individual electron contributions. The ratio for coherent scattering from a beam of electrons interacting in a single thin dielectric, $d \approx \lambda/4$, is $\eta_{\text{coh}} = N^2 \epsilon \eta$. For $d \gg \lambda/4$, we get $\eta_{\text{incoh}} = N \eta$.

As a specific example, we consider an electron beam that delivers $N_0 = 10^{18}$ electrons in a 5×10^{-8} s pulse at an average energy of 5 MeV ($L_e \approx 1,500$ cm, $\gamma_0 \approx 10$), and a dielectric of thickness $d = 1$ mm, $R = 1$ cm and $\epsilon = 1.2$, so that $\gamma^2 \approx 5$ and $\Omega_e/\omega_0 \approx 10$. Taking an infrared laser (10^{14} Hz), for which $\Omega_e \tau_a \approx 6,000$ produces an ultraviolet incoherent scattered wave of relative intensity $\eta_{\text{incoh}} \approx 10^{-2}$. For a thin film of $d = \lambda/4 = 7.5 \times 10^{-6}$ cm, the coherent scattered wave is in the ultraviolet with $\eta_{\text{coh}} \approx 4 \times 10^{-5}$.

The portion of the electron beam that can contribute to the process will in general be limited by the time over which the essential dielectric and material properties of the medium are not destroyed by the laser or electron flux. Estimates for glass indicate no significant change in the electric susceptibility for the above laser and electron parameters. But for a 5-MeV electron beam with $N_0 > 10^{15}$, the physical state of the glass may alter before all the electrons have passed through.

The effect can also be used to generate coherent millimetre and submillimetre waves from intense coherent microwaves, such as from magnetrons, in slow wave or periodic structures (simulated dielectric media). In the case of such simulated dielectric media, where the effect we consider can actually take place in vacuum, the duration and intensity of the scattered radiation are not limited by either the dielectric or the material properties of a medium.

The discussion up to now has involved scattered radiation at energies below those at which atomic resonances occur, for which the assumption of slowly varying electric susceptibility is expected to be valid. The general nature of the physical mechanism underlying the effect we have discussed strongly suggests however that the qualitative features of the effect will persist in the region of anomalous dispersion corresponding to X-ray and γ -ray frequencies, inasmuch as the electric susceptibility for suitable media can be positive in these frequency ranges^{6,7}.

By adjusting such radiation to resonance with the energy required to excite some metastable state, the effect can be applied to achieve substantial population inversion at these higher frequencies. Such selective pumping to the metastable state would improve pumping efficiency over either flash or electron-beam excitation alone. The medium in which the radiation is produced can be, but does not have to be, the same as the system being pumped.

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¹ Kulikov, O. F., Telnov, Y. Y., Filippov, E. I., and Yakimenko, M. N., *Phys. Lett.*, **13**, 344 (1964).

² Bemporad, C., Milburn, R. M., Tanaka, N., and Fotino, M., *Phys. Rev.*, **138**, B1456 (1965).

³ Milburn, R. M., *Phys. Rev. Lett.*, **10**, 75 (1963).

⁴ Arutyunian, F. R., and Tumanian, V. A., *Phys. Lett.*, **4**, 176 (1963).

⁵ Arutyunian, F. R., Goldman, I. I., and Tumanian, V. A., *Soviet Phys. JETP*, **18**, 218 (1964).

⁶ Perelshtein, E. A., and Podgoretskii, M. I., *Soviet J. nucl. Phys.*, **12**, 631 (1971).

⁷ Kolpakov, A. V., *Soviet J. nucl. Phys.*, **16**, 554 (1973).

Intermolecular basis of odour

THREE quite different theories of odour discrimination are in favour at present. The 'site filling theory' of Amoore¹ relates molecular shapes to odour types and identifies some classifications as primary. The penetration and puncture theory of Davies² develops a physical model for impulse transmission. The key physical properties involved are the molecular size and the adsorption coefficient between air and a lipid-water interface. Wright³ has correlated vibrational frequencies in the far infrared with the quality of odour.

All these theories, however, do agree that although there is no clear relationship between the functional group(s) present and odour, the mechanism of interaction with the receptor(s) must be physicochemical involving either electronic, vibrational or rotational forces or any combination of these.

Here I present evidence which implicitly correlates both odour quality and odour thresholds with the depth of the intermolecular potential energy well formed between the odour-producing molecule and the unknown receptor site.

There is a relationship between the depth of the pairwise intermolecular potential energy well and the normal boiling point of a substance⁴; the depth of the well increases linearly with the boiling point (K). The negative derivative of this potential with respect to intermolecular separation is a measure of the electronic interactive force.

In the case of the interaction of a molecule with the receptor site the depth of the potential well formed will depend on the nature of the charge distribution of the odorant molecules. If only one basic type of receptor site is assured, with numerous formal charges, then some correlation should be possible between the normal boiling point of a molecule and its odour type. I have found the normal boiling points for 253 of 418 ethereal, camphorous, minty, almond, aromatic, floral and musk compounds listed by Amoore¹. Table 1 summarises the results giving average

Table 1 Average boiling points and standard deviations of different odour classes

Class*	Average boiling point (K)	s.d. (K)
Ethereal	346	66
Camphorous	450	40
Minty	453	36
Almond	457	45
Aromatic	482	49
Floral	498	48
Musk	636	81

* Pungent and putrid classes were not considered since these can be understood in terms of highly reactive functional groups.

values and standard deviations for each odour class. Ethereal compounds have the lowest boiling points with the musks at the other extreme. Both of these have average values quite far removed from those of the other groups (camphorous, minty and almond) whose average values are so close to one another as to be indistinguishable. This is interesting in that many of the compounds possess more than one of these three odours at a time. The lowest boiling compound of all the molecules is ethylene (ethereal, 169 K). Very low boiling compounds such as CO (81 K), CH₄ (112 K), O₂ (90 K), N₂ (77 K) have small interactive electronic forces with the receptor site and hence are odourless.

A better correlation is achievable if boiling points of compounds with the same functional group are classified separately. Table 2 lists saturated halides in terms of increasing boiling points. If one assumes that for the halides 377 K is the cutoff for ethereal compounds then one obtains

Table 2 Boiling points (b.p.) and odour classifications (o.c.) of alkyl halides

Alkyl halides	b.p.	o.c.*	Alkyl halides	b.p.	o.c.
Methyl chloride	249	E	Pentachloroethane	435	E
Methyl bromide	278	E	1,3-Dibromo 2, 2-dimethyl propane	460	E
Ethyl chloride	285	E	1,1,2-Tribromo 1,2,2-trifluoroethane	390	C
Ethyl bromide	311	E	3-Chloro 2,2,3-trimethylbutane	403	C
Methylene chloride	313	E	Hexachloroethane	458	C
Methyl iodide	318	E	1,1,2,2-Tetrabromo-chloroethane	475	C
Propyl chloride	329	E	1,1,2,2-Tetrabromo-difluoroethane	477	C
Chloroform	334	E	1,1,2-Tribromo-cyclobutane	503	C
Ethyl iodide	345	E	1,2,2,3-Tetra-bromopropane	528	C
Carbon tetra-chloride	350	E			
1,2-Dibromo 1,1,2-trifluoroethane	350	E			
Propyl iodide	375	E			
1,2,2,2-Tetra-bromoethane	377	E			
Bromoform	422	E			

Boiling point at 1 atm (K). Some of the values have been extrapolated from reduced pressure boiling points. With some of the musks we can only give an inequality but these are reasonable.

* Odour classification code. E, ethereal; C, camphorous.

$\lambda^2 = 0.25$ and $P = 0.38$. Similar success is obtained when looking at other functional groups.

In Table 3 the negative log of the odour threshold concentration is computed for a series of saturated alkanes and compared with experimental values⁹. The thresholds (negative log of the concentration) are computed by assuming the correct value for ethane and that the other thresholds should vary by the proportion of their boiling point (K) to that of ethane (K). These results give a high correlation $\lambda^2 = 1.22$ and $P = 0.005$. Since the potential well depth increases with increasing boiling point, fewer molecules would be needed to trigger an impulse. Similar success is obtainable with other classes of molecules.

Table 3 Computed compound with experimental values of the negative log of the threshold concentration for a series of alkanes

Compound	T (K)	Calculated threshold*	Experimental threshold†
Ethane	185	—	3.08
Propane	231	3.85	3.31
Butane	273	4.55	4.28
Pentane	309	5.14	5.32
Hexane	342	5.69	5.57
Heptane	371	6.18	5.79
Octane	399	6.64	6.21
Nonane	423	7.04	6.33
Decane	446	7.43	7.10
Undecane	468	7.79	6.83
Dodecane	488	8.12	6.66
Tridecane	507	8.44	6.64

* Negative log of the threshold concentration (mol l^{-1}) calculated by taking $(T_b(\text{K}) \text{ system}) / (T_b(\text{K}) \text{ ethane}) \times (\text{negative log of ethane threshold})$. (T_b = normal boiling point).

† Negative log of experimental threshold concentration (mol l^{-1}) determined from values in ref. 5.

The theory outlined above fits in with the Davies theory, since it may be the mechanism by which the odorant molecule opens up the space in the cell membrane to induce charge flow. The theory above also gives support to Wright's vibrational correlations since the vibrational energy level separations reflect the shape of the potential energy curve and hence the rate of energy transfer. It appears that the difference between minty, camphorous, and almond odours has more to do with the shape of the potential energy curve than with the depth of the well. In these terms the nearly infinite nuances of odour can be understood.

Randebrock⁶ has suggested that the α -helix protein

molecule may be a receptor site for odour. It should now be possible using electrostatic models based on quantum mechanical charge densities for such molecules to see if a correlation of potential energy surface and odour can be established for this or any other suspected receptor molecule.

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¹ Amore, J. E., *Molecular Basis of Odour* (Charles C. Thomas Co., Springfield, Illinois (1970)).

² Davies, J. T., *J. theor. Biol.*, **8**, 1 (1965).

³ Wright, R. H., *Nature*, **209**, 571 (1966).

⁴ Kauzmann, W., *Kinetic Theory of Gases*, 88–90 (Benjamin, New York, 1966).

⁵ Laffort, P., and Dravnieks, A., *J. theor. Biol.*, **38**, 335 (1973).

⁶ Randebrock, R. E., *Nature*, **219**, 503 (1968).

Coevolution of Danaid butterflies with their host plants

MALE butterflies of the nymphalid subfamily Danainae possess pheromone disseminating organs (hairpencils) the secretions of which function as female flight arrestants or aphrodisiacs^{1,2} and in some case as female attractants¹. The hairpencil secretions of a number of Danaid species contain dihydropyrrolizine derivatives³⁻⁸ which are obtained from pyrrolizidine alkaloid plants by adult feeding^{6,7}. A dihydropyrrolizine ketone isolated⁸ from the hairpencils of *Danaus gilippus herenice* Cramer has been shown to be the flight arrestant or aphrodisiac pheromone in this species^{3,9} and we believe that the related dihydropyrrolizines found on the hairpencils of other species may perform a similar function. We propose here one possible explanation for the unusual dependence of male Danaid butterflies on pyrrolizidine alkaloid plants.

Many Danaid species apparently store in their body tissues vertebrate heart poisons (cardenolides) sequestered by their larvae from food plants^{10,11} which are found primarily in the cardenolide containing sections of the Asclepiadaceae and Apocynaceae¹². The Danainae's association with cardenolide plants is considered to be an important factor protecting them from vertebrate predators.

The pyrrolizidine alkaloid plants most commonly sought out by adult males are species of the Boraginaceae which contain the 1,2-unsaturated allylic ester type alkaloids¹³ for which metabolism into the hairpencil dihydropyrrolizines is readily envisaged. Other sources of this particular type of pyrrolizidine alkaloid which are visited by male Danainae, are the tribes Senecioneae and Eupatorieae of the Compositae and the genus *Crotalaria*¹⁴ of the Leguminosae¹⁵.

We suggest that the Danaid's use of, and dependence on, pyrrolizidine alkaloids may have developed during a period when these alkaloids were constituents of their larval food plants. Based on current views of the phylogeny of the plants concerned we propose that the early larval food plants contained both pyrrolizidine alkaloids and cardenolides and that they, perhaps under pressure of insect predation (see below), split into a cardenolide and a pyrrolizidine alkaloid branch. The Danainae then retained protection from predators by evolving as larval feeders on the cardenolide plants with the males visiting pyrrolizidine alkaloid plants to obtain their pheromones.

Our proposal is consistent with the phylogenetic classifications of Cronquist¹⁶ and Takhtajan¹⁷ if the ancestral food plants occurred early in or before the evolution of the subclass Asteridae and diversified according to the scheme shown in

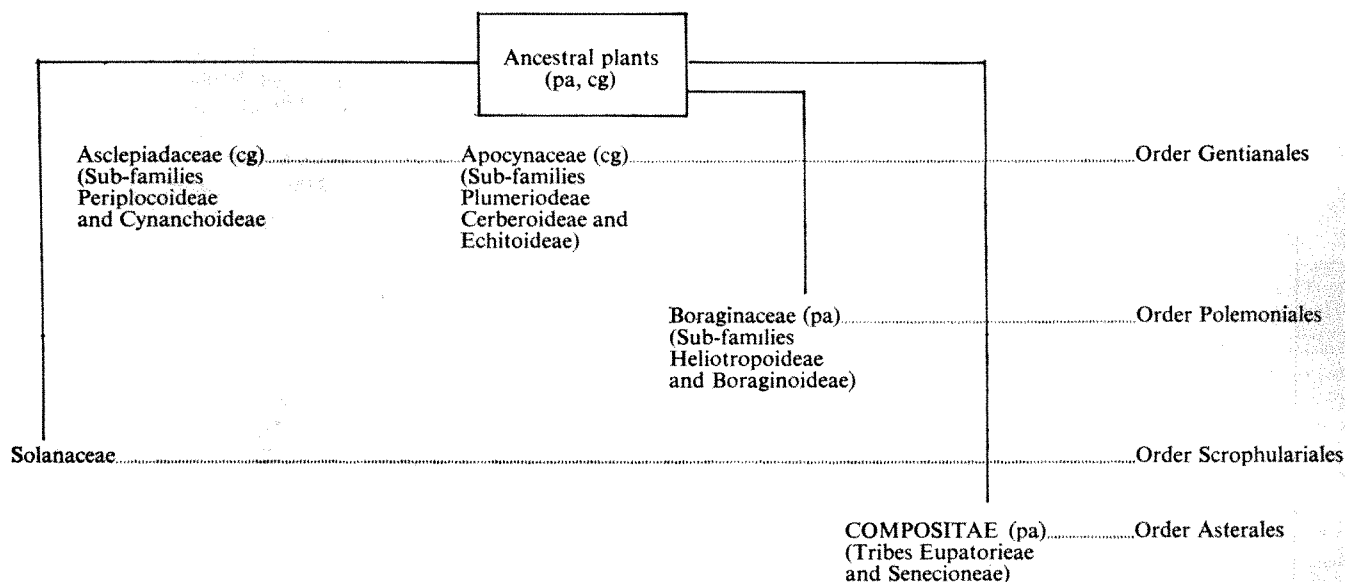


Fig. 1 Proposed origin of the pyrrolizidine alkaloid- and cardiac glycoside-containing sections of the plant families in the subclass Asteridae with which Danaid and Ithomiid butterflies associate showing the distribution of cardiac glycosides (cg) and pyrrolizidine alkaloids (pa).

Fig. 1. Of the pyrrolizidine alkaloid plants which male Danaids visit, only *Crotalaria* species are found outside this subclass. They may be an earlier branch of the postulated ancestral host plants (compare with Takhtajan¹⁶) or represent an independent development of this particular type of pyrrolizidine alkaloid.

Divergence of the original food plants might have occurred as a result of the dependence of the early Danaids on pyrrolizidine alkaloids and cardenolides. Because of their importance to the Danainae both of these substances, or related marker substances, probably provided the cues for locating, and the stimulus for ovipositing on, the early larval food plants. This would have favoured the survival of plants which were naturally deficient in one or other of these chemicals.

The possibility of co-occurrence of cardenolides and pyrrolizidine alkaloids in progenitor species is exemplified by *Urechites karwinskyi*, Mueller, (Apocynaceae) (also referred to as *Fernaldia pandurata* (A.D.C.) Woodson¹⁷). A dihydropyrrolizine closely related to the Danaid hairpencil dihydropyrrolizines has been isolated from this toxic plant¹⁸ and other toxic species of the genus are known to contain cardenolides^{19,20}. We regard as highly significant the fact that *U. karwinskyi* is used as a larval food plant by a primitive Ithomiid butterfly, *Tithorea harmonia salvadores* Staudinger, whose larvae bear a close affinity to those of certain Danainae²¹. The subfamily Ithomiinae (Nymphalidae) is closely related to the Danainae²¹ and in common with male Danaids, male Ithomiids are attracted to, and feed on *Heliotropium indicum* L. (Boraginaceae), which contains pyrrolizidine²². Their larvae feed primarily on Solanaceae¹² but notable exceptions are species of the genus *Tithorea* whose

larvae feed, like certain of the Danainae, on Apocynaceae²¹. The *Tithorea harmonia salvadores*–*Urechites karwinskyi* association may therefore be a conservative development from, and provide a model for, the ancestral association we have proposed.

By considering the pyrrolizidine plants visited by adult males to have evolved from earlier larval food plants the present behaviour of the Danaids and the development of the hairpencil chemicals as female flight arrestants and attractants can be more readily understood. Thus, it is envisaged that mating of early Danaids occurred in the vicinity of the larval food plants and that males subsequently developed the means of storing and disseminating volatile pyrrolizidine alkaloid-derived plant substances then used by the females to locate the larval food plants. By disseminating these substances in the vicinity of a female in flight they would be able to make her settle and thus effect mating in a place remote from the larval food plant. Such a development would have offered an expanded habitat for the insects and would have become essential for their survival during divergence of the original food plants when the females were being selected for their ability to find and oviposit on cardenolide plants, which gave their offspring protection from predators, while the males were seeking out pyrrolizidine plants.

Males make up over 90% of the butterflies attracted to pyrrolizidine plants but a small proportion of females of some species also visit them^{6,23} (Table 1). This may be the remnant of a behaviour pattern and olfactory acuity associated with location of the earlier larval food plants which has not yet been completely lost from the gene pool of the females. *Tithorea* species again seem to be exceptional and, in agreement with the

Table 1 Typical collections of Ithomiinae attracted to *Heliotropium indicum* L.

Species	Males	Females
* <i>Ithomia iphianassa iphianassa</i> D.-H.	166 (98.2%)	3 (1.8%)
* <i>Hymenitis darcetis</i> H.-S.	294 (99.7%)	1 (0.3%)
* <i>Pteronymia beebei</i> Fox and Fox	329 (94%)	21 (6.0%)
† <i>Tithorea harmonia megara</i> Latr.	3 (60%)	2 (40%)
‡ <i>Tithorea terracina pinthias</i> Godman and Salvin	2 (20%)	8 (80%)

* Collected at Estacion Rancho Grande, Parque Nacional Henri Pittier, Aragua, Venezuela; July–August; 1972–73.

† Collected at Wm. Beebe Research Station, Arima, Trinidad; July 1962.

‡ Collected at Cerro Campana, Panama; April 1971.

conservative development we have proposed for Tithorea, both males and females are attracted (Table 1).

The existence in the past of plants containing pyrrolizidine alkaloids and cardenolides might explain Rothschild's observation²⁴ that certain moths of the families Arctiidae and Ctenuchidae also utilise both these phytochemicals.

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- ¹ Brower, L. P., Brower, J. van Z., and Cranston, F. P., *Zoologica*, **50**, 1 (1965).
- ² Pliske, T. E., and Eisner, T., *Science*, **164**, 1170 (1969).
- ³ Meinwald, J., Meinwald, Y. C., Wheeler, J. W., Eisner, T., and Brower, L. P., *Science*, **151**, 583 (1966).
- ⁴ Meinwald, J., Thompson, W. R., Eisner, T., and Owen, D. F., *Tetrahedron Lett.*, 3485 (1971).
- ⁵ Edgar, J. A., Culvenor, C. C. J., and Smith, L. W., *Experientia*, **27**, 761 (1971).
- ⁶ Edgar, J. A., Culvenor, C. C. J., and Robinson, G. S., *J. Aust. ent. Soc.*, **12**, 144 (1973).
- ⁷ Edgar, J. A., and Culvenor, C. C. J., *Nature* (in the press).
- ⁸ Meinwald, J., Meinwald, Y. C., and Mazzocchi, P. H., *Science*, **164**, 1175 (1969).
- ⁹ Schneider, D., and Seibt, U., *Science*, **164**, 1173 (1969).
- ¹⁰ Reichstein, T., von Euw, J., Parsons, J. A., and Rothschild, M., *Science*, **161**, 861 (1969).
- ¹¹ Brower, L. P., *Scient. Am.*, **220**, 22 (1969).
- ¹² Ehrlich, P. R., and Raven, P. H., *Evolution*, **18**, 586 (1965).
- ¹³ Bull, L. B., Culvenor, C. C. J., and Dick, A. T., *The Pyrrolizidine Alkaloids* (North Holland, Amsterdam, 1968).
- ¹⁴ McCann, C., *J. Bombay nat. Hist. Soc.*, **51**, 752 (1952).
- ¹⁵ Cronquist, A., *The Evolution and Classification of Flowering Plants* (Nelson, London, 1968).
- ¹⁶ Takhtajan, A., *Flowering Plants Origin and Dispersal* (Oliver and Boyd, Edinburgh, 1969).
- ¹⁷ Woodson, R. E., *Ann. Mo. bot. Gdn*, **19**, 45 (1932).
- ¹⁸ Borges del Castillo, J., España de Aguirre, A. G., Bretón, J. L., González, A. G., and Trujillo, J., *Tetrahedron Lett.*, 1219 (1970).
- ¹⁹ Hunger, A., *Helv. chim. Acta*, **34**, 398 (1951).
- ²⁰ Hassall, C. H., *J. chem. Soc.*, 3193 (1951).
- ²¹ Gilbert, L. E., and Ehrlich, P. R., *J. lepid. Soc.*, **24**, 297 (1970).
- ²² Beebe, W., *Zoologica*, **40**, 27 (1955).
- ²³ Lever, R. A., *Proc. R. ent. Soc., Lond.*, **A**, **11**, 95 (1936).
- ²⁴ Rothschild, M., von Euw, J., and Reichstein, T., *Proc. R. Soc. Lond., B*, **183**, 227 (1973).

'Fimbriae' in the fungus *Ustilago violacea*

LONG fine hairs, called fimbriae¹, or pili², are commonly found attached to the cell walls of Gram-negative bacteria², but do not seem to have been reported in other organisms. We report here that the yeast-like sporidial cells of the anther smut fungus *Ustilago violacea* also produce fimbriae. At present the use of the term fimbriae for these fungal hairs merely denotes a close morphological and developmental similarity to the bacterial structures; however, preliminary investigations of the fungal 'fimbriae' indicate that there may be chemical and functional similarities too.

The fimbriae of *U. violacea* can readily be observed when sporidia from a log-phase population are washed at least three times in particle-free distilled water, and then air dried on a Formvar-coated grid before shadowing with

tungsten oxide at an angle of 18–25°. The fimbriae resemble bacterial fimbriae in the following ways.

(1) They are of similar dimensions, being about 1–10 μm long and 60–70° A in diameter. They are curved and flexible and do not branch or taper. The apparent branching of the fimbriae in Fig. 1 is common and occurs when they twine together to form multistranded 'cables' which may dissociate into the individual strands. They resemble the curved fimbriae of *Vibrio* spp.³ rather than the rigid, straight type I characteristic of *Escherichia coli*² and *Salmonella*⁴. We cannot be certain whether or not they have the hollow helical structure characteristic of these type I and F fimbriae, but apparently absent from the *Vibrio* forms.

(2) Fimbriae originate below the cell wall and penetrate through it. Freeze-etched preparations show particles of about 70–100 Å on the outer surface of the plasma membrane and corresponding projections on the inner surface of the cell wall. Sectioned walls are traversed by fibrils 70–100 Å in diameter which apparently are fimbriae passing through the cell wall.

(3) As in bacteria, populations of cells in logarithmic growth are the most fimbriated, while stationary phase cells are hairless. Broth cultures are much more fimbriated than cultures from nutrient agar plates. But, even in heavily fimbriated populations, the quantity of fimbriae on individual cells appears to vary greatly from 0 to more than 200. Similar variation has been found in bacteria². The fimbriae seem to be susceptible to mechanical damage and may contract into tight coils on the surface of most cells in particular regions of a grid. Similar coils are produced on all cells by treatments with various reagents including the fixatives glutaraldehyde and formalin.

(4) The fimbriae can be removed easily from cells by violent mechanical agitation in a blender, by sonication or by centrifugation through a viscous liquid (for example, 40% sucrose). In bacteria, fimbriae regenerate very rapidly, beginning about 10 min after reinoculation into nutrient medium². Similar rapid regeneration occurs in *U. violacea* as the fimbriae begin to appear in less than 1 h and reach normal length (about 3–7 μm) in less than the cell cycle time of 5 h. Again as in bacteria the fimbriae are constantly produced and shed by cells so that many free unattached fimbriae can be observed in all cultures.

(5) Type I bacterial fimbriae are composed mainly of a protein called pilin². The fimbriae of *U. violacea* are completely digested by treatment with pronase and thus contain protein throughout their length. Treatments with ether, DNase or RNase do not affect them, and they are not stained by phosphotungstic acid (PTA) or ruthenium red. We conclude that they may be largely and perhaps entirely composed of protein.

We are examining other yeasts and yeast-like fungi to determine whether such fimbriae are common. No species we have investigated so far reveals the long fimbriae of *U. violacea* and indeed even the strains of this species maintained by the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, show fewer fimbriae. However, short hairs and other extensions are produced by some yeasts in genera such as *Lipomyces*, *Nadsonia* and *Saccharomyces*. Thus the presence and structure of surface hairs may have significance for the taxonomy of yeast-like fungi. We have observed short hairs in *Saccharomyces cerevisiae* which are probably extracellular extensions of the microfibrils that Moor and Mühlethaler observed arising from particles on the plasma membrane and penetrating into the wall⁵. These hairs are more frequent on flocculent strains than on non-flocculent forms.

What role do the fimbriae play in the growth of the smut cell? Even though bacterial fimbriae were described about 20 yr ago the functions of the several kinds are largely unknown, except for the F fimbriae which are thought

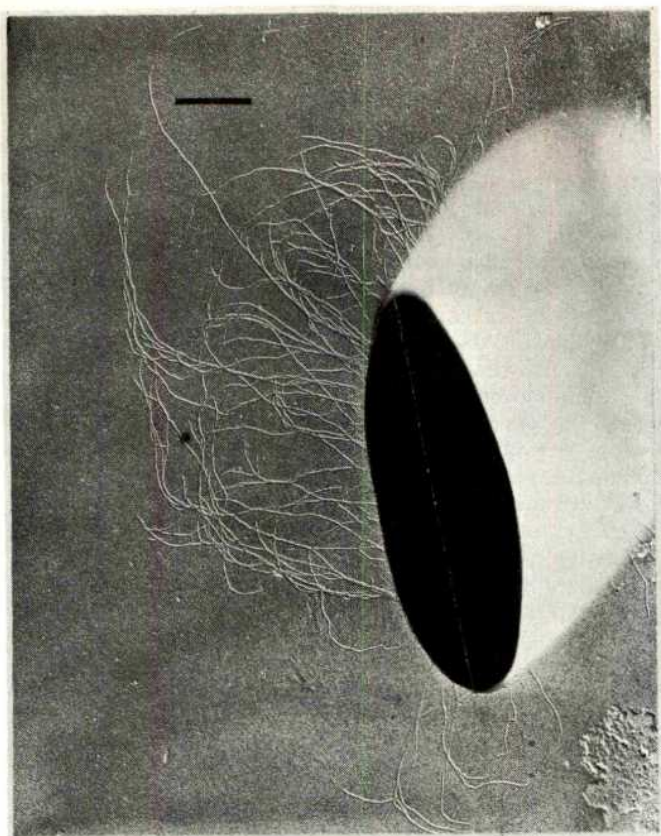


Fig. 1 Metal shadowed preparation of a sporidium of *Ustilago violacea*, showing many long flexible fimbriae. The apparent branchings are always associated with reduction in width and are due to dissociation of multistranded 'cables'. The bar represents 1 μ m.

to transport DNA molecules from F⁺ or Hfr cells to F⁻ recipients. Brinton² has suggested that the other fimbriae are involved in transport of metabolites or informational macromolecules, colicin-like killing of other cells, or simply adhesion to surfaces. He has also suggested that some fimbriae are rod shaped viruses being released from the cell in a manner similar to the release of the DNA male phages.

The function of fungal fimbriae is at present a matter of speculation. The suggestions made above for bacteria are equally applicable to the yeast-like *Ustilago* cells. One possible role would be in the mechanism of conjugation. We have shown that development of the conjugation bridge depends on mutual information transfer at an early stage in courtship^{5,6}. We speculated that such information transfer was mediated 'probably through hairs on the glycocalyx'. The fimbriae described here provide a firm morphological basis by which this information transfer could occur, as we have evidence showing that fimbrial connections are formed between the cells in the early stages of the conjugation process. We are investigating the synthesis and structure of the fimbriae in *U. violacea* and their possible role in conjugation. A full account of this work together with a description of fimbriae in other yeast-like species will be published elsewhere.

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¹ Duguid, J. P., Anderson, E. S., and Campbell, I., *J. path. Bact.*, **92**, 107-138 (1966).

² Brinton, C. C., *Trans. N.Y. Acad. Sci.*, Ser. II **27**, 1003-1054 (1965).

³ Tweedy, J. M., Park, R. W., and Hodgkiss, I., *J. gen. Microbiol.*, **51**, 235-244 (1968).

⁴ Moor, H., and Mühlethaler, K., *J. Cell Biol.*, **17**, 609-628 (1963).

⁵ Day, A. W., and Cummins, J. E., *J. Cell Sci.*, **14**, 451-460 (1974).

⁶ Cummins, J. E., and Day, A. W., *Cell cycle controls* (edit. by Cameron, I., Padilla, G., and Zimmerman, A.) (Academic Press, New York, in the press).

In vivo hybridisation of human tumour and normal hamster cells

It is exceedingly rare for neoplasms to be invasive and metastatic when grafted in xenogeneic hosts¹. During the past 7 yr, we have on numerous occasions observed the production of invasive and metastatic tumours in hamsters soon after injecting human cancer cells of diverse histopathology into their cheek pouches²⁻⁷. We have interpreted this biological phenomenon as due to the *in vivo* fusion of the human tumour with normal hamster host cells²⁻⁸, although the involvement of oncogenic viruses was not excluded. Recently, other evidence supporting the hybridisation of tumour with normal cells *in vivo* has appeared⁹. The significance of such events in the neoplastic process, however, has yet to be appreciated.

It is our view that such *in vivo* cell hybridisations in xenogeneic and perhaps also in isogenic systems are a means by which neoplastic cells can progress to more advanced states of malignancy, as exhibited by the properties of invasiveness and metastasis⁸⁻¹⁰. Utilising advances made in methods for individual chromosome identification, we have now obtained karyological evidence suggesting the *in vivo* fusion of transplanted human cancer cells with putatively normal hamster cells, resulting in the formation of very lethal neoplasms.

As performed in previous studies²⁻⁷, a portion of an astrocytic glioma (Fig. 1a) from the brain of a 44-yr-old female was injected as a fine cell suspension into the cheek pouches of nine male golden hamsters (*Mesocricetus auratus*) weighing 50-60 g. No immunosuppressive host-conditioning measures were used. Fourteen days later, a large tumour measuring 1.1 cm in diameter was confirmed in only one animal's cheek pouch. The microscopic appearance of the tumour graft did not resemble that of the original human neoplasm (Fig. 1b). Four weeks after transplantation, this animal expired with widespread metastasis to all major organs, including the lungs (Fig. 1c) and the adrenals (Fig. 1d). Aliquots of the first passage cheek pouch tumour were then grafted to other hamsters, in their cheek pouches or intraperitoneally, as well as explanted in cell culture. Our cell culture techniques have been described elsewhere¹¹. Both *in vivo* and *in vitro*, a continuously propagable tumour cell line resulted, and has been termed GB-749. Either growing in the cheek pouch or in the ascites form, GB-749 has been uniformly lethal for its hamster host.

GB-749 cells from the fifth ascites and fifteenth cheek pouch tumour passages were placed in culture for 6 and 18 d, respectively, before collecting for cytogenetic analysis; the cancer patient's peripheral blood cells were treated similarly, except for the addition of phytohaemagglutinin, according to standard cytogenetic methods¹². Counting the chromosomes of 50 GB-749 cells of each passage revealed that between 52 and 60% of the cells had a modal range of 58-60 chromosomes. Twenty cells of each transplant generation were karyotyped after banding the chromosomes by the trypsin-Giemsa method¹³, and all of the aneuploid tumour cells examined contained both hamster- and human-like chromosomes. The karyotype of a representative GB-749 cell is reproduced in Fig. 2a, showing chromosomes with banding patterns typical for both human and hamster chromosomes.

Table 1 Frequency of human and hamster chromosomes (single or pairs) in 20 karyograms of GB-749 hybrid tumour cells

		Fifth ascites passage																				
Chromosome No.	X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hamster	18	17	12	4	11	20	20	19	14	13	20	19	20	16	19	19	20	17	19	19	15	2
Human	0	2	13	4	0	1	1	2	0	1	0	7	3	0	0	0	0	0	10	0	0	14

		Fifteenth cheek pouch passage																				
Chromosome No.	X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hamster	10	10	17	5	13	19	20	18	16	19	18	19	20	18	20	20	20	17	18	20	20	4
Human	0	0	10	8	0	0	0	1	0	5	1	1	0	1	0	1	1	0	7	0	0	15

Table 1 shows the frequency of each hamster and human chromosome identified in the fifth and fifteenth hamster transplant passages of GB-749. Chromosomes with a hamster-like banding pattern predominated in both cell generations. A significant number of the human-appearing chromosomes, however, No. 2, 3, 11, 18, and 21, were present among the 20 karyotypes examined at random.

The resemblance of the banding patterns of the human-like chromosomes most frequently found in GB-749 to their counterparts in the patient's peripheral lymphocytes is demonstrated in Fig. 2b. At times, the hamster's chromosome No. 9 looked very similar to the human chromosome No. 11 and 12, but the ones chosen in this figure clearly indicate the presence

of the human 12 chromosome in GB-749. Of additional interest is the occurrence of pronounced satellites on the short arms of the 21 chromosome, both in the patient's peripheral blood cells (H) and in the GB-749 tumour (T) produced in hamsters and serially propagated in these animals and in cell culture. In addition, this chromosome had a centromeric banding pattern typical for the human 21 and distinct from that of any normal hamster chromosome of this size and form. Our recent identification of small acrocentric chromosomes, however, with similar distinguishing features in mouse melanoma/hamster cheek pouch hybrids produced *in vitro*, and in two other presumptive human/hamster hybrid tumours established *in vivo*¹⁰, raises the question of whether this might represent a marker chromosome for such tumours in the hamster, and not necessarily be of human derivation. We are cognisant that rearrangements of hamster chromosomes could have resulted in banding patterns similar to those of the chromosomes identified as human by this morphological method.

The frequency of spontaneous cell fusion *in vitro* has been reported to be very low for most cell types combined, and has been estimated to occur at 1×10^{-6} to 5×10^{-6} (ref. 14). The increased frequency of somatic cell fusion induced with the aid of myxoviruses^{15,16} raises the possibility that the *in vivo* hybridisation suggested in this human tumour transplantation and in previous studies²⁻⁷ may have been influenced by tumour- or host-borne fusing agents.

It is usual for many somatic cell hybrids to lose selectively the chromosomes of one of the parents while the chromosomes from the other remain dominant in number. In the case of human/rodent cell combinations, it is the human chromosomes which are preferentially lost¹⁷⁻¹⁹. Although this is the only reported case of a putative *in vivo* heterospecific hybridisation involving karyological analysis of the hybrid progeny, it seems that a number of relationships found in cell fusion *in vitro* are true for the *in vivo* situation. Our human/hamster cell fusion results indicate that pronounced chromosomal segregation occurs following hybrid formation, where the human chromosomes are preferentially lost. Further, malignancy, when associated with the human parental cells which experienced chromosomal loss, can be expressed in the hybrid progeny, even after a relatively long sojourn in the animal host.

The animal host would be expected to eliminate any human chromosomes in the hybrid tumour cells, particularly if these could restrict the hybrid tumour's viability. Yet, human chromosomes seem to have been retained in the hybrid tumour for at least up to 15 serial transplant passages, or a minimum of 18 weeks. Whether or not the portion of the human genome controlling malignancy required the retention of any particular human chromosomes or chromosomal fragments for the expression of malignancy in the hybrid, or the loss of some inhibitory genes, cannot be concluded on the basis of these data. It is nevertheless intriguing to follow the chromosomal constitution of a human/hamster hybrid tumour during extensive propagation in hamsters, and to explore to what extent the human genome is functional and being recognised by the hamster host.

As *in vivo* hybridisation seems to have permitted a human tumour to become adapted and unusually lethal in the hamster, we suggest that similar mechanisms may be implicated in human cancer, particularly when such fusing agents as parainfluenza viruses are prevalent in man. In the autochthonous tumour-

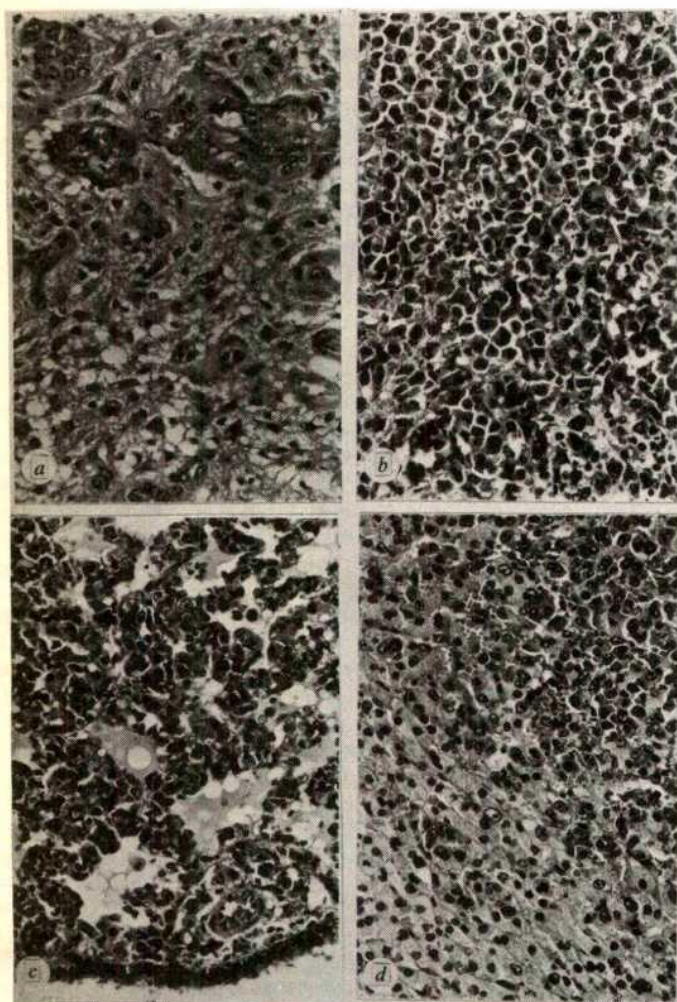


Fig. 1 Microscopic morphology of original human cerebral tumour before heterotransplantation and of the human/hamster hybrid tumour, GB-749 (hematoxylin and eosin, $\times 208$). *a*, Patient's poorly differentiated astrocytic glioma showing pleomorphic tumour cells and a proliferation of the vascular endothelium; *b*, GB-749 tumour cells growing in the hamster cheek pouch; *c*, GB-749 metastasis from hamster cheek pouch to lungs; *d*, subcapsular focus of GB-749 tumour cells in adrenal 4 weeks after transplantation to the cheek pouch.

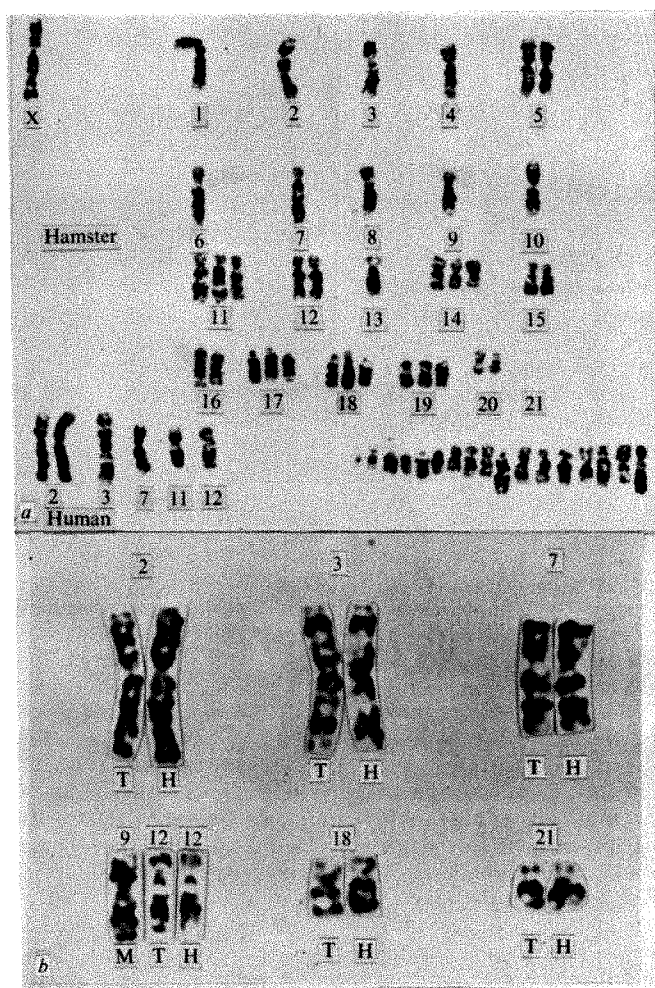


Fig. 2 *a*, Karyotype of giemsa-banded chromosomes of a GB-749 tumour cell showing a simultaneous presence of hamster-like, human-like, and a series of unidentifiable chromosomes (lower right); *b*, Comparison of banded chromosomes selected from several GB-749 tumour cells (T) and the cancer patient's peripheral lymphocytes (H). Human chromosomes No. 12 in GB-749 (T) and in the patient's blood (H) are also compared with hamster chromosome No. 9 (M).

bearing host, fusions of neoplastic with normal cells might provide the tumour with a means for reducing any tumour-specific antigenicity and thereby allow it to escape immunological surveillance mechanisms. In these terms, tumour progression by fusion would permit the neoplasm to circumvent the host's immunological controls. Conversely, fusing the tumour with other cells *in vitro* may provide a more suitable immunogen for the evocation of an immunological reaction against a tumour of advanced malignancy. Although still speculative, such considerations may offer other avenues for the immunological control of cancer.

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¹ Goldenberg, D. M., *Cancer Res.*, **32**, 813 (1972).

² Goldenberg, D. M., Müller, E., and Witte, S., *Eur. J. Cancer*, **3**, 315 (1967).

³ Götz, H., and Goldenberg, D. M., *Experientia*, **24**, 957 (1968).

- ⁴ Goldenberg, D. M., and Fisher, E. R., *Proc. Am. Ass. Cancer Res.*, **10**, 30 (1969).
- ⁵ Goldenberg, D. M., *Tenth int. Cancer Cong., Houston*, abstract, 316 (1970).
- ⁶ Goldenberg, D. M., *Expl molec Path.*, **14**, 134 (1971).
- ⁷ Goldenberg, D. M., Bhan, R. D., and Pavia, R. A., *Cancer Res.*, **31**, 1148 (1971).
- ⁸ Goldenberg, D. M., *Klin. Wschr.*, **46**, 898 (1968).
- ⁹ Wiener, F., Fenyö, E. M., Klein, G., and Harris, H., *Nature new Biol.*, **238**, 155 (1972).
- ¹⁰ Goldenberg, D. M., and Pavia, R. A., in *Biological Characterisation of Human Tumours* (edit. by Davis, W., and Maltoni, C.), (Excerpta Medica, Amsterdam, in the press).
- ¹¹ Goldenberg, D. M., and Pavia, R. A., *Cancer Res.*, (in the press).
- ¹² Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Expl Cell Res.*, **20**, 613 (1960).
- ¹³ Seabright, M., *Lancet*, **i**, 971 (1971).
- ¹⁴ Littlefield, J. W., *Expl Cell Res.*, **41**, 190 (1966).
- ¹⁵ Okada, Y., and Tadokoro, J., *Expl Cell Res.*, **26**, 108 (1962).
- ¹⁶ Harris, H., and Watkins, J. F., *Nature*, **205**, 640 (1965).
- ¹⁷ Weiss, M. C., and Green, H., *Proc. natn. Acad. Sci., U.S.A.*, **58**, 1104 (1966).
- ¹⁸ Boone, C. M., and Ruddle, F. H., *Biochem. Genet.*, **3**, 119 (1969).
- ¹⁹ Kao, F. T., and Puck, T., *Nature*, **228**, 329 (1970).

Evolution of X-chromosome inactivation in mammals

It is now well established that in the somatic cells of mammals only a single X chromosome is active in coding for proteins, no matter how many are present^{1,2}, but the mechanism and evolutionary origin of this phenomenon still remain unsolved problems. Cooper³ suggested that the random inactivation of X chromosomes derived from either parent in eutherian mammals had evolved from a more primitive inactivation of the paternally derived X chromosome as seen in marsupials, and a possible mechanism for this evolution has been put forward⁴. Lifschytz and Lindsley⁵ further suggested that the inactivation seen in somatic cells had evolved from inactivation of both sex chromosomes in male gametogenesis, a phenomenon that is general in gametes of the heterogametic sex of many species.

All of these proposals have obvious merit, but in detail criticisms can be levelled at all. It may therefore help to consider the possible evolution of X chromosome inactivation more fully. It is reasonable to suppose that monoallelic activity of the X chromosome is connected in some way with its function as a sex chromosome. In germ cells of normal females both X chromosomes are clearly active⁶⁻⁸. Moreover, they are required to be so for normal ovarian function in both human and mouse^{9,10}. In males, if more than one X chromosome is present, as in XX and XXY males of various species, the germ cells die at the spermatogonial stage^{2,11}. The explanation that this is due to activity of all X chromosomes in male germ cells as well as female has recently been confirmed by the finding that loss of an X chromosome enables germ cells to survive in these animals¹². But, as previously mentioned, later in male gametogenesis the X seems to become inactive. Thus, the cycle of X chromosome activity is: somatic cells, single; female germ cells, double; late male germ cell, none.

Thus, the X chromosome clearly has a role in the germ cells. By contrast, sex determination in mammals is effected through the Y chromosome. In the presence of the Y chromosome the embryonic gonad is induced to become a testis¹³.

The testis then secretes testosterone which, in combination with the product of a certain gene, activates all genes required for male differentiation, so that the animal develops as a normal male¹⁴. In the absence of the Y the gonad becomes an ovary, testosterone is not secreted, and differentiation follows the female pattern. Thus, there is a simple switch mechanism controlling sex determination and

differentiation¹⁴. Mutation of the gene involved in response to testosterone leads to the syndrome of testicular feminisation, in which a genetic male has testes but no other signs of maleness, and fails to respond to testosterone. In the mouse the locus of this gene, called the *Tfm* gene, is on the X chromosome¹⁵. Hence, by Ohno's law of the conservation of the mammalian X chromosome¹⁶ it is probably X-linked in all mammals, and thus gives another function of the X chromosome in sex differentiation.

The sex chromosomes of present day mammals are typically clearly distinguishable, with a small Y and fairly large X, the variably active part of the X constituting roughly 5% of the genome¹⁶, although some species have other material (constitutive heterochromatin or autosomal material) attached to the X. By contrast, the sex chromosomes of lower vertebrates are commonly not distinguishable¹⁶. Thus, in evolution the mammalian Y must have lost material, and the X has perhaps gained some.

Concurrently, sex determining processes have evolved. Lower vertebrates respond to hormones in a way comparable to the mammalian response to testosterone, but in contrast to mammals sex determination is more labile, and sex reversal can be achieved by hormone treatment. In view of the response to testosterone one may suppose that a gene comparable to the *Tfm* gene was present early in evolution, before the sex chromosomes became morphologically distinct. The next stage was the formation of a small differential segment, containing in the Y the genes involved in testis formation. At this stage, the locus of the *Tfm* gene will have been present on both sex chromosomes, and genes concerned in ovarian function will have been distributed over both X and Y, interspersed with many genes not directly involved in sexual development (homologues of present X-linked genes). After this, the Y chromosome lost much of its material, including the homologues of the X-linked genes. There was then a dosage difference between males and females, which was corrected by X chromosome inactivation in somatic cells.

It is however difficult to envisage exactly how this inactivation first arose. As seen at present it is an all-or-none phenomenon. The inactivated chromosome (except for a possible small region escaping inactivation) is totally and irreversibly unresponsive to stimuli evoking transcription, and it seems unlikely that it could have evolved to this state by a gradual process. There seems no intermediate stage. But total inactivation of one X chromosome when previously two had been active would in effect result in functional monosomy for X-linked genes and monosomy in mammals is usually lethal¹⁷. Thus, the evolution of X inactivation would be easier to envisage if some duplication of genes had occurred first. This could have been achieved

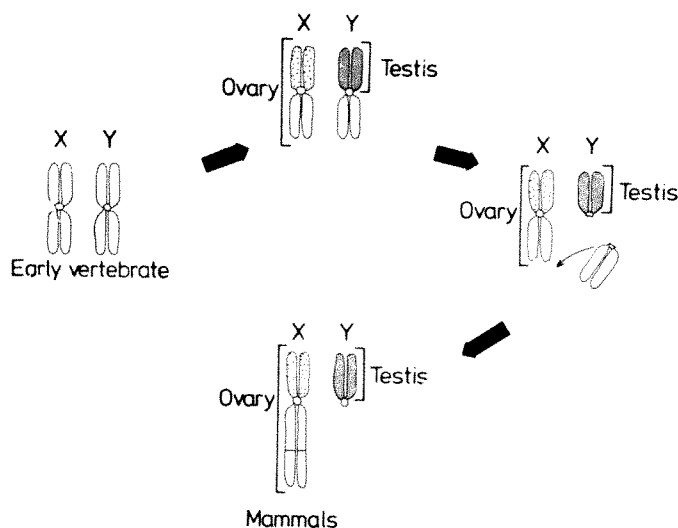


Fig. 1 Suggested evolutionary origin of mammalian sex chromosomes. The position of the centromere, and the relative lengths of the dotted, shaded and white regions are purely diagrammatic and not to scale. The white regions are those postulated to be subject to inactivation in present mammals.

if the material lost from the Y chromosome had been transferred to the X, giving in effect a tandem duplication of the major part of the original X chromosome (Fig. 1). Female offspring which received such a duplicated X chromosome, either homozygously, or heterozygously with the original, would have excess X-linked genes, and hence would probably not be viable, unless the excess material were inactivated. Thus, one must also postulate that a mechanism for inactivation of X chromosomes had already evolved before the proposed Y-X transfer occurred. This, one must suppose, was the form of inactivation seen in late male germ cells, with the mechanism now modified so that it operated in somatic cells, but only on excess material. Such a system could have been selectively advantageous if the additional X chromosomal material was active and beneficial in female germ cells.

From this hypothesis of the evolutionary origin of X-chromosome inactivation at least two predictions can be made. One is that there should be a small region of the present mammalian X chromosome which is not subject to inactivation. This would be the original differential segment of the X, which by definition could have had no homologue on the Y. Therefore, it could not have been transferred from the Y to the X, and hence was never present in excess.

This does indeed seem to be the case in the human X chromosome. The Xg blood group locus is apparently not subject to inactivation in a normal X chromosome¹⁸. Moreover human XO individuals have a range of malformations, which seem to be due to deficiency of gene(s) located in the short arm of the X¹⁹ and in individuals with supernumerary X chromosomes the finger-ridge count, the stature and mental ability seem to vary additively with number of X chromosomes present²⁰. All these facts could be explained if a part of the short arm of the human X chromosome was not subject to inactivation, because it represented the original differential segment. It is not clear, however, whether such a region exists in other species. Chromosomally XXY males are known in a number of species and no clear pattern of abnormalities, except sterility, emerges². Females with the XO constitution are known in the pig²¹, the rhesus monkey²², and the cat²³ and in each there was at least one abnormality also seen in human XOs. But in XO animals of several rodent species (mouse, black rat *Rattus rattus*²⁴, *Akodon*²⁵ and *Microtus oregoni*²⁶) no somatic abnormalities were noted, and some were fertile. Possible explanations for an apparent lack of

Table 1 Human X-linked diseases existing in more than one form

Probably at least two gene loci			
Colour blindness	30380 Deutan	30390 Protan	
Haemophilia	30670 Haemophilia A	30690 Christmas disease	
Muscular dystrophy	31010 Becker	31020 Duchenne	
Retinal degeneration	31270 Retinoschisis	31060 Norrie's disease	
Either alleles or multiple loci			
Adrenal cortical defect	30010	30020	
Agammaglobulinaemia	30030	30040	
Amelogenesis imperfecta	30110	30120	
Cerebral sclerosis	30270	31160	
Charcot-Marie-Tooth disease	30280	30290	
Congenital cataract	30220	30230	
Deafness	30440	30450, 30460, 30470	
Diabetes insipidus	30480	30490	
Ocular albinism	30050	30060	
Retinitis pigmentosa	30310	30320, 30330, 31260	
Thrombocytopenia	30100	31390, 31400	

The numbers are the catalogue numbers given by McKusick²⁹.

the original differential segment in rodents are that in the course of evolution it has been transferred to an autosome, or has been duplicated and then become subject to inactivation.

A second and important prediction is that there should be some evidence of duplication of gene loci on present mammalian X chromosomes. It is to be expected that during evolution the originally duplicate genes will have mutated and diverged, but some evidence of similarity should still remain. Only the human and the mouse X chromosomes are genetically at all well known, the human having far more known X-linked genes than the mouse. McKusick²⁷ pointed out that several X-linked diseases exist in more than one form. The various forms could either be due to different mutant alleles at the same gene locus, or to the involvement of more than one locus for each disease. This point can only be tested conclusively by chromosome mapping. From the latest evidence²⁸ it is highly probable that there are at least two gene loci involved in each of four X-linked diseases: colour blindness (protan and deutan), haemophilia (A and B), muscular dystrophy (Duchenne and Becker), and retinal degeneration (retinoschisis and Norrie's disease) (Table 1). In addition, there are at least eleven other diseases listed by McKusick²⁹, which exist in more than one form, and hence are possibly caused by duplicate loci. McKusick lists 150 X-linked conditions, 86 of which he considers well established. This must be only a small fraction of the probably several hundred genes on the human X chromosome. Hence it is hardly to be expected that both members of every pair of duplicate genes would have been discovered, and the present evidence must be considered quite good. The weakest point is that the similarity between pairs of diseases has been judged at the clinical level, and molecular evidence for the similarity or otherwise of the proteins involved is lacking. By contrast, in the mouse only about 28 X-linked genes are known, thought to be at about 21 loci³⁰, and there is as yet no evidence of duplication.

Thus, the supporting evidence from man is sufficient to make the hypothesis of evolution of X chromosome inactivation through Y-X transfer well worthy of further consideration. To reiterate, the hypothetical sequence of events was: (1) formation of a small differential segment, carrying testis-forming genes in the Y, from previously undifferentiated sex chromosomes; (2) inactivation of the sex chromosomes during male gametogenesis, but full activity of both homologues in female germ cells; (3) transfer of a large part of the homologous region of the Y to the X, and concomitant alteration of the inactivation process, so as to inactivate all excess X chromosomal material in somatic cells, while retaining full activity in female and early male germ cells, and complete inactivity in late male germ cells; (4) slow divergence of the two halves of the duplicated X by mutation and selection.

Further evidence could be obtained from studies of X chromosome anomalies in a range of mammalian species. Human X chromosome linkage data could be re-examined with the possibility of duplicate loci in mind, and at the molecular level proteins coded by X-linked genes could be studied for evidence of duplications.

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¹ Lyon, M. F., *Nature*, **190**, 372-373 (1961).

² Lyon, M. F., *Biol. Rev.*, **47**, 1-35 (1972).

³ Cooper, D. W., *Nature*, **231**, 292-294 (1971).

⁴ Brown, S. W., and Chandra, H. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 195-199 (1973).

⁵ Lifschytz, E., and Lindsley, D. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 182-186 (1972).

⁶ Epstein, C. J., *Science*, **163**, 1078 (1969).

⁷ Epstein, C. J., *Science*, **175**, 1467-1468 (1972).

⁸ Gartler, S. M., Liskay, R. M., Campbell, B. K., Sparkes, R., and Gant, N., *Cell Differ.*, **1**, 215-218 (1972).

⁹ Hamerton, J. L., *Nature*, **219**, 910-914 (1968).

¹⁰ Lyon, M. F., and Hawker, S. G., *Genet. Res.*, **21**, 185-194 (1973).

¹¹ Cattanach, B. M., Pollard, C. E., and Hawkes, S. G., *Cytogenetics*, **10**, 318-337 (1971).

¹² Lyon, M. F., in *Reproductive Physiology and Genetics* (edit. by Fuchs, F., and Coutinho, E. M.) (Plenum, New York, in the press).

¹³ Welshons, W. J., and Russell, L. B., *Proc. natn. Acad. Sci. U.S.A.*, **45**, 560-566 (1959).

¹⁴ Ohno, S., *Nature*, **234**, 134-137 (1971).

¹⁵ Lyon, M. F., and Hawkes, S. G., *Nature*, **227**, 1217-1219 (1970).

¹⁶ Ohno, S., *Sex Chromosomes and Sex-linked Genes* (Springer, Berlin, 1967).

¹⁷ Cattanach, B. M., and Moseley, H., *Cytogenet. Cell Genet.*, **12**, 264 (1973).

¹⁸ Ducos, J., Marty, Y., Sanger, R., and Race, R. R., *Lancet*, **ii**, 219-220 (1971).

¹⁹ Ferguson-Smith, M. A., *J. med. Genet.*, **2**, 93-156 (1965).

²⁰ Penrose, L. S., *Lancet*, **i**, 298-300 (1967).

²¹ Nes, N., *Forskning og forsok i Landbruket*, **19**, 393-440 (1968).

²² Weiss, G., Weick, R. F., Knobil, E., Wolman, S. R., and Gorstein, F., *Folia Primat.*, **19**, 24-27 (1973).

²³ Norby, D. E., Hegreberg, G. A., Thuline, H. C., and Findley, D., *Cytogenet. Cell Genet.* (in the press).

²⁴ Yong, H. S., *Nature*, **232**, 484-485 (1971).

²⁵ Bianchi, N. O., and Contreras, J. R., *Cytogenetics*, **6**, 306-313 (1967).

²⁶ Ohno, S., in *Second International Conference on Congenital Malformations*, 40-42 (International Medical Congress, New York, 1963).

²⁷ McKusick, V. A., *O. Rev. Biol.*, **37**, 69-175 (1962).

²⁸ Race, R. R., and Sanger, R., *Blood Groups in Man* (Blackwell, Oxford, 1968).

²⁹ McKusick, V. A., *Mendelian Inheritance in Man* (Johns Hopkins Press, Baltimore, 1971).

³⁰ *Mouse News Lett.*, **49**, (1973).

A possible role for prolactin in control of steroid secretion by the human Graafian follicle

PROLACTIN constitutes part of the luteotrophic complex necessary for the maintenance and secretory activity of the corpus luteum in the rat^{1,2}, mouse³, rabbit⁴, hamster⁵, ferret⁶, pig⁷ and sheep⁸. Recent evidence suggests, however, that prolactin may have little to do with luteal function in women⁹⁻¹⁵. Nevertheless, it is still possible that prolactin could play a 'permissive' role, as in other species, where alterations in peripheral blood levels, within limits, have little effect on luteal activity. In an attempt to gain more insight into the possible role of prolactin in controlling ovarian activity in women, we have studied the production of steroids by human granulosa cells growing in tissue culture, and the effects of the addition or neutralisation of human prolactin in the culture media. We report that the production of progesterone by human granulosa cells *in vitro* requires low physiological concentrations of prolactin whereas high concentrations are inhibitory.

Ovaries, endometrial biopsies and peripheral blood samples were obtained from patients aged 21-48 yr who were undergoing surgery for gynaecological disorders. The stage of the menstrual cycle was assessed from the date of the last menstrual period, the concentrations in plasma of luteinising hormone (LH), follicle stimulating hormone (FSH) and progesterone, endometrial histology, and the presence or absence of a corpus luteum. Only follicles from morphologically normal ovaries were used as the source of granulosa cells in this study.

Antral follicles were dissected out of the ovary within 2 h of ovariectomy and the fluid aspirated by syringe. The follicular fluid and peripheral plasma sample were stored at -20°C until assayed for prolactin and progesterone by

specific radioimmunoassays (RIA). Prolactin concentration is expressed as ng ml^{-1} Friesen prolactin of which $1 \text{ ng} \equiv 20 \mu\text{U}$ MRC Standard 71/222. The Friesen prolactin preparation was 90% pure and the human pituitary hormone contaminants were found by RIA to be negligible: growth hormone (HGH), HLH, HFSH, all $<0.4\%$ and the concentration of human placental lactogen (HPL) undetectable. The collapsed follicle was slit open and the granulosa cells scraped into culture medium. An aliquot of the cell suspension was counted and the viability determined¹⁶. The follicle wall was placed in fixative for subsequent histological examination to ensure that the basement membrane was still intact. The cells were layered on to 18 mm^2 coverslips and cultured for 10–14 d as previously described¹⁷. The culture medium was replaced every 24 h and stored at -20°C until assayed for progesterone. The variation ($\pm \text{s.e.m.}$) in the production of progesterone between replicate cultures for all treatments was found to be 6.77 ± 1.45 .

Neutralisation of prolactin in the culture medium by the addition of rabbit anti-human prolactin serum, at a dilution which did not cross react with HPL, HGH, HLH or HFSH, caused a significant decrease in progesterone production as compared to the cultured control (Fig. 1). To

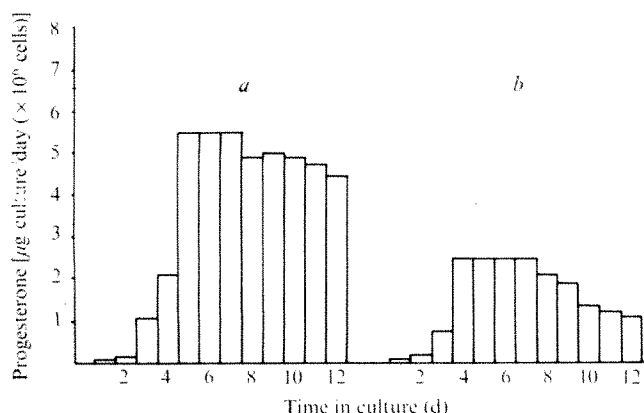


Fig. 1 Progesterone production by luteinised granulosa cells in a culture medium where the endogenous prolactin was neutralised with anti-human prolactin serum. The cells were collected from a single late follicular phase preovulatory follicle. The concentration of endogenous prolactin in the culture medium before the addition of rabbit anti-human prolactin serum was 5.1 ng ml^{-1} . After precipitation of the prolactin-anti-prolactin complex the concentration was $<0.2 \text{ ng ml}^{-1}$. The concentrations of progesterone in the control were determined with either normal rabbit serum, or rabbit anti-bovine serum albumin added to the culture medium at the same dilution (1/500) as the antiserum used in the anti-rabbit culture treatment. There were no significant differences in the production of progesterone between the control culture which were found to be within the precision achieved in replicate cultures. *a*, Control; *b*, anti-prolactin, $P < 0.01$.

confirm that free prolactin in the culture medium had been completely neutralised by the antiserum, the anti-prolactin-prolactin complex was precipitated out using a solid phase second antibody; the concentrations of LH and FSH in the medium were unaltered whereas prolactin was undetectable.

Addition of human prolactin to the culture medium had no effect on progesterone production if the final prolactin concentration did not rise above 20 ng ml^{-1} ; the mean concentration of prolactin in the peripheral blood of women during the cycle is $15 \pm 1 (\pm \text{s.e.m.})^{10}$. When the concentrations in the culture medium were increased from 25 to 100 ng ml^{-1} , there was a progressive decrease in the daily progesterone production (Fig. 2). This inhibitory effect persisted, even when the LH and/or FSH concentrations

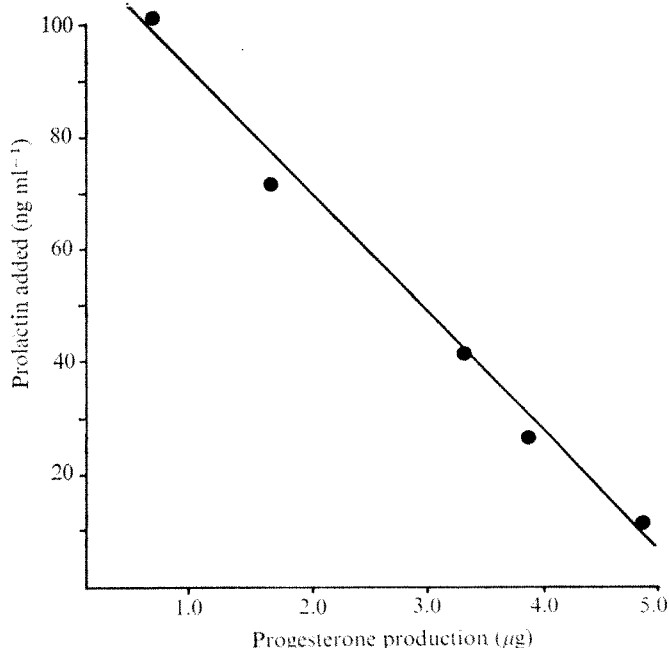


Fig. 2 Relationship between the concentration of prolactin and the total progesterone production by human granulosa cells *in vitro*. Granulosa cells were collected from one late follicular phase preovulatory follicle. Each point is the mean result of duplicate cultures. Total progesterone production is that achieved by 10^6 granulosa cells in 11 consecutive daily changes of culture medium.

were increased 50-fold, and it could be obtained with granulosa cells collected from follicles at any stage of the menstrual cycle. In contrast the soluble non-protein fraction of the added prolactin solution did not inhibit the production of progesterone by human granulosa cells *in vitro* as compared with the controls.

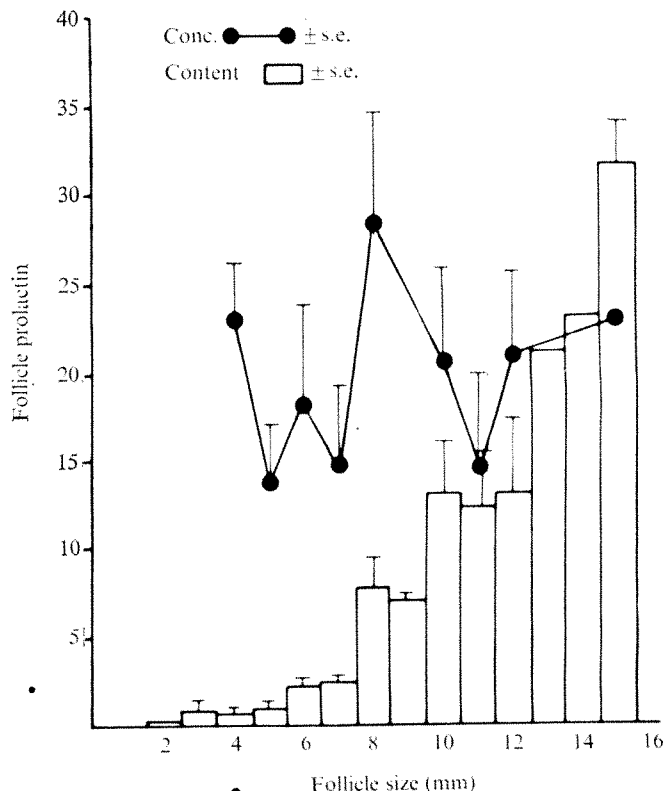


Fig. 3 Changes in concentration and content of prolactin in follicular fluid with respect to follicle size. Vertical bars represent 1 s.e.m. Circles show prolactin concentration (ng ml^{-1}); bars show prolactin content (ng).

Prolactin was assayed in 110 samples of follicular fluid collected at various stages of the cycle. The mean concentration (\pm s.e.m.) was 20 ± 5 ng ml⁻¹ for all sizes of follicle. The increase in the prolactin content of the follicle was correlated with the volume of follicular fluid (Fig. 3). The prolactin concentrations during the late follicular phase of the cycle were, however, significantly lower than at any other time of the cycle with the exception of the early luteal phase (Fig. 4).

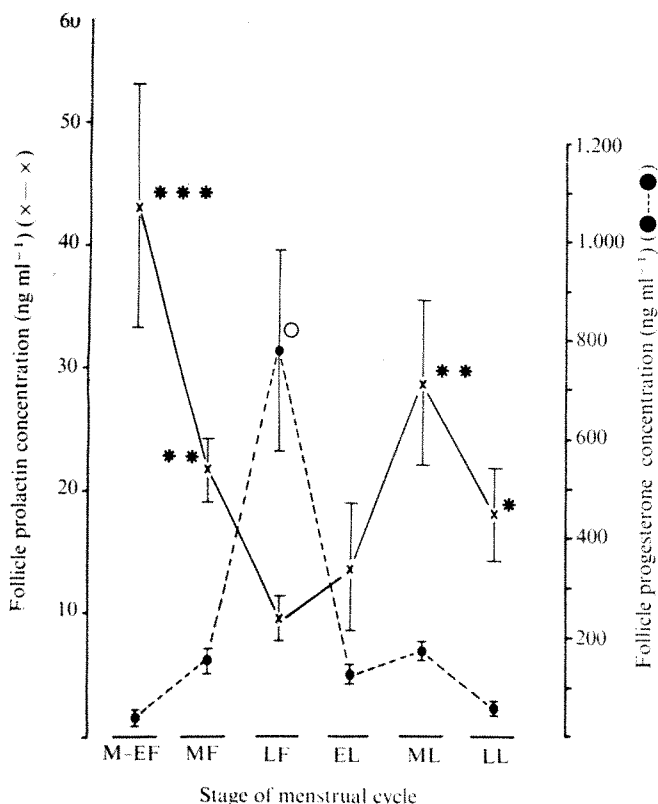


Fig. 4 Changes in the follicular fluid concentrations of prolactin and progesterone related to the stage of the menstrual cycle. The vertical bars represent 1 s.e.m. (M-EF; MF; LL; represent menstruation-early follicular, mid follicular and late follicular phases, and EL, ML and LL represent early, mid and late luteal phases.) *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ° $P < 0.001$.

These *in vitro* experiments suggest that high concentrations of prolactin within the follicular fluid may actually depress progesterone secretion by the granulosa cells. This might provide an explanation for the fact that galactorrhoea with high prolactin levels is commonly associated with amenorrhoea¹⁸, and that if the prolactin levels are lowered by treatment with CB 154, ovulation and menstruation recur^{19,20}. It is well recognised that post-partum lactation can inhibit ovulation in women and other animals^{18,21}, although the mechanism has never been explained.

Although there are now numerous descriptive accounts of the changing hormone levels in blood and urine throughout the menstrual cycle, we do not really understand what factors are responsible for the formation, maintenance and regression of the human corpus luteum. This is an area of great potential interest for the development of new forms of contraception. The *in vitro* results presented here cast doubt on the simplistic view that LH is the only gonadotrophin necessary for luteal maintenance¹⁵, and suggest that prolactin is also involved. Furthermore, it seems probable that the gonadotrophic content of the follicular fluid may have important consequences for the secretory activity of the granulosa cells both before and after ovulation.

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- ¹ Evans, H. M., Simpson, M. E., Lyons, W. R., and Turpeinen, K., *Endocrinology*, **28**, 933-945 (1941).
- ² Astwood, E. B., *Endocrinology*, **28**, 309-319 (1941).
- ³ Kovacic, N., *J. Reprod. Fert.*, **8**, 165-186 (1964).
- ⁴ Spies, H. G., Hilliard, J., and Sawyer, C. H., *Endocrinology*, **83**, 354-367 (1968).
- ⁵ Greenwald, G. S., and Rothchild, I., *J. Anim. Sci.*, **27**, Suppl. 139-162 (1968).
- ⁶ Donovan, B. T., *J. Endocr.*, **27**, 201-212 (1963).
- ⁷ Du Mensil du Buisson, F., *Le Corps Jaune*, 221-237 (Masson et Cie, Paris, 1973).
- ⁸ Denamur, R., Martinet, J., and Short, R. V., *J. Reprod. Fert.*, **32**, 207-220 (1973).
- ⁹ Hwang, P., Guyda, H., and Friesen, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1902-1906 (1971).
- ¹⁰ McNeilly, A. S., Evans, G. E., and Chard, T., in *Human Prolactin*, 231-232 (Excerpta Medica, Amsterdam, 1973).
- ¹¹ Tyson, J. E., and Friesen, H. E., *Am. J. Obst. Gynec.*, **116**, 377-387 (1973).
- ¹² Midgely, A. R., jun., and Jaffe, R. B., in *Proc. 4th Int. Congr. Endocr.* (Excerpta Medica, Amsterdam, 1972).
- ¹³ Robyn, C., Delvoye, P., Nokin, J., Vekemans, M., Badawi, M., Perez-Lopez, F. R., and L'Hermite, M., in *Human Prolactin*, 167-188 (Excerpta Medica, Amsterdam, 1973).
- ¹⁴ Del Pozo, E., in *Human Prolactin*, 218-221 (Excerpta Medica, Amsterdam, 1973).
- ¹⁵ Vande Wiele, R. L., Bogumil, J., Dyrenfurth, I., Ferin, M., Jewelewicz, R., Warren, M., Rizkallah, T., and Mikhail, G., *Rec. Prog. Hormone Res.*, **26**, 63-103 (1970).
- ¹⁶ Paul, J., in *Cell and Tissue Culture* (Churchill Livingstone, Edinburgh, 1972).
- ¹⁷ Channing, C. P., *J. Endocr.*, **45**, 297-308 (1969).
- ¹⁸ Perez, A., Vela, P., Masnick, G. S., and Potter, R. G., *Am. J. Obstet. Gynec.*, **114**, 1041-1047 (1972).
- ¹⁹ Besser, G. M., Parke, L., Edwards, C. R. W., Forsyth, I. A., and McNeilly, A. S., *Br. Med. J.*, **3**, 669-672 (1972).
- ²⁰ Del Pozo, E., Brun del Re, R., Varga, L., and Friesen, H., *J. clin. endocr. Metab.*, **35**, 767-771 (1972).
- ²¹ Bonte, M., and van Balen, H., *J. biosoc. Sci.*, **1**, 97-100 (1969).

Anatomy of an identified serotonin neurone studied by means of injection of tritiated 'transmitter'

THERE is an identifiable serotonin-containing neurone in each cerebral ganglion of the snail *Helix pomatia*¹. Electrophysiological evidence suggests that each neurone sends a process into each cerebro-buccal connective^{2,3}, and that monosynaptic connections are made in each buccal ganglion with other large identifiable neurones³. Because this neuronal system seems to offer a unique opportunity to study the proposed transmitter role of serotonin^{3,4}, it is important to obtain direct information on the localisation and morphology of the presynaptic endings of the serotonin cell. This is not possible with dye⁵ or metal ion⁶ injection techniques, which do not seem to mark processes of the

serotonin neurone at distances of more than a few millimetres from the neurone perikaryon.

In this study we injected tritiated serotonin, or its precursor 5-hydroxytryptophan, into the perikaryon of one of the identified symmetrically placed, serotonin neurones and, after allowing time for the radioisotope to pass along the processes of the neurone, the tissue was prepared for light and electron microscope autoradiography.

The tips (diameter 1 to 3 μm) of glass microelectrodes were filled with a solution of uniformly tritiated serotonin (5-hydroxytryptamine creatinine sulphate) or 5-HTP (DL-5-hydroxytryptophan) (Radiochemical Centre, Amersham). Solutions were prepared by evaporating to dryness 1 mCi of radioactive solution and redissolving in a small volume (0.01 ml) of distilled water. (The isotope specific activities were: serotonin 11 Ci mmol^{-1} and 5-HTP 7 Ci mmol^{-1} ; concentrations of the injection solutions were approximately 0.9 mM and 1.4 mM, respectively). Electrodes were selected by measuring isotope release from the tip into 0.2 ml of snail physiological solution⁷ after passing 10 nA positive square wave pulses of 100 ms duration at 5 Hz for 10 min. Electrodes releasing sufficient isotope to yield 5,000 to 20,000 c.p.m. (measured in a total volume of 10 ml including 9.8 ml of scintillation fluid, counted on a Packard Liquid Scintillation Spectrometer) were found to be suitable for intracellular iontophoresis.

Either the left or right giant serotonin neurone was impaled and positive pulses applied to the electrode for 1 to 2 h; generally 10 to 20 nA pulses of 100 ms duration at 5 Hz were used. The preparation was continuously washed with physiological solution. The direction of flow was from the buccal ganglia and lip nerves towards the cerebral ganglia, thus avoiding contamination of the areas suspected of containing processes and endings of the neurone. During the period of injection, the resting potential of the neurone decreased to between 0 and 10 mV, but

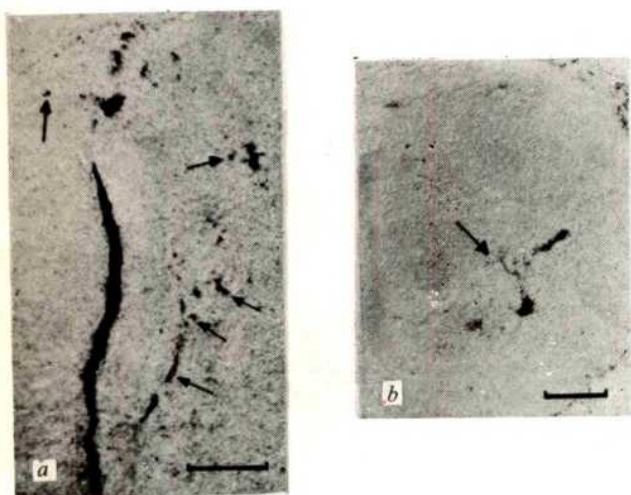


Fig. 1 Micrographs of autoradiographs of a right giant serotonin neurone injected with tritiated serotonin. The areas shown in *a* and *b* are indicated in Fig. 2. *a*, Axon of the serotonin neurone which runs in the contralateral cerebral ganglion. Approximately 25 small branches of this axon (some arrowed), which are shown in different degrees of oblique and transverse section, can be recognised as dark spots. The two larger dark spots at top and right are cross sections of the main axons which run into the lip nerves. In *b*, the largest dark spot is the main axon of the serotonin cell in the ipsilateral buccal ganglion cut in transverse section. Branches of this axon (one fine branch arrowed) terminate within the buccal ganglion neuropile, which is in the middle of the photograph. Scale bars represent 25 μm .

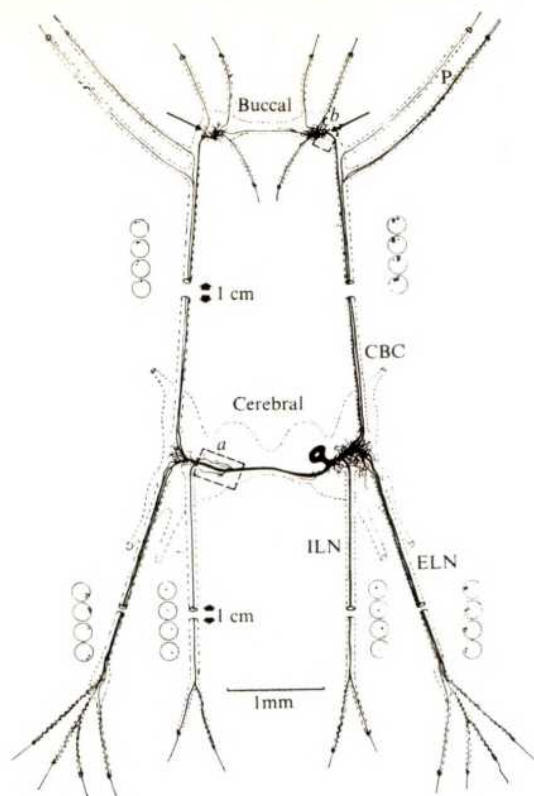


Fig. 2 Diagram of the processes of a right giant serotonin neurone injected with tritium-labelled serotonin. The cerebral and buccal ganglia are joined by connective nerve trunks (CBC). The position of identified neurones in each buccal ganglion which receive synaptic input from the serotonin neurone is indicated with an arrow. Axon branches pass close to, and there are numerous small terminal processes in the immediate vicinity of, these follower neurones. Each cerebro-buccal connective contains two axon processes of the serotonin cell which run close together within the connective. Axon processes pass into the nerves supplying the buccal mass and into the pharyngeal nerves (P), which supply the muscles which control feeding. Two or three separate axon processes of the serotonin neurone pass into each external lip nerve (ELN). The axon processes within these nerves run to muscles in the mouth of the animal. It thus seems that all the peripheral axon branches of the serotonin neurone are somehow associated with feeding. The size and depth of the axon processes observed in transverse sections of each nerve are relatively constant. This is indicated by the diagrams of the cross sections of the nerve trunks, where each circle beside a particular trunk illustrates the situation in a different ^3H -serotonin injected preparation. The processes are located close to the periphery of the CBC and ELN but centrally within the internal lip nerve (ILN). The axon processes of the serotonin neurone are not round in cross section, but have infoldings which vary in shape from section to section. The many small branches within the right and also possibly the left, cerebral ganglia, are thought to be dendrites receiving synaptic input. The areas indicated by *a* and *b* are illustrated in Fig. 1.

cells partly recovered after injection (the resting potential returned to about -20 to -40 mV). The preparation was left for 10–15 h at 4°C before processing for autoradiography.

For light microscope autoradiography, the tissue was fixed for 2 h in 2.5% glutaraldehyde solution, dehydrated in a series of ethanol solutions and embedded in wax. Radioactivity was monitored in the histological solutions. Little if any wash out of the isotope could be detected, that is, less than 100 c.p.m. ml^{-1} of any histological solution. Serial sections (8 μm) were prepared and mounted on glass slides. The sections were covered with Kodak AR 10 stripping film and developed in Kodak D 19 after storage in the dark for

two weeks. For electron microscope autoradiography, tissue was fixed in glutaraldehyde and osmium solutions and embedded in Epon. Thin sections were covered with a monolayer of Ilford L4 emulsion by a loop technique⁸, exposed for three weeks, and subsequently developed in Kodak Mikrodol-X.

Similar results were obtained in all of the experiments. Five light microscope experiments (three left and one right serotonin neurones were injected with ^3H -serotonin and one right neurone was injected with ^3H -5-hydroxytryptophan) and two electron microscope experiments (two left serotonin neurones injected with ^3H -serotonin) were made. The radioactive material was sufficiently well bound to withstand the histological treatment and it was readily detected in the autoradiograms (Figs 1 and 3). Apart from the region in the immediate vicinity of the perikaryon of the serotonin neurone, where there was some spillage and binding of the isotope to connective tissue, it appeared that all the radioactivity detected in the autoradiograms was strictly located within the giant serotonin neurone. The main axons could be easily followed in serial sections with the light microscope and fine terminal branches could be traced at a distance of 1 cm or more from the perikaryon, for example in the buccal ganglia (Fig. 1b), where there was a complete lack of silver grains over the outer connective tissue capsule. The processes of the right serotonin neurone traced by this method are shown diagrammatically in Fig. 2. The processes of the left serotonin neurone form a pattern which is the mirror image of the right neurone.

The results of the light microscope autoradiography showed that axon processes of each giant serotonin neurone pass to the region of the buccal ganglia where the identified follower neurones are located. In these regions many fine terminal branches were seen. In some sections, silver grains were seen directly adjacent to the axon hillocks of the identified follower neurones. This morphological evidence therefore agrees with the electrophysiological results suggesting the presence of monosynaptic connections of the serotonin neurones and these follower neurones. The distribution of fine branches in the neuropile of each buccal ganglion was very similar to that observed after uptake of exogenous ^3H -serotonin by the isolated buccal ganglia⁹.

The pattern of processes was remarkably similar in each preparation. The presence of two or more axon processes running parallel for considerable distances along some nerve trunks is an interesting, but so far unexplained phenomenon, also noted by other workers¹⁰. There was a constancy in the relative sizes of the different processes within a particular nerve trunk and their positioning with respect to the centre or periphery of the particular nerve trunk (see Fig. 2). It is apparent that there is an extensive overlapping of the fields of innervation of each giant serotonin cell, as was suggested by electrophysiological experiments^{3,4}.

When a cerebro-buccal connective or external lip nerve was ligatured, there was a build up of radioactivity on the side of the constriction nearer the perikaryon. This was shown by an increase in density of silver grains within the axon of the serotonin cell against the ligature. This experiment suggested that the labelled serotonin was actively transported along the axons. The minimum rate of movement of the isotopically labelled material within the serotonin cell axon was 1.0 mm h^{-1} .

By electron microscope autoradiography it was possible to study the axons of the serotonin neurone within the cerebro-buccal connectives and some of its terminal processes within the neuropile of the buccal ganglia (Fig. 3). The identification of groups of silver grains over processes of the serotonin neurone was comparatively easy, because after the relatively short time periods of radiographic exposure employed, background silver grains were almost completely absent. Identification of processes from the serotonin neurone with the electron microscope was also

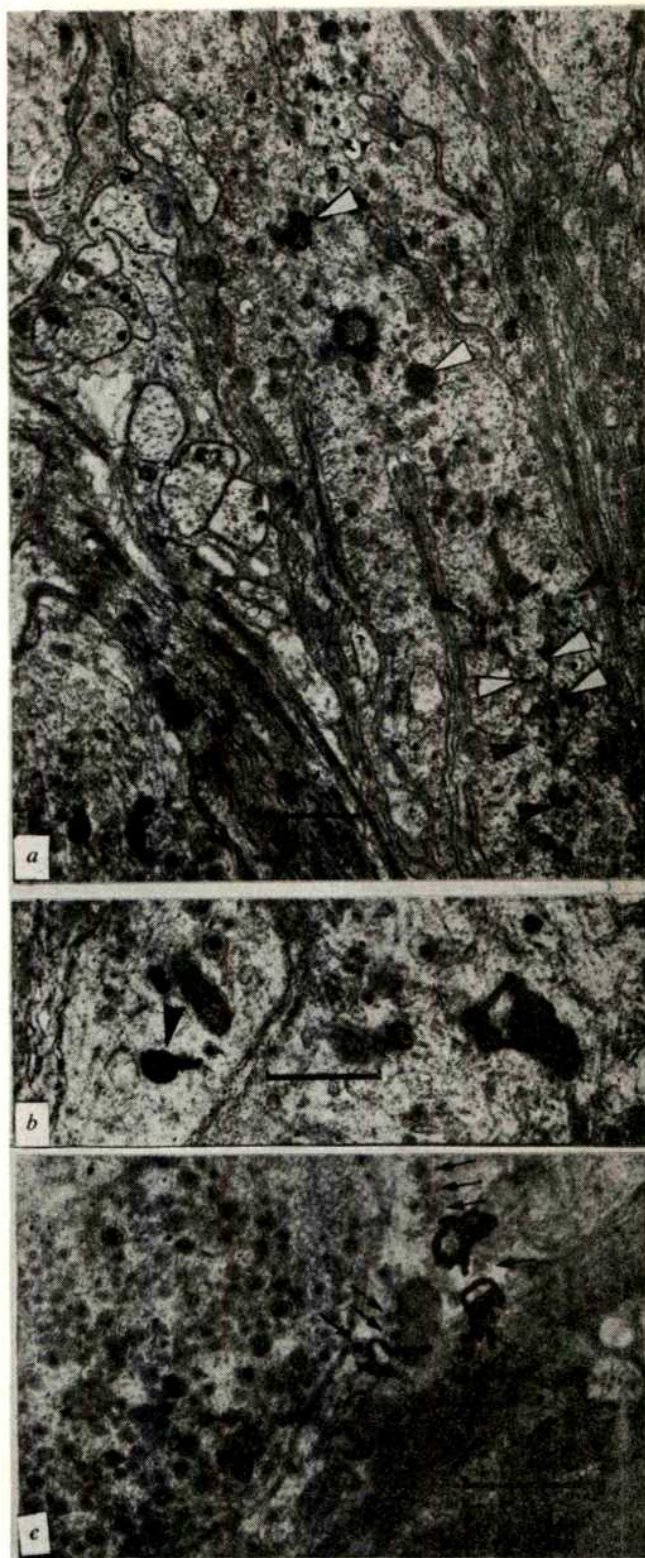


Fig. 3 Electron microscope autoradiograms of cross sections of the cerebro-buccal connective and buccal ganglia showing axons of a serotonin neurone injected with tritium-labelled serotonin. In *a* the silver grains are marked with white arrows for clarity; the black arrows point to groups of dense-cored vesicles. In *b* the black arrow points to a silver grain which overlies a dense-cored vesicle. The silver grain to the right in *b*, and also the silver grain second from top in *a*, seem to be associated with larger electron-dense structures. *c*, Presumed terminal process of an injected serotonin neurone within the neuropile of a buccal ganglion. The arrows point to dense-cored vesicles. The star marks a silver grain which does not obviously overlie the same structure as the other silver grains, although it is possible that this is the case, and that the labelled ending is shown at two levels within the same section. Scale bars represent *a*, μm ; *b*, $0.5 \mu\text{m}$; *c*, $0.5 \mu\text{m}$.

aided by comparison with adjacent thicker tissue sections processed for light microscope autoradiography.

All the labelled axon processes contained vesicles with electron-dense cores (Fig. 3). These vesicles had an average diameter of 100 nm, and were morphologically identical to those which are thought to sequester serotonin in the perikaryon of the serotonin neurone¹¹ and those seen in a small proportion of nerve endings in the buccal ganglia which selectively accumulate exogenous tritium-labelled serotonin⁸. Some silver grains overlay such vesicles (Figs 3b and c).

None of the terminal processes observed resembled classical synapses in containing membrane thickenings, however 'typical' synapses are only rarely seen in the central nervous system of this animal¹¹. Because the fine terminal processes of the serotonin neurone in the neuropile regions contain many vesicles (Fig. 3c), they probably represent, or are close to, the release sites of the transmitter.

The results suggest that the technique of injecting tritiated 'transmitter', or a readily metabolised precursor, may be of general value in mapping the axons, and locating and studying the structure of the presynaptic terminals of individual neurones of known transmitter type. The technique is similar to that of injecting radioactive amino acids before autoradiography, which has been shown to be valuable in studying the anatomy of, and axonal transport within, the dendrites of single mammalian spinal motor neurones^{12,13}. In the latter situation, however, there was evidence for a transfer of radioactivity between glia and other neurones, and it was not established that any of the proteins presumed to be labelled after incorporation of the radioactive amino acids might have been destined for the presynaptic terminals^{12,13}. On the other hand, it might be expected that injected transmitter will be treated as natural transmitter and transported to and concentrated within presynaptic terminals. (Some experimental results, not described here, obtained after the injection of leucine into identified snail neurones support this view). Further, the results show that no noticeable damage is caused by the iontophoresis of tritium labelled serotonin to the fine structure of the neurone. This is not the case with other intraneuronal staining techniques^{5,6,14,15}. Finally, the results show for the first time that the presynaptic endings of a specified neurone of known transmitter type can be studied in direct relation to its perikaryon.

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- ¹ Cottrell, G. A., and Osborne, N. N., *Nature*, **225**, 470-472 (1970).
- ² Kandel, E. R., and Tauc, L., *J. Physiol., Lond.*, **183**, 269-280 (1966).
- ³ Cottrell, G. A., and Macon, J., *J. Physiol., Lond.*, **236**, 435-464 (1974).
- ⁴ Cottrell, G. A., *Nature*, **225**, 1060-1062 (1971).
- ⁵ Stretton, A. O. W., and Kravitz, E. A., *Science*, **162**, 132-134 (1968).
- ⁶ Pitman, R. M., Tweedle, C. D., and Cohen, M. J., *Science*, **176**, 412-415 (1972).
- ⁷ Meng, K., *Zool. Jber. Neapel*, **68**, 539-566 (1960).
- ⁸ Caro, L. G., *J. Cell Biol.*, **41**, 918 (1969).
- ⁹ Pentreath, V. W., and Cottrell, G. A., *Nature new Biol.*, **239**, 213-214 (1972).
- ¹⁰ Willows, A. O. D., Dorsett, D. A., and Hoyle, G., *J. Neurobiol.*, **4**, 207-237 (1973).
- ¹¹ Pentreath, V. W., Osborne, N. N., and Cottrell, G. A., *Z. Zellforsch. mikrosk. Anat.*, **143**, 1-20 (1973).
- ¹² Globus, A., Lux, H. D., and Schubert, P., *Brain Res.*, **11**, 440-445 (1968).

- ¹³ Schubert, P., Lux, H. D., and Kreutzberg, G. W., *Acta Neuropath. Berl., Suppl.* V, 179-186 (1971).
- ¹⁴ Christensen, B. N., *Science*, **182**, 1255-1256 (1973).
- ¹⁵ Gillette, R., and Pomeranz, B., *Science*, **182**, 1256-1257 (1973).

Calcium ionophore X-537A increases spontaneous and phasic quantal release of acetylcholine at frog neuromuscular junction

TRANSMITTER release from nerve endings is believed to be triggered by the influx of Ca^{2+} , which is thought to enter the terminal as a result of an increase in conductance produced by the action potential¹. At the neuromuscular junction the evidence for the role of Ca^{2+} is largely indirect, based on changes in endplate potential (e.p.p.) amplitude following variations in $[\text{Ca}^{2+}]_0$. Miledi² showed that microinjection of Ca^{2+} into the presynaptic terminal at the synapse of the squid giant axon causes a brief period of enhanced quantal release. Ca^{2+} also affects the rate of spontaneous quantal release at the neuromuscular junction; miniature end-plate potential (m.e.p.p.) frequencies are very low in solutions with reduced Ca^{2+} (ref. 3-5). The mode of action of Ca^{2+} within the terminal remains largely a matter of speculation. Now a new tool has become available: an ionophore, X-537A, that transfers both univalent and divalent cations across lipid bilayers and cell membranes⁶⁻¹⁰. Using X-537A it may be possible to change intracellular Ca^{2+} levels while observing changes in quantal release. The ionophore might also be used to transfer other divalent metal ions into the terminal, to see how effective they are compared with Ca^{2+} .

Using conventional methods, e.p.p.s and m.e.p.p.s were recorded intracellularly from the sartorius and cutaneous pectoris muscles of the frog *Rana pipiens*. Initial experiments were performed in our usual Ringer, based on an analysis of plasma¹¹, containing (mM): NaCl, 100; KCl, 2.0; CaCl_2 , 2.5; MgCl_2 , 3.0, and Tris-maleate buffer (pH 7.4), 8.0. In later experiments the Ringer was modified to contain only one metal chloride salt; in each case the concentration of the divalent ion is specified. All experiments were performed at 17° C. To record e.p.p.s, 3×10^{-6} g ml⁻¹ d-tubocurarine

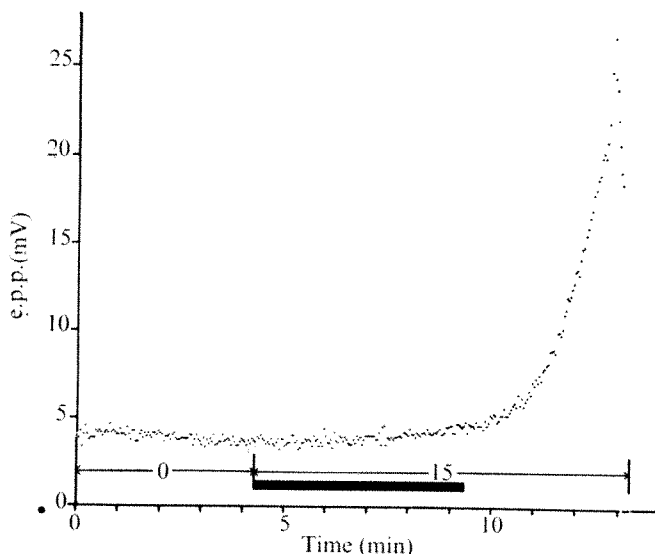


Fig. 1 The effect of X-537A on e.p.p. amplitude in a sartorius muscle. The sciatic nerve was stimulated supramaximally once every 2.5 s. The shaded region indicates the period during which the solution was being changed at a rate of 10 ml min⁻¹. The volume of the bath was about 5 ml. The numbers show the concentration of X-537A in μM .

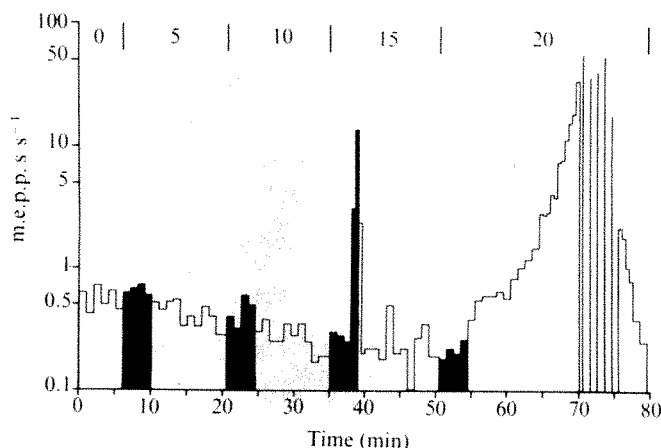


Fig. 2 The effect of a series of increasing concentrations of X-537A, on the frequency of m.e.p.p.s. recorded from the sartorius muscle in 2.5 mM Ca^{2+} -Ringer. The width of each bar represents the period during which m.e.p.p. count was made. The shaded regions indicate the periods during which a solution change took place. The figures across the top show the X-537A concentration.

chloride was added to the Ringer. To increase the amplitude of m.e.p.p.s 1×10^{-6} g ml^{-1} neostigmine bromide was added.

The effect of X-537A on e.p.p. amplitude in 2.5 mM Ca^{2+} -Ringer is shown in Fig. 1. In Ringer the mean amplitude was 3.9 ± 0.3 mV (s.d., $n=103$). As Ringer containing 15 μM X-537A flowed into the chamber, the amplitude of the e.p.p. began gradually to increase. Within 205 s of the completion of the solution change, it reached a maximum of 26.9 mV. Then it declined precipitately and disappeared abruptly. In two other experiments similar responses were observed in 10 and 15 μM X-537A, though in these examples it took somewhat longer to reach a maximum e.p.p. and the increase was less marked. (10 μM X-537A: control 1.6 ± 0.4 mV ($n=101$) to a maximum of 5.5 mV. 15 μM X-537A: 1.1 ± 0.3 mV ($n=102$) to a maximum of 4.4 mV). Again the e.p.p.s disappeared abruptly within 4 min of reaching the maximum amplitude. Similar results were obtained from preparations in which transmission was blocked by increasing the Mg^{2+} concentration to 20 mM. The X-537A does not cause an increase in m.e.p.p. amplitude; in fact it may produce a decrease. For example, the mean m.e.p.p. amplitude in 2.5 mM Ca^{2+} -Ringer was 2.5 ± 0.85 mV (s.d., $n=50$; corrected to a standard membrane potential of 90 mV¹²). After 3 min in 15 μM X-537A the m.e.p.p. amplitude was 1.4 ± 0.35 mV ($n=50$). Another experiment gave a similar result. This means that the increase in e.p.p. amplitude produced by the drug must be caused by an increased release of acetylcholine from the motor nerve.

The effects of increasingly larger concentrations of X-537A on the frequency of m.e.p.p.s recorded in 2.5 mM Ca^{2+} -Ringer are shown in Fig. 2. X-537A at 5 or 10 μM did not elicit a change. During exposure to 15 μM there was a brief acceleration of m.e.p.p. frequency, lasting only about 90 s. With 20 μM there was a massive increase, followed by a decline to extremely low levels. In 2.5 mM Ca^{2+} , 3.0 mM Mg^{2+} -Ringer, 10 μM X-537A produced a notable increase in four of seven trials; 20 μM produced a notable increase in five of seven experiments. It is not uncommon for concentrations lower than those required for massive release to elicit a slight, transitory increase in m.e.p.p. frequency.

Figure 3 shows that the addition of X-537A to a Ringer solution containing no divalent cation does not change the m.e.p.p. frequency. The subsequent addition of 2.5 mM Ca^{2+} leads to a marked, transitory increase in m.e.p.p. rate. (In control experiments, the addition of 2.5 mM Ca^{2+} to a preparation in Ca^{2+} -free Ringer leads to about a two-fold, sus-

tained increase in m.e.p.p. frequency.)

Calcium is not the only effective metal. For example, the addition of 1.5 μM X-537A to a preparation soaked in 2.5 mM Ba^{2+} -Ringer led to a transitory increase in m.e.p.p. frequency which lasted for 55 min, the maximum frequency was 128 times the control level.

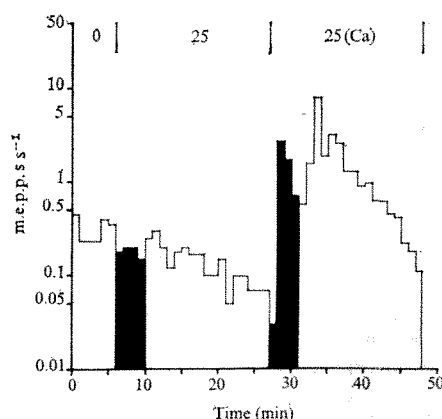


Fig. 3 The effects of Ca^{2+} on the response to X-537A. The cutaneous pectoris muscle was first soaked for 24 h in the refrigerator in a Ringer made without divalent cations but containing 1.0 mM MgEGTA. Then, as shown in the figure, the muscle was soaked in the same Ringer containing 25 μM X-537A during the period indicated as 25. The solution contained 25 μM X-537A and 2.5 mM Ca^{2+} but no MgEGTA during the period indicated as 25 (Ca).

A rough estimate of the relative effectiveness of different metals in accelerating spontaneous quantal release in the presence of X-537A can be drawn from Fig. 4. The apparent sequence is: $\text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Mn}^{2+} \approx \text{Co}^{2+} \approx \text{Ni}^{2+} > \text{Mg}^{2+}$. For those metals for which both physiological and chemical data are available, the effectiveness in promoting spontaneous quantal release seems to parallel the ability of X-537A to carry the metal into a hydrophobic layer⁶.

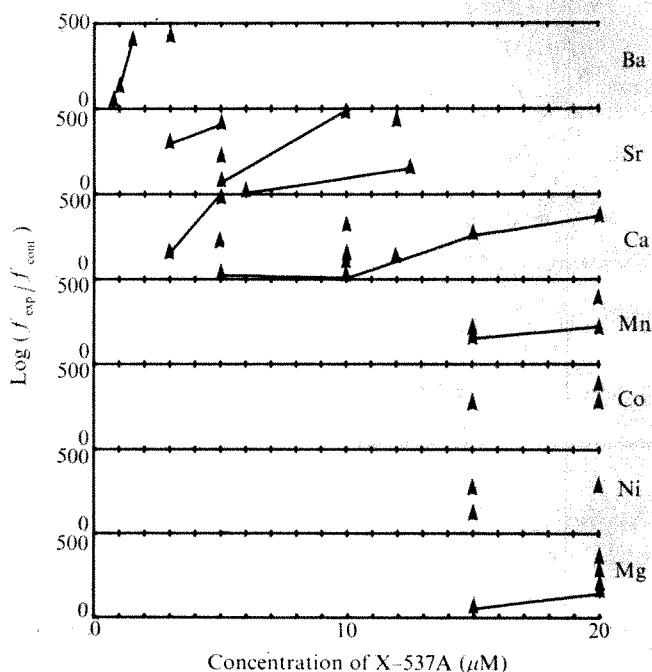


Fig. 4 The relative effectiveness of different metals in increasing m.e.p.p. frequency in the presence of X-537A. In each experiment, the muscle was first soaked in Ringer containing 2.5 mM of the indicated metal. The m.e.p.p. frequency in this solution is (f_{cont}). The maximum m.e.p.p. frequency in the presence of the indicated concentration of X-537A is (f_{exp}). The muscles were usually soaked for two hours in the metal Ringer before observations were begun. Values obtained from the same endplate are connected by lines.

When the preparation is exposed to effective concentrations of X-537A and a metal, the period of enhanced m.e.p.p. frequency lasts for 5–55 min. After the period of rapid release: (1) m.e.p.p.s are rarely seen; (2) in Ca^{2+} -Ringer, nerve stimulation does not elicit an e.p.p.; (3) tetanic nerve stimulation does not produce the usual increase in m.e.p.p. frequency; (4) a second application of X-537A does not produce a second increase in m.e.p.p. frequency. The reasons for the disappearance of m.e.p.p.s and for the abrupt failure of transmission are still uncertain. The compound action potential in the sciatic nerve is not affected by 25 μM X-537A, but we have not yet tested conduction in the terminals immediately above the end-plate.

In conclusion the Ca^{2+} -transporting antibiotic, X-537A, together with a divalent metal ion can elicit a substantial, transitory increase in m.e.p.p. frequency and in acetylcholine release elicited by nerve stimulation. The steep dose-effect curve suggests that the relation between intracellular metal concentration and rate of release is not linear. This finding may bear on the well known nonlinear relation between $(\text{Ca}^{2+})_i$ and quantal release from stimulated terminals^{13–15}. Once brought into the terminal by the ionophore, or by nerve stimulation¹⁶ each of several divalent metals increases the frequency of quantal release.

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- ¹ Katz, B., *The release of neural transmitter substances*, 60 (Liverpool University Press, 1969).
- ² Miledi, R., *Proc. R. Soc.*, **B183**, 421 (1973).
- ³ Blioch, Z. L., Glagoleva, I. M., Liberman, E. A., and Nenashin, V. A., *J. Physiol., Lond.*, **199**, 11 (1968).
- ⁴ Quastel, D. M. J., Hackett, J. T., and Cooke, J. D., *Science*, **172**, 1034 (1971).
- ⁵ Kita, H., and Van der Kloot, W., *Life Sci.*, **10**, 1423 (1971).
- ⁶ Pressman, B. C., *Fedn Proc.*, **32**, 1698 (1973).
- ⁷ Scarpa, A., Baldassare, J., and Inesi, G., *J. gen. Physiol.*, **60**, 735 (1972).
- ⁸ Kita, H., and Van der Kloot, W., *Physiologist*, **16**, 365 (1973).
- ⁹ Levy, J. W., Cohen, J. A., and Inesi, G., *Nature*, **242**, 461 (1973).
- ¹⁰ Repke, O. I., and Katz, A. M., *Physiologist*, **16**, 432 (1973).
- ¹¹ Danforth, W. H., and Helmrich, E., *J. biol. Chem.*, **239**, 3133 (1964).
- ¹² Katz, B., and Thesleff, S., *J. Physiol., Lond.*, **137**, 267 (1957).
- ¹³ Jenkinson, D. H., *J. Physiol., Lond.*, **138**, 434 (1957).
- ¹⁴ Dodge, jun., F. A., and Rahamimoff, R., *J. Physiol. Lond.*, **193**, 419 (1967).
- ¹⁵ Van der Kloot, W., and Kita, H., *J. Membrane Biol.*, **14**, 365 (1973).
- ¹⁶ Kita, H., and Van der Kloot, W., *Nature new Biol.*, **245**, 52 (1973).

Arginase affects lactogenesis through its influence on the biosynthesis of spermidine

THE activity of arginase in mammary gland increases markedly during lactation^{1–3}. Since lactating mammary gland, unlike liver, lacks other enzymes of the urea cycle³, the function of arginase in this gland is somewhat restricted. Results of earlier studies^{3,4} have suggested that the enzyme is involved in proline formation, in concert with ornithine aminotransferase, for increased synthesis of milk protein.

Another possible role for arginase is participation in the biosynthesis of spermidine. This possibility appears particularly attractive because the concentration of spermidine increases markedly in lactating tissue, as do the activities of

ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase⁵. In the presence of the latter two enzymes, ornithine formed by arginase can be converted to spermidine through putrescine⁶. Moreover, recent studies⁷ on the development of mammary gland *in vitro* have demonstrated that spermidine, together with insulin and prolactin, produces a marked increase in the synthesis of milk proteins, which is similar to that produced by the combination of insulin, glucocorticoid and prolactin. The involvement of spermidine in milk protein formation is further supported by the observation⁷ that the combination of these three hormones enhances the cellular concentration of spermidine before the accelerated synthesis of the milk proteins, casein and α -lactalbumin.

We have investigated the mechanism whereby the interplay of the hormones stimulates spermidine formation during mammary development. The data demonstrate that prolactin, in the presence of insulin, increases the activity of arginase in mammary epithelium. It appears that arginase may have a crucial function in milk protein synthesis by participating in the biosynthesis of spermidine.

The hormonal regulation of arginase activity was examined by culturing mouse mammary explants in a chemically defined medium containing several combinations of insulin, hydrocortisone and prolactin (Table 1). These hormones effect morphological and biochemical changes associated with mammary gland development⁸. Although mammary gland is primarily composed of epithelial cells and a large mass of fat cells during pregnancy, the activity of arginase in the gland was almost completely recovered in the homogenate of isolated epithelial cells⁹. After 3 d of culture, the initial level of arginase activity was maintained in the presence of insulin. Hydrocortisone by itself, or prolactin alone, or the combination of the two could not effectively maintain the initial level, and the enzyme activity decreased markedly as it did in the absence of any added hormones. In the presence of insulin, prolactin produced a

Table 1 Effect of various combinations of insulin, hydrocortisone, and prolactin on the activity of arginase in mammary explants in culture

Culture conditions	Arginase activity (μg urea formed per 10 min per mg wet wt tissue)
Single incubation	
Uncultured control	1.02 \pm 0.05
No hormone	0.40 \pm 0.08
Insulin	1.00 \pm 0.07
Hydrocortisone	0.38 \pm 0.04
Prolactin	0.41 \pm 0.04
Hydrocortisone + prolactin	0.38 \pm 0.05
Insulin + prolactin	3.20 \pm 0.08*
Insulin + hydrocortisone	1.28 \pm 0.04*
Double incubation	
Control	1.24 \pm 0.08
Insulin	1.31 \pm 0.05
Prolactin	0.47 \pm 0.04
Insulin + hydrocortisone	1.40 \pm 0.09
Insulin + prolactin	3.10 \pm 0.08*
Insulin + hydrocortisone + prolactin	4.43 \pm 0.07*

Mammary explants from midpregnant C3H/HeN mice were cultured in Medium 199 (Gibco) containing the indicated combination of hormones as described previously¹⁰. The final concentration of hormones was 5 μg ml⁻¹ for insulin and prolactin, and 0.5 μg ml⁻¹ for hydrocortisone. Single incubation was carried out for 72 h. Double incubation was carried out by first culturing mammary explants in insulin-containing medium for 72 h and then transferring explants to medium containing the indicated combination of hormones. After transfer the explants were cultured for another 72 h. Control in the double incubation system refers to explants cultured with insulin for the initial 72 h.

The activity of arginase was determined by measuring the formation of urea from arginine as described earlier¹¹. Each value represents the mean \pm s.e.m. of six separate determinations on three separate cultures.

* The increase is significant over control ($P < 0.01$).

Table 2 Effect of various culture conditions on α -lactalbumin activity in mammary gland explants

Culture conditions	α -Lactalbumin activity (pmol lactose formed per 30 min per mg wet wt tissue)
Uncultured control	20 \pm 5
Three hormones in regular M199	90 \pm 7
Three hormones in arginine-deficient M199	32 \pm 4
Three hormones + L-ornithine in arginine-deficient M199	95 \pm 6
Three hormones + putrescine in arginine-deficient M199	91 \pm 6
Three hormones + spermidine in arginine-deficient M199	85 \pm 5
Three hormones + proline in arginine-deficient M199	30 \pm 5
Three hormones + lysine in arginine-deficient M199	22 \pm 4
Three hormones + spermine in arginine-deficient M199	10 \pm 4

Mammary explants from midpregnant C3H/HeN mice were cultured for 48 h in the presence of insulin, hydrocortisone and human placental lactogen. The final hormone concentration was 5 μ g ml⁻¹ for insulin and hydrocortisone, and 1 μ g ml⁻¹ for human placental lactogen. Human placental lactogen was previously shown to be as effective as prolactin in stimulating milk protein synthesis in mammary explants¹⁹. Arginine-deficient Medium 199 contained 0.2 mM L-arginine, whereas regular Medium 199 contained 4 mM L-arginine. The final concentration of other added amino acids was 0.05 mM L-ornithine, 4 mM L-proline, and 5 mM L-lysine. The final concentration of putrescine, spermidine and spermine was 4 mM.

The activity of α -lactalbumin was determined by measuring the formation of lactose from uridine-diphospho-¹⁴C-galactose (290–300 mCi mmol⁻¹, New England Nuclear Corp.) and glucose as described previously¹⁹. Each value represents the mean \pm s.e.m. of four separate determinations on two cultures.

large increase in the enzyme activity, whereas hydrocortisone consistently produced a slight increase.

Insulin stimulates cell proliferation in mammary epithelium during the first 2 d of culture, but by the third to fourth day the proliferative activity disappears¹⁰. These non-proliferative cells are still viable since they synthesise milk proteins in response to insulin, glucocorticoid and prolactin^{11,13}. Table 1 shows that addition of prolactin to the culture of such cells also resulted in increased arginase activity. The stimulatory effect of prolactin was enhanced by hydrocortisone although the steroid alone, or in combination with insulin, produced little increase in arginase activity in post-mitotic cells. It is clear that the effect of prolactin again required the presence of insulin, since no stimulation occurred when insulin was omitted during the second incubation. These results indicate that stimulation of arginase activity by prolactin is not a consequence of the increase in the number of mammary epithelial cells since proliferation of mammary epithelium was essentially completed when prolactin was added.

We next assessed the possibility that arginase participates in milk protein synthesis by supplying ornithine for the formation of spermidine (Table 2). When mammary explants were cultured in medium in which the concentration of arginine was reduced from 4 mM to 0.2 mM, the triple hormone combination produced very little increase in the synthesis of α -lactalbumin. However, addition of ornithine, the product formed by arginase, to such a medium restored the increase in α -lactalbumin. Addition of the polyamines, putrescine and spermidine, was also effective, whereas another polyamine, spermine, and amino acids such as proline and lysine did not effect an increase in α -lactalbumin. The effect of arginine deprivation is specific in the sense that in experiments where the concentration of other amino acids, such as leucine, was similarly reduced in the culture medium the three hormones produced an increase in α -lactalbumin similar to that observed in the regular medium 199 (not shown). These results can best be explained by the possibility that arginine may be converted, through ornithine and putrescine, to spermidine, which is required for milk protein synthesis^{7,14}. Preliminary studies indicate that ³H-arginine is indeed converted to ³H-spermidine and that prolactin stimulates this conversion by enhancing the activity of arginase (our unpublished

results). These results strongly suggest that arginase plays an important role in the biosynthesis of spermidine. Earlier studies^{14,15} on cultured mammary explants showed that insulin stimulates the activity of ornithine decarboxylase and that glucocorticoid increases the activity of S-adenosyl-L-methionine decarboxylase. Thus it appears that insulin, glucocorticoid, and prolactin interact to stimulate a group of enzymes which are involved in the biosynthesis of spermidine during the development of mammary epithelium. In view of the observation^{7,14} that spermidine is necessary for milk protein synthesis, such interaction may represent a fundamental mechanism for the regulation of lactogenesis by these hormones.

Finally, it should be emphasised that our studies *in vitro* do not exclude a possible role of arginase for proline formation, as suggested earlier³. It is possible that in addition to a role in spermidine formation, arginase may also participate in proline synthesis in intact animals where the proline supply may not be abundant.

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- Folley, S. J., and Greenbaum, A. L., *Biochem. J.*, **41**, 261–268 (1947).
- Greengard, O., Sahib, M. K., and Knox, W. E., *Archs Biochem. Biophys.*, **137**, 477–482 (1970).
- Yip, M. C. M., and Knox, W. E., *Biochem. J.*, **127**, 893–899 (1972).
- Mephram, T. B., and Linzell, J. L., *Nature*, **214**, 507–508 (1967).
- Russell, D. H., and McVicker, T. A., *Biochem. J.*, **130**, 71–76 (1972).
- Tabor, H., and Tabor, C. W., *Adv. Enzymol.*, **36**, 203–268 (1972).
- Oka, T., *Science*, **184**, 78–80 (1974).
- Topper, Y. J., *Rec. Prog. Hormone Res.*, **26**, 287–308 (1970).
- Oka, T., and Perry, J. W., *J. biol. Chem.*, **249**, 3586–3591 (1974).
- Stockdale, F. E., and Topper, Y. J., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 1283–1289 (1966).
- Turkington, R. W., Lockwood, D. H., and Topper, Y. J., *Biochim. biophys. Acta*, **148**, 475–480 (1967).
- Mills, E. S., and Topper, Y. J., *J. Cell Biol.*, **44**, 310–328 (1970).
- Oka, T., and Topper, Y. J., *J. biol. Chem.*, **246**, 7701–7707 (1971).
- Oka, T., and Perry, J. W., *J. biol. Chem.* (in the press).
- Aisbitt, R. P. G., and Barry, J. M., *Biochim. biophys. Acta*, **320**, 610–616 (1973).
- Juergens, W. G., Stockdale, F. E., Topper, Y. J., and Elias, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **54**, 629–634 (1965).
- Schimke, R. T., *J. biol. Chem.*, **239**, 3809–3817 (1964).
- Vonderhaar, B. K., Owens, I. S., and Topper, Y. J., *J. biol. Chem.*, **248**, 467–471 (1973).
- Turkington, R. W., and Topper, Y. J., *Endocrinology*, **79**, 175–181 (1966).

Cytosol-binding protein of thyroxine and triiodothyronine in human and rat kidney tissue

DURING the past few years, it has become increasingly apparent that nonpeptide hormone action in the respective target organs occurs in the nucleus. This was first shown for steroid hormones^{1–4} and more recently for thyroid hormones⁵. Specific nuclear acceptor molecules for hormones have been shown in target tissues, associated with nuclear proteins^{6–8}. The question arises whether hormone molecules, on their way to the nuclear binding sites, traverse the cytoplasm in a more or less random fashion or whether they are bound by specific cytoplasmic receptor molecules.

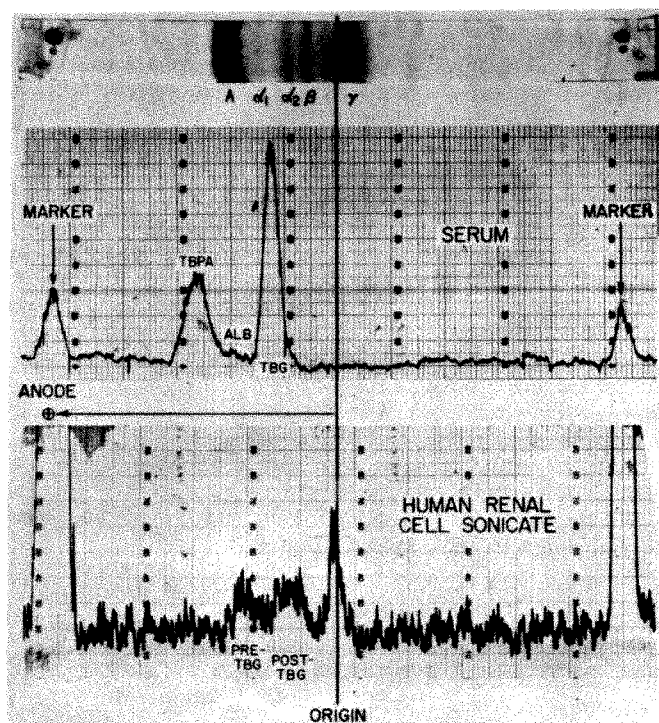


Fig. 1 Comparison of radioactive scans of human serum and human renal cell binding proteins. Electrophoresis was done in glycine-acetate, pH 8.6. The label is $^{125}\text{I}-\text{T}_4$.

Accordingly we have sought specific cytoplasmic proteins that bind thyroid hormones and are distinctly different from the serum protein carriers such as thyroxine-binding α -globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin, the major carriers of thyroid hormones in the circulations of rats as well as humans. In particle-free cytosol preparations from rat kidneys we have found four separate protein peaks, three of which bind both thyroxine (T_4) and triiodothyronine (T_3). One peak seems to bind T_3 only. Thus, the cytoplasmic transport of thyroid hormones seems to depend on receptor molecules.

Male Sprague-Dawley rats weighing in excess of 250 g were used. Cytosol was prepared from perfused rat kidneys and livers. The aorta was cannulated and the kidneys perfused *in situ* for 20–30 min with ice-cold isotonic saline solution until the venous effluent was crystal clear. Subsequent protein determinations by the Oyama-Eagle modification⁹ of the Lowry method¹⁰ confirmed the absence of appreciable protein in the effluent irrigation solution. The kidneys were removed and homogenised in three volumes of saline solution in an all glass homogeniser. In some cases the homogenate was spun at 800g to obtain a supernatant free of nuclei and gross membrane fragments. The supernatant or the homogenate, packed in ice, was sonicated for 3 min with a Savant Instrument Model 1000 Insonator. The sonicated material was centrifuged for 1 h at 105,000g in a Beckman L3-50 ultracentrifuge. The temperature throughout this procedure was 0°–4°C. After centrifugation the supernatant was decanted and stored at –20°C until used.

The cytosol proteins were labelled with $^{125}\text{I}-\text{T}_3$ or with $^{131}\text{I}-\text{T}_4$ (Industrial Nuclear) and subjected to paper electrophoresis. The electrophoretic systems were 0.2 M glycine-acetate, pH 8.6 (refs 11, 12), 0.125 M sodium borate buffer, pH 10.0, or 0.05 M sodium glycinate buffer, pH 10.0. The paper strips were dried after electrophoresis and scanned with a Nuclear-Chicago Actigraph III (Model 1004) strip scanner. In case of dual labelling, the strips were scanned rapidly and the area containing radioactivity was cut into 0.5 cm segments, which were then counted in a Packard Auto-Gamma spectrometer. Cultures of human kidney and liver cells were purchased from Microbiological Associates and treated as described earlier¹³.

The total protein content of the effluent perfusate was less than 2 mg ml⁻¹ after 20 ml of effluent had been collected, and less than 1 mg ml⁻¹ after 150 ml had been collected, which usually required 20–30 min of perfusion. The liver perfused through the portal vein exhibited blanching, but effluent fluid was not analysed.

It was not unexpected therefore, that cytosol preparations subjected to electrophoresis showed no peak with the mobility of serum TBG. In all cases, cytosol-binding proteins (CBP) were readily evident on scanning of dried paper electrophoretic strips; the scanning was done before staining with bromphenol blue to avoid elution of the tracer.

The dialysis of the cytosol against at least 100 volumes of buffer or saline solution overnight before electrophoresis revealed retention of more than 60% of the radioactivity by the cytosol solution in the dialysis bag, signifying unequivocal protein binding.

Gel filtration studies on Sephadex G-200 showed a protein peak of radioactivity compatible with a molecular weight of about 70,000. To investigate the existence of cytosol binders in human kidney tissue, similar electrophoretic studies were carried out on sonicated human renal cells which had been incubated with $^{125}\text{I}-\text{T}_4$ as previously reported¹³. A typical scan is illustrated in Fig. 1, which shows 'pre-TBG' and 'post-TBG' peaks, so designated because of their mobility, as well as origin radioactivity. The low level of radioactivity in human renal cell sonicates reflected the tracer amounts of hormone added to the cell cultures, approximately 2×10^{-8} M T_4 .

The amount of origin radioactivity in different human and rat cell studies was quite variable, occasionally absent, and not clearly associated with any obvious experimental detail.

Experiments were also performed on perfused rat liver cytosol as well as human liver cells (Chang liver) grown in culture¹³, with findings of cytosol binding proteins in all cases.

As Fig. 2 shows, labelled T_3 was apparently bound by different cytosol-binding proteins than T_4 , as illustrated in electrophoretic strips run simultaneously.

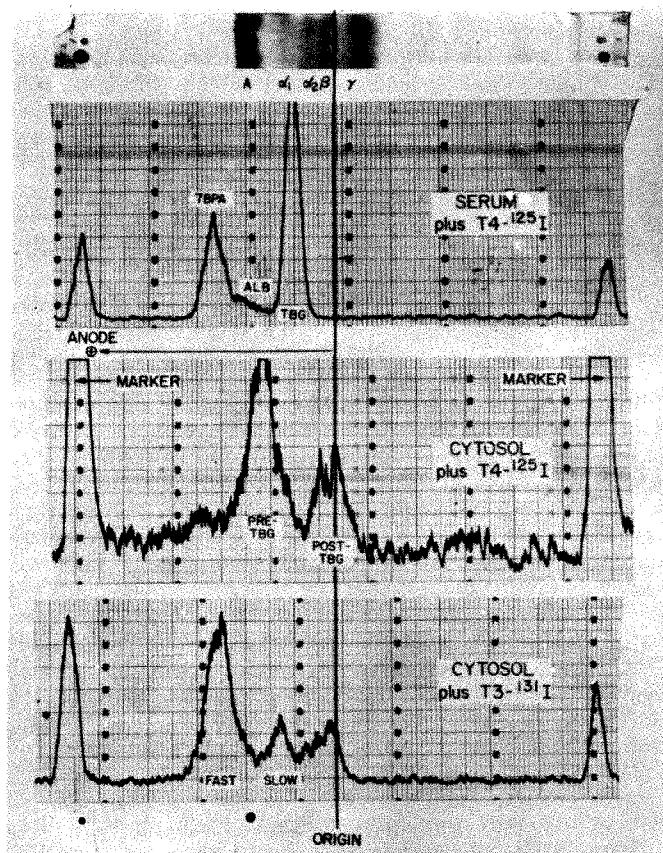


Fig. 2. Rat renal cytosol binding proteins for T_4 and T_3 . Electrophoresis was done in glycine-acetate, pH 8.6.

Since it could be argued that the different ligands could conceivably cause slightly different electrophoretic mobilities, experiments were carried out in which both labelled hormones as $^{125}\text{I}-\text{T}_3$ and $^{131}\text{I}-\text{T}_4$ were added to the same rat renal cytosol preparation. A peak with greatest anionic mobility was found to bind T_3 but not T_4 , even with increments of nonradioactive T_4 as high as 20 mg per 100 ml (2.6×10^{-7} M) as illustrated in Fig. 3. Whereas the glycine-acetate system at pH 8.6 used in the foregoing studies provided reasonable separation of peaks, we found that the sodium borate and sodium glycinate buffer systems at pH 10.0 provided much more rapid migration and greater separation of cytosol binding proteins (although these alkaline buffers are less satisfactory for study of serum protein carriers).

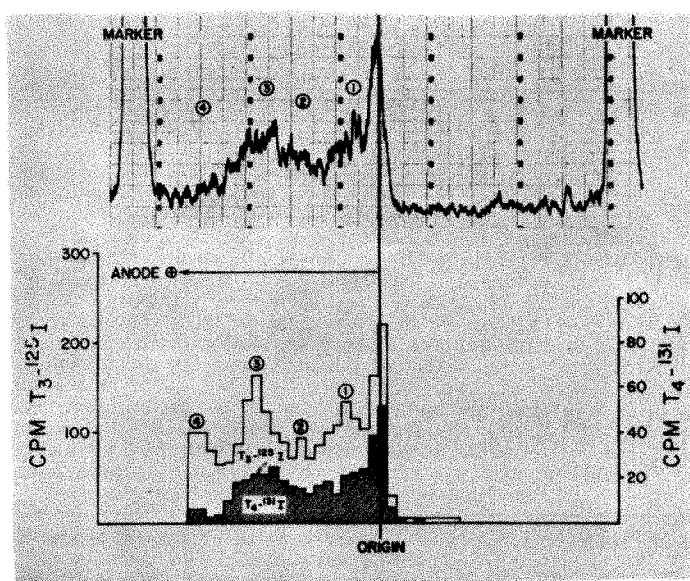


Fig. 3 Rat renal cytosol binding proteins for T_4 and T_3 . Cytosol contained both $^{125}\text{I}-\text{T}_3$ and $^{131}\text{I}-\text{T}_4$. Electrophoresis was done in sodium glycinate buffer, pH 10.0. The radioactive scan above reflects mainly ^{131}I , and ^{125}I to a lesser degree. In the histogram below with discrimination between the labels, in addition to origin radioactivity, the labelled T_3 is distributed in four peaks, appropriately numbered, with 4, which has moved farthest toward the anode at the left, clearly showing T_3 , but no appreciable T_4 radioactivity. In contrast, the other peaks bound both radioactive hormones. In the run illustrated, the concentration of T_3 added was $0.16 \mu\text{g}$ per 100 ml (2.4×10^{-9} M), while the T_4 was $11 \mu\text{g}$ per 100 ml (1.4×10^{-7} M). Similar results, that is, peak 4 showing T_3 but not T_4 radioactivity were also seen with the same T_3 concentration, but T_4 concentrations of 1.1, 6.1, and also $21.1 \mu\text{g}$ per 100 ml (1.4×10^{-8} M, 7.9×10^{-8} M and 2.7×10^{-7} M).

The basic finding is the demonstration of binding of thyroid hormones by cytosol with retention of the ligand hormones after overnight dialysis. The demonstration of electrophoretic peaks suggests that it may become possible to isolate specific cytosol binding proteins and determine their binding constants by equilibrium dialysis and other methods. The findings are in general agreement with the preliminary descriptions of cytosol binding proteins by others¹⁴⁻¹⁷.

The use of strongly alkaline (pH 10.0) buffers represents a novel technical approach, in that it enhanced the sensitivity of ordinary paper electrophoresis with conventional rather than 'reverse' or countercurrent flow¹⁸. Since the overall picture is not different from those runs with lower pH, it is reasonable to assume that the only effect of the high pH was increased separation.

Hormone action at the molecular level seems to involve the interaction between the hormone and the nonhistone or acid soluble protein associated with nuclear chromatin, when small molecule (nonpeptide) hormones are considered. The evidence amassed during the past decade includes more evidence for the

action of the steroid hormones than for the thyroid hormones. Thus numerous studies have dealt with the sex steroids^{19,20} cortisol²¹ and aldosterone^{22,23}. The thyroid hormones, on the other hand, have been considered to have a primary action on the cell nucleus since the demonstration of nuclear localisation of labelled thyroid hormone by Siegal and Tobias²⁴ in cultured human renal epithelial cells. Further evidence concerning nuclear binding of the thyroid hormones has been found in our laboratory, in which nuclear uptake *in vitro* has been shown, and further details have been reported by others⁵⁻⁷. Since there is also evidence of mitochondrial mediation of thyroid hormone action²⁵⁻²⁸, it is interesting that we have found mitochondrial protein binding of both T_4 and T_3 , in studies now in progress.

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- Clark, J. H., Anderson, J. N., and Peck, E. J., *Steroids*, **22**, 707-718 (1973).
- Anderson, J. N., Peck, E. J., and Clark, J. H., *Endocrinology*, **92**, 1488-1495 (1973).
- O'Malley, B. W., and Strott, C. A., *Handbook of Physiology, Endocrinology II*, part I, 591-602 (1973).
- O'Malley, B. W., 1973, *New Engl. J. Med.*, **284**, 370-377 (1971).
- Oppenheimer, J. H., Koerner, D., Schwartz, H. L., and Surks, M. I., *J. clin. endocr. Metab.*, **35**, 330-333 (1972).
- Surks, M. I., Koerner, D., Dillman, W., and Oppenheimer, J. H., *J. biol. Chem.*, **248**, 7066-7072 (1973).
- DeGroot, L. J., Refetoff, S., Strausser, J., and Barsano, C., Program, American Thyroid Association, Forty-ninth Meeting, 1973, T-14.
- Schutz, G., Beato, M., and Fiegelson, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1218-1221 (1973).
- Oyama, V. I., and Eagle, H., *Proc. Soc. exp. Biol. Med.*, **91**, 305-307 (1956).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).
- Sterling, K., and Tabachnik, M., *Endocrinology*, **68**, 1073-1075 (1961).
- Inada, M., and Sterling, K., *J. clin. Invest.*, **46**, 1442-1450 (1967).
- Sterling, K., Brenner, M. A., and Saldanha, V. F., *Science*, **179**, 1000-1001 (1973).
- Refetoff, S., Matalon, R., and Bigazzi, M., *Endocrinology*, **91**, 934-947 (1972).
- Spaulding, S. W., and Davis, P. J., *Biochim. biophys. Acta*, **229**, 279-283 (1971).
- Hamada, S., and Ingbar, S. H., Program, Endocrine Society, Fifty-third Annual Meeting, 1971, A-109.
- Hamada, S., Torizuka, K., Miyake, T., and Fukase, M., *Biochim. biophys. Acta*, **201**, 479-492 (1970).
- Robbins, J., *Archs Biochem. Biophys.*, **63**, 461, 469 (1956).
- Clark, J. H., Anderson, J. N., and Peck, E. J., in *The receptor for reproductive hormones* (edit. by O'Malley, B. W., and Means, A. R.), 15-59 (Plenum, New York, 1973).
- O'Malley, B. W., Schroeder, W. T., and Spelsberg, T. C., in *The receptor for reproductive hormones*, (edit. by O'Malley, B. W., and Means, A. R.), 174-196 (Plenum, New York, 1973).
- Higgins, S. J., Rousseau, G. G., Baxter, J. D., and Tomkins, G. M., *J. biol. Chem.*, **248**, 5873-5879 (1973).
- Herman, T. S., Fimognari, G. M., and Edelman, I. S., *J. biol. Chem.*, **243**, 3849-3856 (1968).
- Marver, D., Goodman, D., and Edelman, I. S., *Kidney Int.*, **1**, 210-223 (1972).
- Siegel, E., and Tobias, C. A., *Science*, **153**, 763-765 (1966).
- Tapley, D. F., and Basso, N., *Biochim. biophys. Acta*, **36**, 486-498 (1959).
- Primack, M. P., Tapley, D. F., and Buchanan, J., *Biochim. biophys. Acta*, **244**, 349-352 (1971).
- Primack, M. P., Tapley, D. F., and Buchanan, J., *Endocrinology*, **91**, 840-844 (1972).
- Babior, B. M., Creagan, S., Ingbar, S. H., and Kipnes, R. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 98-102 (1973).

Aryl hydrocarbon (benzo(a)pyrene) hydroxylase in human peripheral blood monocytes

MICROSOMAL mixed-function oxygenases metabolise a variety of exogenous compounds, including drugs, pesticides and carcinogens, as well as endogenous substances such as steroids^{1,2}. One oxygenase, aryl hydrocarbon hydroxylase (AHH), is important in both the detoxification of some carcinogenic polycyclic hydrocarbons and the activation of others to more carcinogenic forms¹⁻⁴. Hydroxylase activity and cytochrome P-450 have been found in various tissues of animals^{1,2} and man⁵⁻¹². AHH activity increases in many tissues after treatment with polycyclic hydrocarbons. In certain inbred mouse strains this increase appears to be regulated by a single autosomal dominant gene¹³, and a correlation between enzyme levels and susceptibility to methylcholanthrene-induced tumorigenesis has been reported¹⁴. Data obtained by Shaw and his colleagues point to genetic regulation of AHH in human populations¹⁵.

With the exception of peripheral blood lymphocytes, which must be stimulated with mitogens before assay, none of the human tissues in which AHH has been detected can be obtained readily for clinical and epidemiological study. Detection of AHH in macrophages of the human lung¹⁰, rat liver¹⁶ and guinea pig peritoneum¹⁷ suggested that monocytes might also contain the enzyme. We report here that AHH is present in monocytes from human peripheral blood. This observation may facilitate studies in human populations relating hydroxylase activity and the metabolism of chemical carcinogens.

Mononuclear leukocytes were obtained from heparinised human blood by the method of Boyum¹⁸. At least 99% of the cells isolated were mononuclear and excluded trypan blue. After incubation in cell culture for 24 h, the leukocytes were treated with medium that either contained or lacked the polycyclic hydrocarbon benz(a)anthracene (1 $\mu\text{g ml}^{-1}$). After incubation for a further 17 h, the culture medium was removed and the surface of the plastic culture dish was rinsed with Dulbecco's phosphate-buffered saline. The adherent cell population was more than 95% viable and contained 98% monocytes, judged by cellular morphology and by the ability of the cells to phagocytose 1 μm latex spheres (Dow) suspended in Eagle's minimum essential medium containing 10% pooled human AB serum. An average of 46×10^6 monocytes was obtained from 500 ml of heparinised blood. Treatment with benz(a)anthracene did not affect the yield, viability or differential cell count of these preparations. Monocytes were assayed for AHH activity by the method of Nebert and Gelboin¹⁹ as modified by Kellerman *et al.*¹⁵.

AHH is present in human peripheral blood monocytes and its activity is increased following treatment with benz(a)anthracene (Table 1). In twelve different donors, AHH activity ranged from 0.3 to 1.4 units per 10^6 cells and increased 4 to 33-fold after incubation with benz(a)anthracene. The activity of replicate samples from a single monocyte pool varied by less than 5%. AHH from human peripheral blood monocytes has properties which are typical of the microsomal mixed-function oxygenases (Table 2), that is, a requirement for NADPH and inhibition by carbon monoxide^{11,18}. In addition, hydroxylase activity is inhibited by 7, 8-benzoflavone, a potent inhibitor of the complex²⁰.

The presence of AHH in human peripheral blood monocytes further implicates the reticuloendothelial system in the metabolism of polycyclic hydrocarbons, suggests one explanation for the immunogenicity of these compounds and may provide a convenient assay for the study of AHH in human populations. Peripheral blood monocytes contribute to macrophage pools throughout the body and accumulate at sites of acute and chronic inflammation²¹. Circulating monocytes could provide large local concentrations of the enzyme at inflammatory foci. Cantrell *et al.*¹⁰ obtained

larger numbers of alveolar macrophages with higher hydroxylase activity per cell from the lungs of smokers than from those of nonsmokers. The infiltration of inflammatory cells evoked by cigarette smoke, by various carcinogens and by cocarcinogenic promoters might influence the local metabolism of polycyclic hydrocarbons. Reticuloendothelial cells in the liver, lung and lymphoid tissue might also participate in the detoxification or activation of certain carcinogens. Agents that stimulate or depress the reticuloendothelial system, such as BCG can modify chemical carcinogenesis²². The functional state of the reticuloendothelial system might alter the metabolic fate of carcinogenic compounds.

Table 1 Aryl hydrocarbon hydroxylase activity in human peripheral blood monocytes

	Specific activity*	
	Donor 1	Donor 2
Control	0.5	1.4
BA-treated	12.9	19.3

Monocytes were suspended in 0.85 ml of a solution (pH 7.55) containing 67.5 $\mu\text{mol KH}_2\text{PO}_4$, 66.7 $\mu\text{mol KOH}$, 4.2 $\mu\text{mol MgCl}_2$ and 25 μmol nicotinamide. A solution (100 μl) containing 0.7 mg NADH, 0.7 mg NADPH and 0.7 mg bovine serum albumin was added. The reaction was started by the addition of 100 nmol of the substrate benzo(a)pyrene, in 0.050 ml acetone. Incubations were for 20 min at 37°C under the illumination of a single 25 W red bulb. The reaction was stopped by the addition of 1.0 ml acetone and the mixture was extracted with 3.0 ml hexane. A 2.5 ml sample of the organic layer was extracted with 1.0 ml 1 N NaOH, and the fluorescence of the alkali phase was measured in an Aminco-Bowman spectrofluorometer and compared with that of a standard 3-hydroxybenzo(a)pyrene solution. The reaction was linear with respect to the time of incubation (0–20 min) and the number of monocytes that was assayed.

*Units per 10^6 cells. One unit of AHH activity catalyses in 20 min the formation of phenolic products with the fluorescence equivalent to that of 1 pmol of 3-hydroxybenzo(a)pyrene. Each assay contained $1.4\text{--}3.3 \times 10^6$ monocytes.

Table 2 Effect of assay conditions on aryl hydrocarbon hydroxylase activity from human peripheral blood monocytes

Assay system	Specific activity*
Complete	5.0
No NADPH	1.5
90% CO–10% O ₂	1.6
7.8 BF (10^{-4}M)†	2.0

Monocytes were treated with benz(a)anthracene (1 $\mu\text{g ml}^{-1}$) as outlined in the text. AHH was assayed as described in Table 1. *Units per 10^6 cells. Each assay contained 3.3×10^6 monocytes.

†7.8 benzoflavone was added in 0.010 ml dimethylsulphoxide.

Cutaneous application of polycyclic hydrocarbon carcinogens can produce immunologically specific contact hypersensitivity²³. These low molecular weight hydrocarbons probably attain immunogenicity by conjugation with host proteins. Microsomal enzymes might contribute to the formation of immunogenic conjugates by converting unreactive parent compounds to reactive intermediates able to bind covalently to cellular macromolecules. Macrophages derived from peripheral blood monocytes process macromolecular antigens for presentation to lymphocytes in a more immunogenic form²⁴. AHH within macrophages could catalyse the formation of immunogenic conjugates before antigen processing.

A recent study has attempted to relate AHH levels to the development of lung cancer in man. Kellerman *et al.*²⁵ have measured hydroxylase induction in human leukocytes after incubation with phytohaemagglutinin and pokeweed mitogen. Under these conditions the polycyclic hydrocarbon 3-methylcholanthrene induced a greater increase in hydroxylase activity in leukocytes from patients with lung cancer than in leukocytes from a control population. The requirement for mitogens in this system, however, raises the possibility that hydroxylase levels might be a function of the response of leukocytes to the mitogens rather than their response to the 3-methylcholanthrene. Treatment with mitogens is not required to measure AHH in monocytes

from human peripheral blood. Monocytes constitute 2–6% of the leukocytes in peripheral blood and absolute counts range from 1×10^5 to $6 \times 10^5 \text{ ml}^{-1}$ (ref. 26). AHH assays require at least 4×10^6 monocytes for duplicate determinations following incubation with and without a polycyclic hydrocarbon. If all monocytes could be recovered, 7–40 ml of peripheral blood would suffice. While several questions remain regarding the practicality of a monocyte assay for clinical use, including the efficiency of isolation techniques, these cells may provide a convenient source of tissue for the study of AHH in human populations.

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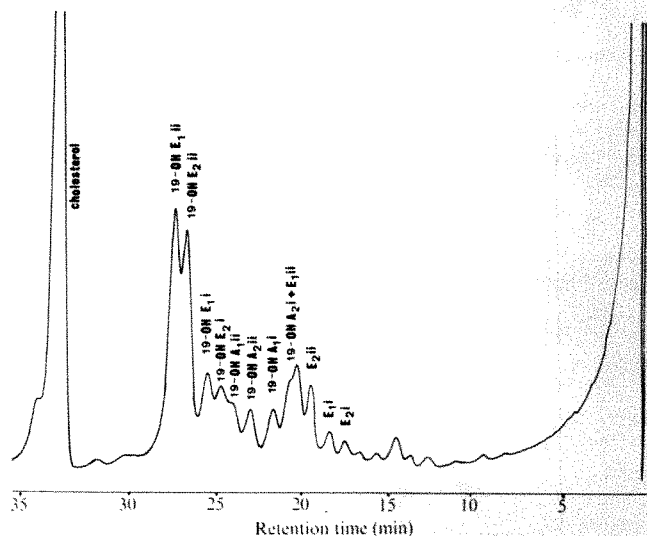


Fig. 1 Gas chromatogram of a semen extract as methyl oximes, methyl esters and trimethyl silyl ethers. The extract was prepared by mixing the semen with four volumes of 1.5 M pyridinium acetate buffer (pH5) containing 5 mg ml⁻¹ methoxyamine hydrochloride and leaving in an ultrasonic bath for 40 min. The prostaglandins were then extracted with ether/ethyl acetate (4:1), methylated (diazomethane) and silylated (*bis*-(trimethylsilyl) acetamide). The oximation gives rise to *syn* and *anti* isomers; in the E compounds the first isomer to elute accounts for approximately 1/4 of the material derivatised⁶. The isomers are denoted by suffixes (in roman numerals) giving the order of elution. Identification of the components was by gas chromatography/mass spectrometry. The column was 1% of Dexsil 300 on chromosorb G and was programmed from 200° at 2° min⁻¹.

concentration of 40 µg ml⁻¹ (ref. 3). In a detailed study of semen PGs we have used a new approach for the measurement of the E prostaglandins, which involves protecting the unstable β-ketol system by oximation in the unextracted semen, thus ensuring minimal degradation to the A or B series. Using this technique we found that the levels of 19-hydroxy derivatives of PGA and PGB are relatively low whereas two new PGs, later identified as 11α,15,19-trihydroxy-9-keto prost-13-enoic acid (19-hydroxy PGE₁) and 11α,15,19-trihydroxy-9-keto prosta-5,13-dienoic acid (19-hydroxy PGE₂) were present at an average total concentration of 100 µg ml⁻¹. These compounds can be isolated together as the methyl ester/methyl oxime derivatives by thin layer chromatography and the individual components can be separated by gas chromatography as the methyl ester/methyl oxime/*tert*-butyl dimethyl silyl ethers⁴.

The nature of the 19-hydroxy prostaglandins E was determined by gas chromatography/mass spectrometry and thin layer chromatography. The compounds were present in gas

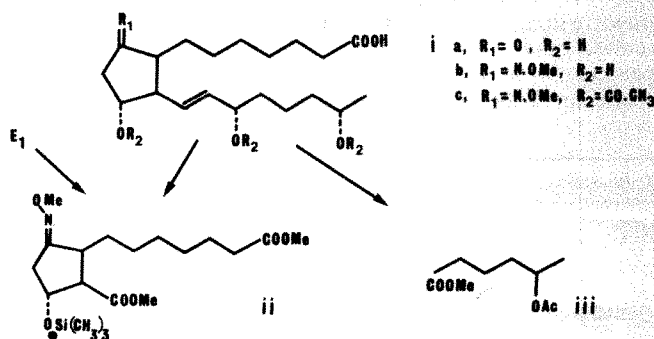


Fig. 2 Oxidative degradation of 19-OH PGE₂ as proof of structure. ia is 19-OH PGE₂, peroxide/permanate oxidation of the derivatised Ib followed by methylation (diazomethane) and silylation (*bis*-(trimethylsilyl) acetamide) gives ii. Oxidation of ic followed by methylation gives iii. Compound ii was also obtained by a similar oxidation of authentic PGE₂.

19-Hydroxylated E prostaglandins as the major prostaglandins of human semen

ALMOST 30 years after the initial discovery of the prostaglandins, Bergstrom *et al.*, succeeded in isolating and identifying prostaglandins (PGs) E₁, E₂, F_{1α} and F_{2α} from sources including human semen¹. It was subsequently claimed² that the 19-hydroxy derivatives of prostaglandins A and B were also present. These compounds were later found at an average

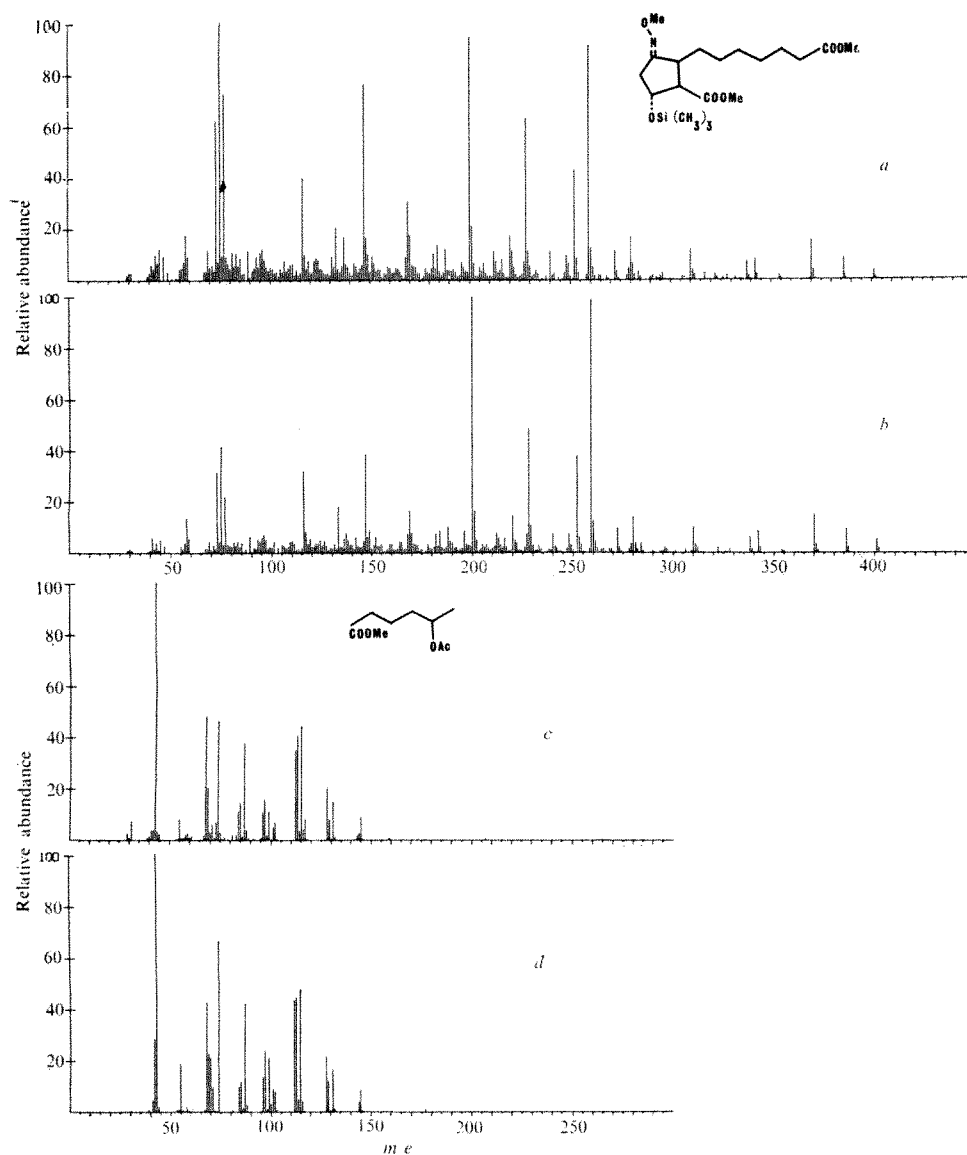


Fig. 3 Mass spectra of oxidation products of 19-OH PGE₁ (a and c) and spectra of authentic origin; (b) was obtained by the oxidation of PGE₁, and (d) was prepared by methylation, borohydride reduction in tetrahydrofuran and acetylation of 5-keto hexanoic acid.

chromatograms of semen extracts as a pair of peaks, (19-OH E₂) followed by (19-OH E₁), of approximately equal size, having retention times longer than those of E and 19-OH A prostaglandins (Fig. 1). The mass spectral evidence (Table 1) was wholly consistent with the assigned structures. The shift of 14 mass units in the molecular ion between a methyl and an ethyl oxime indicated one carbonyl group and a shift of 126 between the molecular ion of the trimethyl silyl ether derivative and that of the *tert*-butyl dimethyl silyl derivative implied three hydroxyl groups. The mass spectra of all derivatives showed many similarities to those of the E prostaglandins; particularly informative was a strong M-159 peak in the spectrum of the methyl oxime/methyl ester/TMS ether repre-

sented the loss of C₁₆ to C₂₀ of the prostaglandin molecule. This ion was particularly strong in the first isomer of the pair arising from oxidation. The loss of 159 mass units from the 19-OH compound corresponds to the loss of 71 mass units from the parent E derivative. This ion is similarly strong in the first isomer of E to elute on gas chromatography⁵. The presence of an ion of *m/e* 117 in all mass spectra of the TMS ether derivative of the 19-hydroxy compounds supported the assignment of the extra hydroxyl group to the 19 position, as this ion is characteristic of the CH (OTMSi) CH₃ group.

The identity of the 19-OH E prostaglandins was confirmed by oxidative degradation. The 19-OH E compounds were isolated as a group by TLC using the methyl ester/methyl

Table 1 Prominent peaks in the mass spectra of the two isomers of 19-OH E₁s = 19-OH ES as methyl oximes, methyl esters, trimethyl silyl ethers

Ion <i>m/e</i>	19-OH prostaglandin E ₂		Probable origin	Ion <i>m/e</i>	19-OH prostaglandin E ₁		Probable origin
	First isomer Relative abundance (%)	Second isomer Relative abundance (%)			First isomer Relative abundance (%)	Second isomer Relative abundance (%)	
627		3	M	598	18	5	M-31
596	18		M-31	508	17	5	M-(90+31)
537		16	M-90	470	60	4	M-159
506	16	27	M-(90+31)	456		8	M-(C ₁ -C ₇)
468	20	7	M-159	418	17	6	M-(180+31)
416	12	8	M-(180+31)	380	45		M-(159+90)
378	25		M-(159+90)	366		74	456-90

Ninety mass units represents loss of (-OTMSi+H), 31 mass units represents loss of -OCH₃ from methyl oximes.

oxime derivatives. These derivatives were recovered from the TLC plate and were oxidised with periodate/permanganate as described by Jacobson *et al.*⁶. This reagent hydroxylates double bonds and cleaves adjacent oxygenated functions. Thus hydroxyl groups are introduced at 13 and 14 and cleavage occurs both between C₁₃ and C₁₄ and between C₁₄ and C₁₅ (Fig. 2). To identify the fragment C₁ to C₁₃ the products of the oxidation of 1b were methylated, silylated and analysed by gas chromatography mass spectrometry. The spectrum of the expected compound (II) correlated well with the same fragment obtained by a similar oxidation of authentic PGE₁ (Fig. 3). To identify the C₁₅-C₂₀ fragment the 19-OH prostaglandins E were first acetylated (to prevent lactone formation after oxidation) and then oxidised as before. Gas chromatography mass spectrometry analysis showed the major product to be 5-acetoxy methyl hexanoate, identified by comparison with the spectrum from authentic material (Fig. 3).

The identity of these two parts of the molecule together with the mass spectral information from the intact compound conclusively identify the material present in the second gas chromatography peak as 19-OH PGE₁. The similarity in the mass spectra and the gas chromatography evidence strongly indicate that the first peak of the pair is 19-OH PGE₂.

These 19-OH prostaglandins have probably been missed before for various reasons. They are converted to the A series if left in semen (almost all 19-OH prostaglandins E had disappeared in a semen sample left at 37° C for 60 h) and they are more difficult to extract from semen than the hydroxylated A or B compounds. We have succeeded in identifying the compounds by immediate oxidation of fresh semen samples followed by extraction. We are now using this technique to establish the range of levels of these compounds in normal males and in cases of infertility.

The biological role of prostaglandins in human semen has yet to be established but it is attractive to suppose that they could be concerned with sperm migration; it has even been claimed that PGE levels are lower in the semen of infertile men³. This leads us to pursue the investigation of the biological activity and physiological significance of these exciting compounds.

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¹ Samuelsson, B., *J. biol. Chem.*, **238**, 3229 (1963).

² Hamberg, M., and Samuelsson, B., *J. biol. Chem.*, **241**, 257 (1966).

³ Bygdeman, M., Fredricsson, B., Svanborg, K., and Samuelsson, B., *Fertil. Steril.*, **21**, 622 (1970).

⁴ Corey, E. J., and Venkateswarlu, A., *J. Am. chem. Soc.*, **94**, 6190 (1972).

⁵ Green, K., *Chem. Phys. Lipids*, **3**, 254 (1969).

⁶ Jacobson, M., Beroza, M., and Jones, W. A., *J. Am. chem. Soc.*, **83**, 4819 (1961).

identified and quantitated³, and the macrophage contents of different experimental tumours were found to range from 4%–56% of the total cell population³. These cells were shown to be of host origin, most if not all being derived from circulating blood monocytes rather than by self-replication of macrophages within the tumour (Unpublished observation).

The experiments described here were designed to investigate whether the macrophage contents of a group of chemically induced rat fibrosarcomata were in any way related to their biological behaviour, and in particular to their metastatic capacity. It is possible that the number of monocytes gaining entry into a tumour is governed solely by such properties as its vasculature, or alternatively, the presence of macrophages may represent part of an immunological

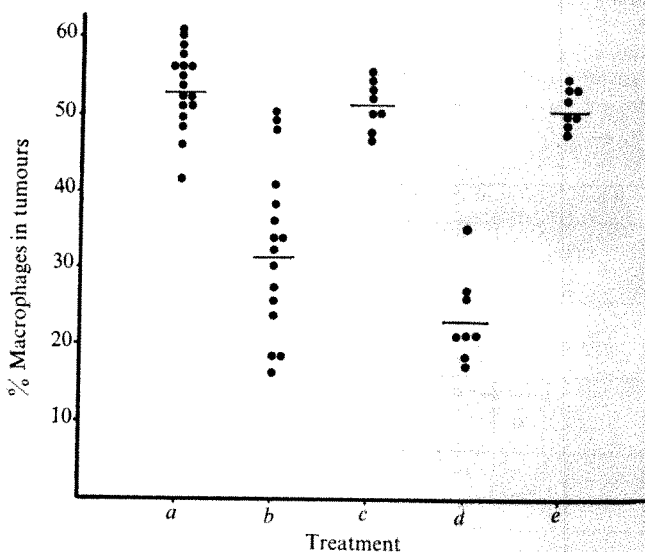


Fig. 1 Effect of immunosuppression on % macrophages in tumours grown in deprived rats. *a*, Controls; *b*, rats with thoracic duct lymph drainage; *c*, sham cannulated rats; *d*, thymectomised and irradiated rats; *e*, sham thymectomised rats. The thoracic ducts of rats were cannulated and tumours implanted 1 d later. Drainage was terminated after 8–10 d when the number of lymphocytes collected over 24 h had fallen to 0.1% of the first day's yield. Sham cannulated rats underwent anaesthesia and laparotomy, but the duct was left intact. Other rats were thymectomised at 4 weeks and then received 3×300 rad whole-body irradiation (X rays) at 2 week intervals. To ensure recovery of the bone marrow, an interval of 4 weeks was allowed between the last dose of irradiation and implantation of the tumour. At this time, treated rats were able to develop normal inflammatory macrophage exudates in response to intraperitoneal oyster glycogen stimulation⁶, indicating normal monocyte production. Thymuses of sham thymectomised rats were exposed but not removed and received no irradiation. The tumour used was a benzpyrene-induced fibrosarcoma (HSBPA) which had been passaged 20–25 times in syngeneic Hooded rats. All tumours were grown intramuscularly (i.m.) in hind limbs, excised 14 d later when they were approximately 2 cm diameter, and their macrophage contents assessed by Evans' methods³. Each point represents one rat, and the horizontal bar is the mean of the group.

reaction to tumour antigens and the degree of macrophage infiltration could then be a measure of the host response.

The macrophage content of tumours grown in immunosuppressed Hooded rats was monitored. For these experiments to be interpretable, it was necessary to suppress immunity without affecting normal bone marrow function, so that the availability of monocytes would not be reduced. The procedures chosen were two methods which removed predominantly T lymphocytes: prolonged thoracic duct lymph drainage⁴ during tumour growth; and thymectomy of rats followed by repeated whole-body irradiation⁵, before tumour implantation. Both procedures caused a significant suppression in T-cell dependent immune reactivity, which was demonstrated by the fact that an allograft (Wistar rat)

Macrophage content of tumours in relation to metastatic spread and host immune reaction

THE presence of infiltrating 'histiocytic', 'mononuclear' or 'round' cells (many of which may be cells of the monocyte/macrophage series) has often been observed in histological sections of certain types of human tumours and has been claimed to indicate good prognosis^{1,2}. Evans, using a variety of functional criteria (such as adhesion to glass in the presence of trypsin and phagocytic ability) has shown that macrophages in tumour cell suspensions can be readily

tumour grew progressively in the treated rats, but was invariably rejected by normal Hooded rats.

Rats immunosuppressed by removal of T cells were inoculated with cells from a syngeneic sarcoma, HSBPA, which when grown in normal animals gives rise to tumours containing approximately 50% macrophages. Figure 1 shows that in the treated rats the mean macrophage content of the tumours was lowered considerably. The rather wide range of variation in the drained rats (Fig 1b) may be a reflection of the total number of lymphocytes removed, since the rate of drainage achieved varied in different animals. The numbers of macrophages in tumours grown in the sham cannulated group were normal, indicating that the stress of surgical trauma and confinement in restraining cages were not responsible for the observed differences. The effect of thymectomy plus irradiation was similar, but the individual values were less widely spread. This may be due to the fact that T cell removal was complete before tumour implantation, whereas in the previous group some degree of host reaction to tumour cells may have been induced before T cells were maximally depleted.

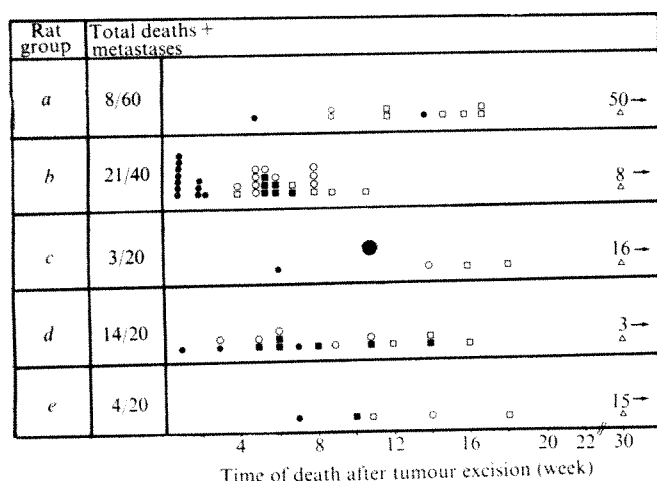


Fig. 2 Effect of immunosuppression on incidence of metastases after excision of HSBPA tumour. HSBPA tumours were grown i.m. in hind limbs of various groups of immunosuppressed rats (lymph drained, *b*, and thymectomised and irradiated, *d*) and appropriate controls (*a*, *c*, *e*) untreated rats *a*, and sham cannulated *c*, or sham thymectomised *c*, for 14 d. The tumour-bearing limbs were then amputated, and the animals kept for observation on the development of metastases for up to 30 weeks. ●, Deaths with no evidence of metastases; ○, deaths with lymph node metastases; □, deaths with lung metastases; ■, deaths with lymph node and lung metastases; △, survivors.

To investigate whether the macrophage content of a tumour can be influenced by raising the immune reactivity of the host, a Hooded rat sarcoma, MC3, was transplanted into August rats. This strain differs from the Hooded strain at minor, but not major histocompatibility loci, and will therefore allow progressive growth of Hooded rat tumours, in spite of a more pronounced host reaction to the grafts. In the syngeneic Hooded rats, the MC3 tumour grows from as few as 100 cells, invariably metastasises, and no protection to a subsequent challenge can be induced by repeated immunisation. In the 'allogeneic combination, (that is, in August rats) larger inocula of 10^6 – 10^8 tumour cells are needed and the tumour remains strictly localised. The average macrophage content of MC3 tumours grown in syngeneic hosts was 8%, whereas in August rats it was raised to 38%.

Non-specific stimulation of the reticuloendothelial system of syngeneic Hooded rats with BCG (Glaxo) before inoculation of MC3 sarcoma cells mixed with PPD reduced the incidence of spontaneous metastases from 100% to 80%, and led to a small rise in the macrophage contents of the

Table 1 Macrophage content, incidence of metastases and immunogenicity of chemically induced rat fibrosarcomata transplanted into syngeneic recipients

Tumour	Mean % macrophages (and range)	Incidence of metastases (%)	Immunogenicity
MC-3	8 (2–12)	100	< 10^3
HSH	12 (10–15)	100	10^3 – 2×10^4
ASBPI	22 (18–26)	50–55	10^5
MCI-M	38 (36–42)	20–30	10^6
HSN	40 (34–44)	30–35	5×10^6 – 10^7
HSBPA	54 (42–63)	10–12	10^7 – 5×10^7

The incidence of metastases was measured after excision of tumours which had been growing i.m. in hind limbs for 14 d. Immunogenicity was assessed as the number of cells required for tumour growth in rats which were immunised by excision of i.m. tumours 14 d previously.

tumours to between 9% and 20%. These experiments suggest that the infiltration of macrophages is associated with an effective immune response of the host to the tumour, and that the variation in the macrophage content of different tumours may be related to their immunogenicity.

Immunosuppression has been shown to facilitate metastatic spread^{7,8} and Fig. 2 shows that when the HSBPA rat fibrosarcoma was inoculated into T-cell depleted rats, the incidence of spontaneous metastases was greatly increased. From these experiments it is not possible to deduce whether the simultaneous decrease in macrophage infiltration (see Fig. 1) was causally related to the subsequent increase in metastases. A role for tumour macrophages in the control of tumour dissemination is suggested, however, by the parallel between macrophage content and the rate of spontaneous metastasis of a series of six different rat sarcomas grown in normal syngeneic recipients (Table 1).

The tendency to metastasise is also related (inversely) to the 'immunogenicity' of the tumours—this being defined as the degree of resistance to tumour challenge (expressed as the number of cells required to produce a tumour) in a suitably pre-immunised syngeneic recipient. Immunogenicity is not synonymous with antigenicity, since the former measures the effectiveness of the host response, which is determined both by the magnitude of the immune mechanism and the success of the escape mechanism. Thus the MC3 sarcoma induces cytotoxic cells⁹ and antibody to approximately the same extent as highly immunogenic tumours. The lack of immunogenicity of tumours such as MC3 has been ascribed to a high rate of shedding of tumour-specific antigen into the circulation¹⁰, which combines with and neutralises cytotoxic lymphocytes before they can reach the tumour. The initiation of an immune reaction by itself is, therefore, not sufficient to induce a macrophage infiltration, there must be an effective immune reaction at the tumour site for this to occur. Thus, as in a delayed hypersensitivity site, while the presence of macrophages is an essential component of such a reaction, these cells do not appear until an interaction of immune lymphoid cells with the eliciting antigen has occurred. We have previously shown¹¹ that tumour-bearing animals become progressively less responsive to DHS skin testing antigens and inflammatory stimuli, and it seems that this may be due to a competition of growing tumours for available monocytes, since the magnitude of the defect was directly related to the number of macrophages sequestered in the tumour of the rat being tested.

What then is the role of macrophages in tumours? Are they ineffectual bystanders brought in by lymphokines released in an immune process effected entirely by lymphoid cells interacting with the tumour? Though this remains a possibility, the clear demonstration of an immunologically specific cytotoxicity of macrophages to syngeneic sarcoma cells¹² makes this unlikely and the available data are more

consistent with the view that macrophages in the tumour play an active part in preventing metastatic spread. They may also contribute by phagocytosing tumour breakdown products, thereby reducing the amount of soluble antigen released into the circulation.

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- ¹ Black, M. M., and Leis, H. P., *N.Y. St., J. Med.*, **70**, 2583-2589 (1970).
- ² Hamlin, I. M. E., *Br. J. Cancer*, **24**, 653-667 (1969).
- ³ Evans, R., *Transplantation*, **14**, 468-473 (1972); *J. natn. Cancer Inst.*, **50**, 271-273 (1973).
- ⁴ Gowans, J. L., *J. Physiol. Lond.*, **146**, 54-69 (1958).
- ⁵ Miller, J. F. A. P., *Br. J. Cancer*, **14**, 93-98 (1960).
- ⁶ Spector, W. G., and Willoughby, D. A., *J. Path. Bact.*, **96**, 389-404 (1968).
- ⁷ Gershon, R. K., Carter, R. L., and Kondo, K., *Nature*, **213**, 674-676 (1967).
- ⁸ Proctor, J., Rudenstam, C-M., and Alexander, P., *Nature*, **242**, 29-31 (1973).
- ⁹ Currie, G. A., and Gage, J. O., *Br. J. Cancer*, **28**, 136-146 (1973).
- ¹⁰ Currie, G. A., and Alexander, P., *Br. J. Cancer*, **29**, 72-75 (1974).
- ¹¹ Eccles, S., and Alexander, P., *Br. J. Cancer* **30**, 42 (1974).
- ¹² Evans, R., *Br. J. Cancer*, **28**, Suppl. 1, 19-25 (1973).

Antibody cell daughters can produce antibody of different specificities

ALTHOUGH it contradicts current dogma, there is considerable indirect evidence for the idea that most antibody diversity is generated only after B cells are stimulated to proliferate by antigens or mitogens¹⁻³. A prediction of this theory, confirmed here, is that there may be rapid production within single clones, of cells producing antibody of different specificities.

We characterised the specificity of antibody from single cells by its cross reactivity, using a modified version of the haemolytic plaque technique⁴. We tested antibody plaque-forming cells (PFC) not against a single type of red cell, but on a mixture of erythrocytes from two different individual sheep. Plaques were either clear (both red cells lysed), partial (only one lysed), or "sombrosos"⁵ with different degrees of lysis of both indicators (Fig. 1). The use of plaque morphology as a specificity marker has been fully justified elsewhere (A. J. C. and L. M. Pilarski, in preparation). It is not influenced by differences in amount of antibody since the morphology of a given plaque is faithfully maintained as it grows (A. J. C. and L. M. Pilarski, in preparation). Nor does it reflect changes in antibody class since, in our experiments, plaques were always direct, produced early in a primary response (1-3 d) and obtained from cultures stimulated with bacterial lipopolysaccharide⁶, all conditions which favour IgM.

Table 1 records that of 911 PFC cultured *in vitro* for 2 d, 93 gave rise to two or more plaque-forming progeny. Procedure B unequivocally established that all PFC came from the initial donor cell. With procedure A, controls showed that the probability of plaque formation by contaminating cells, or by irradiated cells, was very low.

In most cases, plaques produced by all progeny were strikingly homogeneous except for small variations in overall diameter. This seems to serve as a good control of the validity of the marker system. That is, different individual cells from the same clone usually made plaques which were morphologically identical. In 10 cases, however, there was obvious variation in the specificity of the antibody released by daughter cells. Seven of these microclones, (including the six where there were only

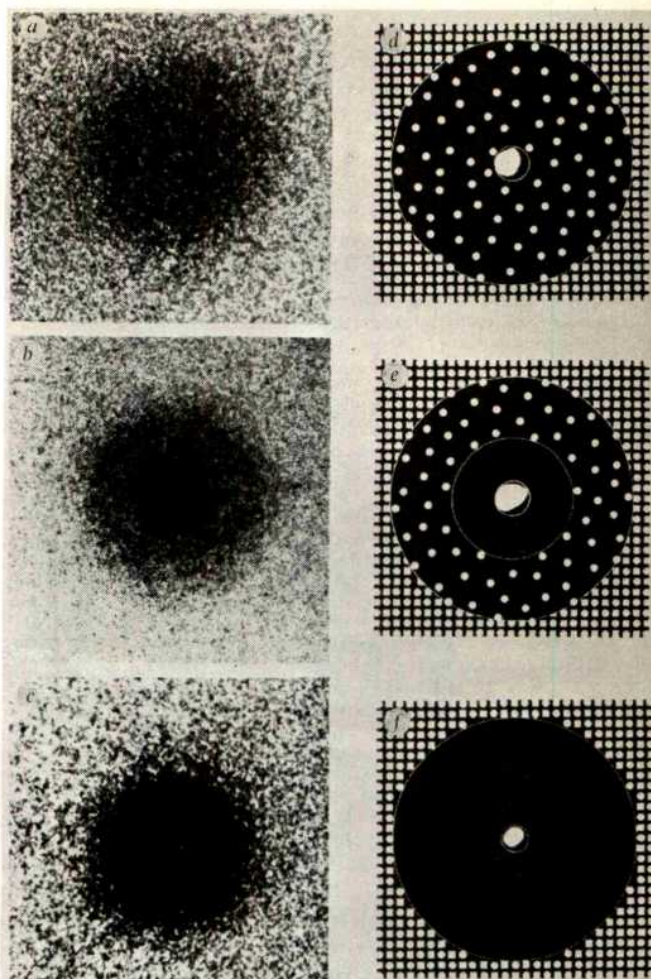


Fig. 1 Photographs (a-c), and diagrammatic representation (d-f), of clear (a, d), sombrero (b, e) and partial (c, f) plaques each produced by a single cell in a mixture of erythrocytes from sheep 1 and sheep 2. Illumination was dark field. In the diagrams, the circles and squares represent red cells of the two different types. A clear plaque is produced by antibody which is equally capable of binding to both indicator red cells. A partial plaque is produced by a different antibody specificity which binds to determinants accessible on one red cell type and not on the other. A sombrero is caused by antibody which binds to both kinds of erythrocyte, but to one better than the other. Mixtures of red cells from two different sheep have been used rather than from sheep and goat or cow as in earlier work^{12,13} so that clear and partial plaques would occur with about equal frequency, to allow the best chance of detecting a change from clear to partial or *vice versa*: the principle is the same whether red cells from two outbred individuals of the same or different species are used.

two plaques) showed two specificity types. Two of the clones contained what seemed to be plaques of three specificities (a clear plaque, a sombrero and partial). The most dramatic example of variation came from the culture of two large and tightly adherent cells (probably daughter cells) taken from the centre of a clear plaque in a microdrop, which on culture yielded a ball of 12 cells. Four of these gave similar plaques with a large clear centre, five produced sombreros with a tiny clear centre, one made a plaque which was possibly intermediate between these two types, one gave an entirely partial plaque, and one was undiagnosed since it was firmly stuck to one of the other cells (see below and Table 2). All of these plaques were similar in overall diameter, and all came from cells which looked macroscopically undamaged and were of similar size.

Sombrero plaques were a common result of these single-cell cultures. The ratio of overall (partial lysis) area to area of clear lysis can vary widely in such plaques, but in most single clones it was strikingly constant: if one plaque had a narrow 'brim' of partial lysis, then all showed exactly the same morphology.

Table 1 Numbers of PFC obtained from cultures of a single initial PFC*

Procedure	Number of individual transfers	Variation shown by clones	Number of cases where x PFC progeny were found												
			x=0	1	2	3	4	5	6	7	8	9	10	11	>11
A	340	No	248	51	18	5	5	1	1	0	1	0	0	0	5†
		Yes			2	2	1								
B	571	No	437	82	30	9	2	2	3	0	0	0	1	0	
		Yes			4										1‡

*Pooled results of all experiments.

†In all of these cases there were one or two large plaques and a large number (up to 60) tiny plaque-like areas with no central white cell, which were possibly caused by fragments of cytoplasm shed from antibody-containing cells.

‡This clone came from two tightly joined cells which were cultured together.

For procedure B, 10^7 spleen cells from one or more normal adult CBA mice were incubated in polyacrylamide rafts, as described by Marbrook and Haskill^{14,15}. Approximately 5×10^6 sterile sheep red cells were added, from any one of several commercial donor sheep. The medium was Eagles minimal essential, plus bicarbonate, with 10% added foetal calf serum. All cultures always contained *Escherichia coli* 0128:B12 lipopolysaccharide (Difco) at a final concentration of $7.5 \mu\text{g ml}^{-1}$. Rafts were incubated at 37°C in an atmosphere of 10% CO_2 and 83% N_2 , at pH 7.3. These cultures were collected at 24 h, when they had developed a total of 100–200 anti-sheep PFC. Cells from these 1 d mass cultures were dispersed under oil in microdrops containing a mixture of red cells from two standard sheep, (1 and 2) together with complement and medium¹⁶. The oil chambers were incubated horizontally for 15–30 min at 37°C , so that plaques could be seen around the rare antibody-forming cells. This period of incubation was kept as short as possible to prevent damage to the cells. For this reason the morphology of the plaque around the donor PFC was not always certain, although there was probably some selection for clear plaques which are easier to see than partials when small. The medium for these short-term incubations in an air atmosphere was 199 plus 10% foetal calf serum, buffered with 0.02 M HEPES. Individual PFC were now isolated from all other cells and micromanipulated out of the drops¹⁶. A single PFC was then transferred with a fine, hand-held micropipette to one of the microculture wells in a new polyacrylamide raft. These wells had each been pre-seeded with about 100 heavily irradiated (5,000 r.) normal CBA spleen cells and 1,000 sterile sheep red cells (again from any one of several commercial donor sheep), the medium being a mixture of equal parts of fresh Eagle's medium and filtered conditioned medium from the donor cultures. The individual PFC was observed at a magnification of $\times 40$ as it was pipetted on to the small pellet in the well. Rafts containing transferred PFC were then incubated for 42–48 h (in rare cases 18–24 h) at 37°C . After culturing, it was possible to identify those cells which had come from the original PFC by their large size, and because they were usually stuck firmly together in small clumps of up to 12 cells. They were sucked out of the well with a micropipette and separated by continuous sucking up and down, where necessary with the addition of trypsin at a final concentration of about $100 \mu\text{g ml}^{-1}$ to the drop for 1–20 min. Not infrequently, two or more cells could not be separated. The cells were then added to a mixture of fresh medium, complement, and red cells from the two standard merino sheep, 1 and 2. The mixture was pipetted into a slide chamber⁴, incubated for 1 h, and the resulting plaques characterised as having clear, partial or sombrero morphology. Procedure A was used for earlier experiments in which a larger number of feeder cells was used and 20–100 random normal cells were transferred and cultured together with the single PFC.

Contrasted with this were the instances of heterogeneous clones. Table 2 records the sizes and relative areas of clear and partial lysis in one representative homogeneous clone, and in two of the clones obtained by procedure B which were classified as showing variation.

The frequency of variation shown by cells in our cultures is obviously much higher than has been previously thought to occur. Assuming an average of two divisions per microclone we estimate about one variation event (mutation or gene switch) per 30 divisions. We have also obtained evidence for a similar rate of variation in limit dilution cultures of spleen cells both *in vitro* and *in vivo* (L. M. Pilarski and A.J.C., in preparation). There are a number of possible reasons why the method we have used has detected many more variants than other methods, the most important being its sensitivity. The plaque technique can pick up a single cell which is different from the rest of a clone. It is also extremely sensitive in that when two closely similar antigens (red cells from two sheep) are used, it seems reasonable that a small change in antibody structure may produce a slight increase or decrease in avidity for one antigen without affecting avidity for the other, resulting in a measurable change in plaque morphology, for example the plaque may change from clear to sombrero. Our conditions (IgM antibody, mitogen stimulation, early stages of clonal development), may also favour variation by comparison with most other clonal work, which has usually been done on protracted cultures of antibody-forming cells or myelomas, (for example refs 7,8). Frequently, the methods used to characterise such antibody as 'monoclonal' would not detect 5% of variants.

The only other attempt to detect clonal variation using the same marker as ours is the work of Nossal and Lewis¹³ who found no variants in the two daughter cells from each of 13 PFC, by testing plaques on a mixture of sheep and goat red cells. According to the rate of variation which we have observed this was too small a number of divisions for a variation event to be expected.

Other groups^{9,10} have recently looked for variation in cultured myeloma cells, using techniques which would probably

Table 2 The morphological characteristics of plaques in three clones, each assayed on the same mixture of red cells from sheep 1 and 2

Clone number	Classification	Diameter* (mm)	Ratio† of areas
1	Homogeneous	0.5	2.4
		0.7	2.0
		0.65	2.1
		0.7	2.0
		0.55	2.2
2	Variation	0.43	2.0
		0.4	7.1
3	Variation	0.55	3.0
		0.50	3.0
		0.50	4.0
		0.55	4.0
		0.50	5.0
		0.60	12.0
		0.38	25.0
		0.45	36.0
		0.55	53.0
		0.45	56.0
		0.53	∞

*Average diameter to which partial lysis extended.

†Ratio of area bounded by border of partial lysis to area of complete lysis.

detect mainly large changes in Ig structure. Nevertheless, Bauml *et al.*⁹ demonstrated a fairly high rate of variation under some culture conditions, leaving open the possibility that small undetected changes in the V region may also have been common.

Our experiments support the idea that antibody diversity can be generated at a high rate after immunocompetent cells are stimulated to proliferate. The experiments describe antibody phenotype only, and do not elucidate the genetic basis for clonal variation. Baltimore¹¹ has recently described a possible mechanism of hypervariation. The small changes in specificity which we observe might well be due to point mutations in the V region (hypermutation), but we cannot entirely exclude rapid switching from one pre-existing gene to another.

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- ¹ Cunningham, A. J., *Contemp. Top. molec. Immun.*, **3**, 1 (1974).
- ² Cunningham, A. J., and Pilarski, L. M., *Eur. J. Immun.* (in the press).
- ³ Cunningham, A. J., and Pilarski, L. M., *Scand. J. Immun.*, **3**, 5 (1974).
- ⁴ Cunningham, A. J., and Szenberg, A., *Immunology*, **14**, 599 (1968).
- ⁵ Cunningham, A. J., and Sercarz, E. E., *Eur. J. Immun.*, **1**, 413 (1971).
- ⁶ Andersson, J., Sjöberg, O., and Møller, G., *Transplant. Rev.*, **11**, 131 (1972).
- ⁷ Askonas, B. A., and Williamson, A. R., *Nature*, **238**, 339 (1972).
- ⁸ Klinman, N. R., *J. Immun.*, **106**, 1345 (1971).
- ⁹ Baumal, R., Birshtein, B. K., Coffino, P., and Scharff, M. D., *Science*, **182**, 164 (1973).
- ¹⁰ Cotton, R. G. H., Secher, O. S., and Milstein, C., *Eur. J. Immun.*, **3**, 135 (1973).
- ¹¹ Baltimore, D., *Nature*, **248**, 409 (1974).
- ¹² Cunningham, A. J., *Aust. J. exp. Biol. med. Sci.*, **47**, 493 (1969).
- ¹³ Nossal, G. J. V., and Lewis, H., *Immunology*, **20**, 739 (1971).
- ¹⁴ Marbrook, J., and Haskill, J. S., in *Cell. Interactions in the Immune Response: 2nd Internat. Convoc., Immunol., Buffalo, N.Y.* 66 (Karger, Basel, 1971).
- ¹⁵ Marbrook, J., and Haskill, J. S., *Cell. Immun.* (in the press).
- ¹⁶ Cunningham, A. J., *Progr. Allergy*, **17**, 5 (1973).

Spontaneous antilymphoma reaction of preleukaemic AKR mice is a non-T-cell killing

THE high incidence of spontaneous leukaemias in AKR mice may be explained by the early appearance and high level of replication of a type C virus. It is often suggested, however, that in addition, AKR mice could be tolerant to their leukaemias. This tolerance would enhance the *in vivo* growth of malignant cells. In fact, AKR mice are not completely devoid of antileukaemic immune response¹⁻³. It would be interesting, therefore, to determine if a quantitative or qualitative defect of one given type of antileukaemic response exists in AKR mice. Previous work has shown that an antibody response directed against the type C virus or against antigens of leukaemic cells can be demonstrated not only in hyperimmune¹ but also in normal AKR mice⁴.

A cell mediated reaction directed against G (Gross)⁵ leukaemia antigen-bearing cells was also detected *in vitro*^{5,7} but this cell-mediated reaction was characterised by inhibition of the growth of G+ cells in monolayers. It is now known, however, that this kind of inhibition can be due to non-T cells⁷⁻⁹ and could be antibody dependent¹⁰. A completely different cell-mediated antitumour reaction can be evidenced in the murine sarcoma virus (MSV) induced tumour system by the chromium release test (CRT) which detects a pure T-cell-mediated^{11,12} and antibody-independent¹³ cytotoxicity of tumour cells. Our work was performed to determine if AKR mice are capable of spontaneously developing such a kind of T-killer cell response.

AKR, C₃H/He, BALB/c and C57BL/6 mice were from our own colony. They have been tested at various ages from 2 weeks to 19 months without any preliminary treatment. Both males and females were used and tested individually. The CRT was done as previously described¹³. The effector cells were spleen cells, and the tumour target cells were lymphoma cells in suspension. A 100:1 effector:target cell ratio was used. The serologically defined tumour antigens and the origin of the target cells are given in Table 1. The specific chromium release was measured after 18 h incubation of target cells in the presence of effector cells. It was expressed as the excess of release when target cells were incubated with AKR spleen cells in place of C₃H/He, BALB/c or C57BL/6 control cells. No difference in the chromium release can be detected when non-AKR cells were used as effector, whatever the age of the donor mice. As previously discussed, an excess of 4% in the chromium release can be considered as significant¹³.

Results of experiments in which the effectors were whole spleen cells are given in Table 1. Individual AKR mice less than 3 months old were constantly negative. Positive CRT was found in 75% of 3-5-month-old AKR mice. The reaction was generally weak (specific release average = 12%), but could occasionally reach a high level (20-30%). Not only syngeneic or allogeneic G+FMRGi- lymphoma cells, but also G-FMRGi+ lymphoma cells could be lysed by the AKR spleen cells. Such a cross reaction was previously observed in MSV tumour system^{13,14}. ERLD lymphoma and P815 mastocytoma, which bear no serologically defined leukaemic antigen⁵, were never lysed by AKR spleen cells. Leukaemia of AKR mice began to appear after 5 months. Positive CRT reactions were observed in 41% of non-leukaemic animals older than 5 months. These results show that positive reactions are less frequent than in younger mice. In addition, positively-reacting animals were more frequent between 5 and 8 months (57%) than after 8 months (30%). CRT reactions were always negative in mice with overt leukaemia (enlargement of spleen, lymph nodes and thymus).

Table 1 Frequency of spontaneous reactions of AKR in CRT

Effector spleen cells	(G+) target cells*			Total of experiment with (G+) target	(G-) target cells*		
	GL 3 (G+FMRGi-)	E δ G2 (G+FMRGi-)	SK 1 (G+FMRGi-)		GiL 4 (G-FMRGi+)	ERLD (G-FMRGi-)	P 815 (G-FMRGi-)
2 weeks to							
3 months old	0/32†	0/6	0/12	0/40	0/6	0/7	0/5
3-5 months old	26/35	3/4	4/5	33/44	6/10	0/3	0/7
Non-leukaemic:							
older than 5 months	21/46	1/4	5/13	27/63	3/12	0/23	0/3
Leukaemic:							
older than 5 months	0/14	0/2	ND‡	0/16	0/4	0/7	ND

*GL 3 and E δ G2 = C57BL/6 Gross virus-induced lymphoma.

SK 1 = AKR spontaneous lymphoma.

GiL 4 = C57BL/6 Graffi virus induced lymphoma.

ERLD = C57BL/6 X-ray induced lymphoma.

P 815 = DBA/2 methyl-cholanthrene induced mastocytoma.

†Number of mice with positive results/number of tested mice.

‡ND = Not done.

Table 2 Effect of anti- θ AKR serum and complement treatment on the activity of spontaneously cytotoxic AKR spleen cells for the GL 3 lymphoma of C57BL/6 mice

Spleen cells from AKR	Specific chromium release (%) in the presence of reactive spleen cells treated by:				
	Medium alone	C ₃ H normal serum with complement	C ₃ H normal serum without complement	C ₃ H anti- θ AKR serum with complement	C ₃ H anti- θ AKR serum without complement
4 months old	25	23	22	27	28
5 months old	19	17	20	22	21
6 months old	11	12	9	13	12
7 months old	8	7	8	10	7
8 months old	15	12	13	14	13

These results suggest that AKR mice can develop a spontaneous antileukaemic reaction which appears during the preleukaemic period and completely disappears with the progression of the disease. It was previously shown that the CRT allows the detection of a pure T-killer cell phenomenon in our MSV tumour system^{11,12} as well as during allograft rejection^{15,16}. Thus, we have attempted to discover whether T cells could be responsible for the spontaneous CRT reactions detected in AKR mice. The whole spleen cell suspensions of individual AKR mice were treated with C₃H/He anti- θ AKR serum diluted at 1:10 and rabbit complement, before they were used in the CRT. Anti- θ AKR serum was obtained by repeated inoculations of AKR thymocytes in C₃H/He mice, the serum was absorbed by C₃H/He thymocytes and tested for its specificity for various lymphoid organs of AKR mice. Controls included in each experiments were first, AKR spleen cells treated by normal C₃H/He serum and rabbit com-

plement. It can be concluded that the positive results obtained in the CRT with AKR spleen cells were due to non-T cells.

To study the nature of the effector cells, spleen cell suspensions from individual AKR mice were treated according to one of the following procedures (Table 4): first, incubation for 30 min at 37° C with carbonyl iron followed by six successive passages over a powerful magnet¹⁶. This procedure removes practically all the macrophages. Second, filtration through anti-immunoglobulin-coated glass bead columns according to the method of Wigzell *et al.*¹⁷. This method removes both the macrophages and the immunoglobulin-bearing lymphocytes, and the eluted suspensions contain about 90% T cells. Third, incubation for 20 min at 37° C with 0.25% trypsin. The reaction was stopped by the addition of five volumes medium containing 10% foetal calf serum. This method allows the eventual removal of antigen-antibody complexes attached to effector cells. The results (Table 4) show that column filtration

Table 3 Effect of anti- θ AKR serum and complement treatment on the activity of anti-C57BL/6 immune AKR spleen cells for the GL 3 lymphoma of C57BL/6 mice

Anti C57BL/6 immune spleen cells from AKR	Specific chromium release (%) in the presence of immune spleen cells treated by:				
	Medium alone	C ₃ H normal serum with complement	C ₃ H normal serum without complement	C ₃ H anti- θ AKR serum with complement	C ₃ H anti- θ AKR serum without complement
2 months old	31	38	35	0	33
5 months old	23	26	24	0	27
8 months old	36	37	34	0	38

plement and, second, AKR spleen cells treated by anti- θ AKR serum without complement.

The results of a typical experiment are summarised in Table 2. They show that the elimination of θ antigen-bearing cells does not decrease the antileukaemic response of AKR spleen cells. Twenty-three positive mice were individually studied giving identical results. It is unlikely that these results would be a consequence of a weak activity of the anti- θ AKR serum used, since this serum lyses all T-cells of AKR mice at dilutions up to 1:100 and because AKR receiving 4×10^7 C57BL/6 spleen cells intraperitoneally have cytotoxic lymphocytes in CRT for C57BL/6 target cells and pretreatment of these cytolytic cells with anti- θ AKR serum diluted at 1:10 and rabbit complement abolish their activity (Table 3). In addition, this last result demonstrates clearly that AKR mice are perfectly able to develop a T-killer cell response against an allogeneic

strongly decreases the CRT activity of spleen cells, and carbonyl iron treatment abolishes it completely. Identical results were obtained with the 18 reactive mice tested. It can be concluded that non-T cells, probably macrophages, are the main effector cells of the spontaneous CRT reactions of preleukaemic AKR mice. Treatment of spleen cells with trypsin suppress the CRT activity (Table 4), and this activity does not reappear even 24 h after trypsinisation. It is likely, therefore, that an antibody-dependent cell-mediated reaction is involved. We are studying the capacity of various AKR sera to block reactive cells and to 'arm' nonreactive cells in CRT.

Three points should be noted. First, an antileukaemic reaction can be detected by the CRT in preleukaemic AKR mice, but this reaction, probably antibody mediated, is unable to protect the mice since it was found in at least 75% of 3-5-month-old AKR mice, whereas the incidence

Table 4 Effect of various treatments on the cytotoxic activity of reactive AKR spleen cells

Spleen cells from AKR	Specific chromium release (%) in the presence of reactive spleen cells treated by:			
	Medium alone	Column filtration	Carbonyl iron and magnet	Trypsin
4 months old	11	0	0	0
4 months old	21	7	0	0
5 months old	16	4	0	ND
7 months old	12	0	0	0
7 months old	9	0	ND	0
8 months old	8	0	0	0

of leukaemia in AKR mice is about 80%.

Second, we have demonstrated^{11,12} in the MSV tumour system that the CRT allows the detection of pure T-cell killing of syngeneic tumour cells. Similar observations were done previously in allogeneic immunisation¹⁵⁻¹⁶. In the same experimental conditions, however, the AKR spontaneous reaction is caused by non-T cells. Immune cytolysis of P 815 tumour cells resulting from non-T cells have been also detected in old NZB mice¹⁸. Furthermore, it is known that in the microtoxity assay (MA) both T and non-T cells^{7,8} and even macrophages⁹ can be involved. It therefore seems necessary to determine precisely the nature of the effector cells for each tumour system, whatever the *in vitro* methods used to study the cell-mediated immunity.

Third, the absence of T-killing in the antileukaemic response in preleukaemic AKR mice must be emphasised since the same animals can respond normally to allogeneic grafts with a high level of specific T-killer cells. The existence of such a reaction not only in young AKR but also in 8-month-old mice is especially interesting and suggests that a decrease with aging in the immune competence of AKR is not involved. It is noteworthy that the same AKR mice which develop anti-allogeneic T-killer cells can have at the same time a non-T cell mediated reaction directed against syngeneic G+ lymphoma. The first reaction is abolished by anti- θ AKR whereas the second remains unchanged. Preliminary results indicate that the absence of T-killer response is not a general property of the leukaemia viruses since resistant mice such as C57BL/6 are able to develop a 'T' response after Friend or Moloney virus inoculation (unpublished data). Further experiments in the Gross system using both sensitive and resistant mice are now in progress to determine whether the absence of T-killer cell response is a property of AKR mice or not. A hypothesis would be that the *Rgv-1* gene¹⁹ which conditions the sensibility to Gross virus induced leukaemias could be responsible for the AKR inability to develop an antileukaemic T-killer cell response. This hypothesis could be reinforced by the fact that an H-2-linked gene, possibly identical to *Rgv-1* mapped in the H-2 complex near the 'Ir region'²⁰ has been recently shown to act like an immune response control gene²¹.

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- ¹ Doré, J. F., Ajuria, E., and Mathé, G., *Revue Étud. clin. biol.*, **15**, 81-84 (1970).
- ² Marklam, R. V., Sutherland, J. C., Cimino, E., Drake, W. P., and Mardiney, M. R., *Revue Étud. clin. biol.*, **17**, 690-698 (1972).
- ³ Vredevoe, D. L., and Hays, E. F., *Cancer Res.*, **29**, 1685-1690 (1969).
- ⁴ Oldstone, M. A., Aoki, A., and Dixon, F. J., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 134-138 (1972).
- ⁵ Wahren, B., and Metcalf, D., *Clin. exp. Immun.*, **7**, 373-386 (1970).
- ⁶ Old, L. J., Boyse, E. A., and Stockert, E., *Cancer Res.*, **25**, 813-819 (1965).
- ⁷ Lamon, E. W., Skurzak, H. M., Klein, E., and Wigzell, H., *J. exp. Med.*, **136**, 1072-1079 (1972).
- ⁸ Plata, F., Gomard, E., Leclerc, J. C., and Levy, J. P., *J. Immun.*, **112**, 1477-1487 (1974).
- ⁹ Owen, J. J. T., and Seeger, R. C., *Br. J. Cancer*, **28**, Suppl. 1, 26-34 (1973).
- ¹⁰ Baldwin, R. W., Price, M. R., and Robins, R. A., *Int. J. Cancer*, **11**, 527-535 (1973).
- ¹¹ Leclerc, J. C., Gomard, E., Plata, F., and Levy, J. P., *Int. J. Cancer*, **11**, 426-432 (1973).

- ¹² Plata, F., Gomard, E., Leclerc, J. C., and Levy, J. P., *J. Immun.*, **111**, 667-671 (1973).
- ¹³ Leclerc, J. C., Gomard, E., and Levy, J. P., *Int. J. Cancer*, **10**, 589-601 (1972).
- ¹⁴ Ortiz de Landazuri, M., and Herbermann, R. B., *Nature new Biol.*, **238**, 18-19 (1972).
- ¹⁵ Cerottini, J. C., Nordin, A. A., and Brunner, K. T., *Nature*, **228**, 1308-1309 (1970).
- ¹⁶ Golstein, P., Shirmacher, U., Rubin, B., and Wigzell, H., *Cell. Immun.*, **9**, 211-225 (1973).
- ¹⁷ Wigzell, H., Sundqvist, K. G., and Yoshida, T. O., *Scand. J. Immun.*, **1**, 75-87 (1972).
- ¹⁸ Greenberg, A. H., and Playfair, J. M. L., *Clin. exp. Immun.*, **16**, 99-110 (1974).
- ¹⁹ Lilly, F., Boyse, E. A., and Old, L. J., *Lancet*, **ii**, 1207-1209 (1974).
- ²⁰ Lilly, F., *Genetics*, **53**, 529-539 (1966).
- ²¹ Sato, H., Boyse, E. A., Aoki, T., Iritani, C., and Old, L. J., *J. exp. Med.*, **138**, 593-606 (1973).

Observations on the mechanism by which T-lymphocytes exert cytotoxic effects

The mechanism by which T lymphocytes kill tumour cells is unknown¹. The killing is more rapid than reported for lymphotoxin². Among possible mechanisms are the involvement of complement components³ or phospholipase A, which could generate lysolecithin in the target cell membrane. To test these possibilities we have examined the rates of release from tumour cells of markers of high and low molecular weight and the effects of nonpenetrating solutes. If killing involves disruption of the structure of the cell membrane, for example by lysolecithin, simultaneous release of markers and no protection by macromolecular solutes would be expected. If lysis is osmotic, markers of low molecular weight should be released before those of high molecular weight and nonpenetrating solutes should protect against the lysis by counterbalancing the intracellular osmotic pressure. In the case of complement lysis, small macromolecules of molecular weight less than 40,000, which

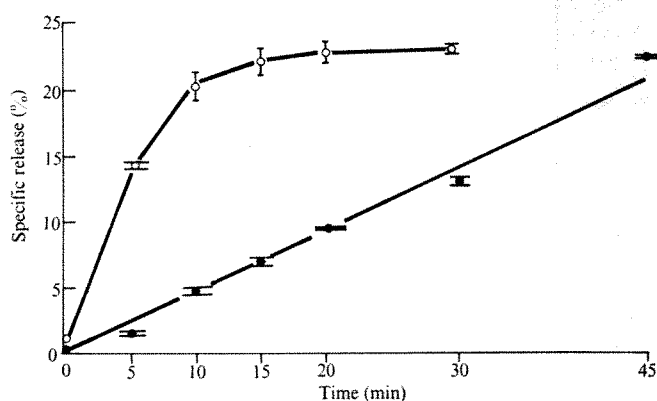


Fig. 1 Time course of the specific release of ⁸⁶Rb (O-O) and ⁵¹Cr (●-●) from mastocytoma cells by sensitised spleen cells. P-815-X2 mastocytoma cells were double labelled: 5×10^6 cells were incubated for 1 h at 37° C with 100 μ Ci of $\text{Na}_2^{51}\text{CrO}_4$ and 1000 μ Ci of ⁸⁶RbCl in 1 ml Eagle's minimal essential medium containing 10% foetal bovine serum. Sensitised spleen cells were obtained from C57BL/6 mice injected intraperitoneally 11 d earlier with 3×10^7 live mastocytoma cells. Mixtures containing 10^7 normal or sensitised spleen cells and 1.5×10^5 labelled mastocytoma cells in 1 ml of the above medium were centrifuged in flat bottomed plastic tubes of 1.25 cm diameter and incubated at 37° C for the indicated lengths of time. Then they were mixed, briefly centrifuged and aliquots of supernatants taken for counting the released labels. Percentage specific release of the two labels was obtained by subtracting the release in the presence of normal spleen cells from the release in the presence of sensitised cells and is expressed as a fraction of the total labels released by freezing and thawing the cells. The values are means of duplicates and the range of variation is indicated.

penetrate through complement lesions, do not inhibit lysis, whereas those of molecular weight above 40,000 protect⁴.

Our experiments have been performed with a widely studied cytotoxicity model, mastocytoma cells from DBA/2 mice and sensitised lymphocytes from C57BL/6 mice¹. As a convenient small marker we have used ⁸⁶Rb⁺, which is not bound in the cytoplasm and behaves in a manner analogous to K⁺, and as a large marker ⁵¹Cr which is bound to cytoplasmic protein. The time course of release of the two markers is shown in Fig. 1. It is clear that the specific release of ⁸⁶Rb⁺ occurs much more rapidly than that of ⁵¹Cr, as previously reported by Henney⁵. In contrast to Henney⁵, but like C. J. Sanderson and G. A. Taylor (unpublished), we find linear release of ⁵¹Cr from the time of contact of effector lymphocytes and target cells. The slope of the curve

Table 1 Effect of dextran on release of ⁵¹Cr and ⁸⁶Rb from mastocytoma cells by sensitised lymphoid cells

Experiment No.	Time (min) when dextran added	Isotope released	% inhibition of specific release	
			15–20 min*	40–45 min
1	5	⁵¹ Cr	70 ± 1.7	44 ± 1.1
1	5	⁸⁶ Rb	14 ± 1.8	0 ± 1.4
2	15	⁵¹ Cr	53 ± 1.9	51 ± 1.6
2	15	⁸⁶ Rb	0 ± 2.1	0 ± 2.0
3	20	⁵¹ Cr	37 ± 0.7	30 ± 0.6
3	20	⁸⁶ Rb	0 ± 0.9	0 ± 1.1

*Time after dextran addition.

The cytotoxicity assays were carried out as in Fig. 2, but with varying time intervals before and after the addition of dextran solution to the cell mixtures

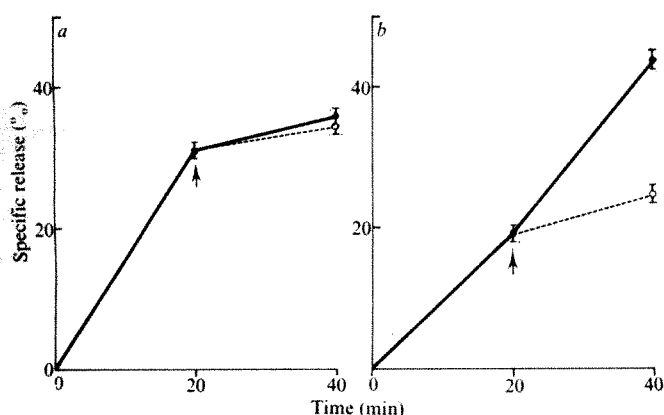


Fig. 2 Effect of dextran on the specific release of *a*, ⁸⁶Rb and *b*, ⁵¹Cr from mastocytoma cells by sensitised lymphoid cells. Spleen cell–target cell mixtures ($10^7 : 1.5 \times 10^5$ in 0.5 ml) were centrifuged and incubated at 37° C for 20 min. At that point (shown by arrow) 0.5 ml of a solution of 24% dextran molecular weight 10,000 daltons was added to some 0.5 ml cell suspensions and all samples were incubated for a further 20 min before the release of the two labels was assessed in the presence (O) or absence (●) of dextran.

of ⁵¹Cr release is related in a linear fashion to the concentration of the effector cells. These observations allow three conclusions. First, the cytotoxic mechanism depends on contact of one effector cell with a target cell. Second, within minutes of the establishment of such a contact the plasma membranes of the target cells show increased permeability to ions but not proteins. Third, loss of cytoplasmic proteins follows a slower, linear time course beginning in some cells within a few minutes of contact and occurring in other cells only after a delay of an hour or more.

Although several nonpenetrating solutes were found to inhibit the lysis of target cells, most experiments were performed with dextran of molecular weight 10,000 (T 10). Representative experiments with dextran T 10 in the extracellular medium are shown in Table 1 and Fig. 2. When the dextran was added at the beginning of the experiment, inhibition of the release of ⁸⁶Rb as well as of ⁵¹Cr was found, presumably because effective contact of the lymphocytes and target cells was prevented. When dextran was added after 5 min there was only slight inhibition of ⁸⁶Rb release (possibly because effective contacts between lymphocytes and all target cells had not yet been established) but there was much greater (70%) inhibition of ⁵¹Cr release. Addition of dextran as late as 20 min still produced highly significant inhibition of ⁵¹Cr release but none of ⁸⁶Rb release. This effect was fully reversible: when medium containing dextran was replaced with medium lacking dextran, the release of ⁵¹Cr proceeded rapidly to completion. Hence the presence of dextran does not affect the increased permeability of target cell membranes to ions but produces a marked and reversible inhibition of the release of cytoplasmic proteins.

The kinetics of release of large and small markers and the protection against loss of large markers by low molecular weight dextran provide strong evidence that the lysis of tumour cells by sensitised T lymphocytes occurs through osmotic effects and that the ion-conducting channels formed in the plasma membrane of the target cells are smaller than those produced by complement. As shown in Table 2, neither the osmotic lysis produced in the same target cells by antibody and complement nor that produced by lysolecithin is appreciably affected by dextran of molecular weight 10,000.

It has been reported that cooling the injured target cells to 0° C in the effector cell independent phase of the reaction prevents ⁵¹Cr release⁶. This observation was interpreted as evidence in support of an enzymic reaction. An alternative explanation is that because of loss of fluidity in the target cell membrane the ion-conducting channels generated following contact with effector lymphocytes are sealed at low temperatures. As shown in Fig. 3, cooling to 10° C inhibits almost completely leakage of ⁸⁶Rb as well as ⁵¹Cr from the target cells. In other experiments ⁸⁶Rb and ⁵¹Cr release were markedly depressed even at 15° C, and returned to control levels when the cells were placed again at 37° C. These observations support the view that membrane fluidity is

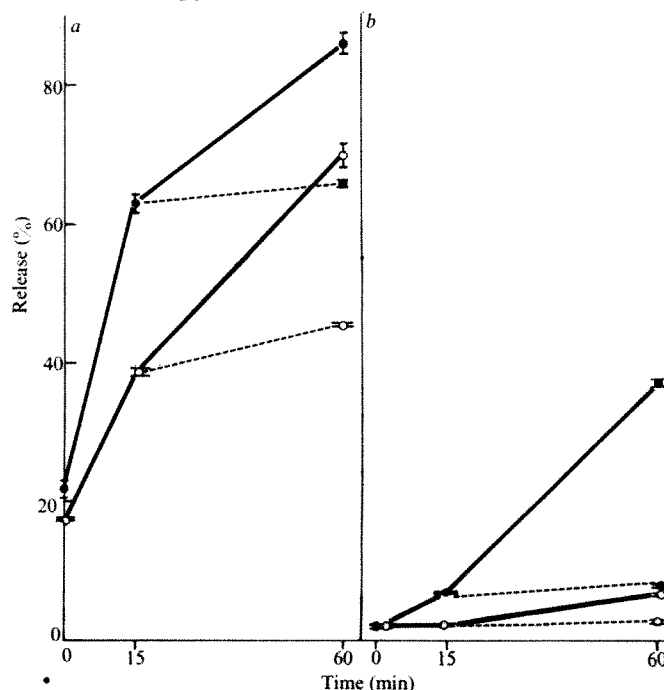


Fig. 3 Effect of lowering temperature on *a*, ⁸⁶Rb and *b*, ⁵¹Cr release from the mastocytoma cells. Spleen cell–target cell mixtures were prepared as in Fig. 1 and incubated at 37° C for 15 min. Then some cell samples were cooled to 10° C and all cell samples were incubated at the appropriate temperatures for up to 60 min. The labels released from the target cells in presence of sensitised (●) or normal (O) spleen cells at 37° C (—) or at 10° C (---).

involved. Even the basal release of ^{86}Rb in the absence of specific effector cells is markedly reduced at $10^\circ\text{--}15^\circ\text{C}$, which is again consistent with a requirement for membrane fluidity.

These observations help to clarify the mechanism by which sensitised T lymphocytes lyse target cells. The first stage is the establishment of effective contact between effector cells and target cells. This requires effector cells which are living and moving: inhibition of motility of the cells by cytochalasin^{7,8} blocks the early but not the later stages of the reaction. The inhibition of target cell lysis by agents generating cyclic AMP⁹⁻¹¹ may exert their effects by depressing movements of effector cells. Movement is required for three reasons: bringing effector cells and target cells together, moving apposed membranes to establish intimate contact of appropriate type, and subsequent movement of the cells apart. Once effective contact of lymphocytes and target cells is established (which begins within a few minutes of their coming together) killing the lymphocytes by specific antibody and complement does not prevent continued lysis of target cells: intact effector cells are not required for the final lytic process¹². In fact, the films of Ginsburg and Ax¹³ show that lysis does not take place while the effector cell and target cell are in contact but occurs only after the effector cell has disengaged itself and moved on. The films also show that the diameter of mastocytoma cells is increased before lysis, which is consistent with the interpretation that osmotic swelling is taking place.

Table 2 Effect of dextran on the lysis of tumour cells by antibody and complement and by lysolecithin

Lytic agent	Dextran	% Specific ^{51}Cr release	% Inhibition by dextran
Antibody	—	91 \pm 0.9	—
Antibody	T 10	86 \pm 0.2	6
Antibody	T 40	46 \pm 2.3	50
Lysolecithin	—	100 \pm 0.2	—
Lysolecithin	T 10	100 \pm 0.1	0

Tumour cells were incubated with a minimum lytic concentration of rabbit antiserum against mastocytoma cells and guinea pig complement (4 haemolytic units) or with lysolecithin ($20\text{ }\mu\text{g ml}^{-1}$) for 1 h at 37°C . Lysis in the presence and absence of dextran (12%) was compared. The ^{51}Cr release by antibody was compared with that produced by freezing and thawing cells, and that by lysolecithin, which was higher, compared in the presence and absence of dextran.

During the second phase of the reaction the plasma membrane of the target cell becomes leaky to ions. The underlying mechanism is not yet certain. An appealing hypothesis is that a gap junction is established between the effector and target cell. The report of Sellin and his colleagues¹⁴ that fluorescein passes from target cells to sensitised lymphocytes supports this interpretation. The electrotonic coupling, involving free passage of ions, of lymphocytes treated with phytohaemagglutinin¹⁵ is also relevant since this lectin can promote lysis following contact between effector cells and target cells¹⁶. Gap junctions are characterised by clusters of particles in apposed membranes demonstrable by freeze-fracturing electron microscopy¹⁷, and it is conceivable that crosslinking of proteins in the membrane of the target cell by specific receptor molecules on the surface of the lymphocyte (by a process analogous to patching¹⁸) may be involved in the generation of gap junctions, and also in the persistence of such leaky complexes, allowing increased passive ion flux in the membrane of the target cell once the lymphocyte has moved away.

The experiments reported in this paper seem to exclude a role of lysolecithin generation or of typical complement-induced membrane lesions in the lysis of tumour cells by sensitised T lymphocytes. A second phase of lysis, which does not require the presence of intact effector cells, is characterised by increased permeability of the target cell

membrane to ions but not to cytoplasmic proteins, and the final lytic event is due to osmotic effects. The nature of the lesion in the target cell membrane is not yet certain, but it may be due to the formation and persistence of a gap junctional complex.

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- Cerottini, J. C., and Brunner, K. T., *Adv. Immun.*, **18**, 67-132 (1974).
- David, J. R., and David, R. R., *Progr. Allergy*, **16**, 300-449 (1972).
- Perlmann, P., Perlmann, H., Müller-Eberhard, H. J., and Manni, J. A., *Science*, **163**, 937-939 (1969).
- Sears, D. A., Weed, R. I., and Swisher, S. N., *J. clin. Invest.*, **43**, 975-985 (1964).
- Henney, C. S., *J. Immun.*, **110**, 73-84 (1973).
- Martz, E., and Benacerraf, B., in *Schering Symposium on Immunopathology* (Cavtat, Yugoslavia) 37-46 (1973).
- Ferluga, J., Asherson, G. L., and Becker, E. L., *Immunology*, **23**, 577-590 (1972).
- Cerottini, J. C., and Brunner, K. T., *Nature new Biol.*, **237**, 272-273 (1972).
- Henney, C. S., and Lichtenstein, L. M., *J. Immun.*, **107**, 610-612 (1971).
- Strom, T. B., Deisseroth, A., Morganroth, J., Carpenter, B., and Merrill, J. P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2995-2999 (1972).
- Johnson, G. S., Morgan, W. D., and Pastan, I., *Nature*, **235**, 54-56 (1972).
- Martz, E., and Benacerraf, B., *J. Immun.*, **111**, 1538-1545 (1973).
- Koren, H. S., Ax, W., and Freund-Moelbert, E., *Eur. J. Immun.*, **3**, 32-37 (1973).
- Sellin, D., Wallach, D. F. H., and Fischer, H., *Eur. J. Immun.*, **1**, 453-458 (1971).
- Hülser, D. F., and Peters, J. H., *Expl Cell Res.*, **74**, 319-326 (1972).
- Holm, G., and Perlmann, P., *J. exp. Med.*, **125**, 721-736 (1967).
- Gilula, N. B., Reeves, O. R., and Steinbach, A., *Nature*, **235**, 262-265 (1972).
- Taylor, R. B., Duffus, W. P. H., Raff, M. C., and DePetris, S., *Nature new Biol.*, **233**, 225-229 (1971).

Rapid mixed lymphocyte culture test based on relative increase in protein synthesis

WE have developed a mixed lymphocyte culture assay for matching prospective recipients of organ transplants to donor cadavers. Its rapidity, combined with the use of frozen cells from a recipient panel, could facilitate the sharing of cadaveric organs among transplantation centres.

Allogeneic lymphocytes mixed in culture stimulate each other and undergo blastogenesis to a degree proportional to the degree of antigenetic disparity between the cells. This mixed lymphocyte reaction (MLR) is a precise means by which the graft *versus* host response can be estimated *in vitro*. MLR is usually evaluated by the measurement of DNA synthesis in 5-8-d-old mixed lymphocyte cultures (MLC). Although the MLC test has been used for the study of some aspects of histocompatibility in man, its use for matching donors to recipients for cadaveric organ transplantation has been limited by the length of the test. A more rapid test (72 h) measuring stimulation of DNA synthesis in MLC has been reported¹, and a method for early evaluation (11-24 h) of mixed leukocyte interaction in mice and

Table 1 MLR after inhibition of protein synthesis in stimulating lymphocytes

Lymphocytes	c.p.m. ^3H -leucine 1 h pulse	c.p.m. ^3H -thymidine 8 h pulse (5 d culture)
C	1220	307
D	1170	342
D _m	1008	96
D _e	112	42
D × D _e	1216	324
C × D	6978	2176
C × D _e	1357	298
C × D _m	5109	1585

The effects of emetine on stimulation of DNA and protein synthesis in MLR. Suspensions of lymphocytes (C) and (D) were prepared as described in the text (2×10^6 cells ml^{-1}). Lymphocytes D were arbitrarily selected as stimulating cells and were treated with $25 \mu\text{g ml}^{-1}$ mitomycin C for 30 min at 37°C and used in MLC after excess mitomycin was removed by three washes with medium. Similarly, lymphocytes D were treated with 1 mg ml^{-1} emetine for 30 min and used as D_e after three washes. Both mitomycin-treated (D_m) and emetine treated (D_e) cells remained viable (97%) as tested by trypan blue exclusion. Lymphocytes C and D (D_e and D_m) were incubated alone or mixed together and incubated in leucine-deficient medium in the presence of ^3H -leucine $0.001 \mu\text{mol ml}^{-1}$ for 1 h, collected and counted for ^3H incorporation. They were also cultured alone or mixed together and cultured for 5 d in chemically defined medium³ containing 5% calf serum. On the fifth day cells were collected 8 h after the addition of $10 \mu\text{Ci ml}^{-1}$ of ^3H -thymidine. Incorporation of ^3H -thymidine was measured by scintillation counting. A mixture of DD_e was used to rule out possible carry-over of excess emetine by D_e.

guinea pigs based on the measurement of protein synthesis has been described²⁻⁴. We now report a rapid MLC assay (1 h) to discriminate between isogeneic and allogeneic human lymphocytes by measuring the relative increase in the rate of protein synthesis in MLR. The assay allows the use of frozen lymphocytes from a panel of prospective recipients.

Using a synthetic medium⁵ free of leucine, we compared the changes in the rate of ^3H -leucine incorporation into proteins of allogeneic lymphocytes. Leukocyte-rich plasma was obtained from heparinised blood after red blood cell sedimentation, and the leukocytes were isolated by centrifugation (10 min at 150g). Lymphocytes were separated from granulocytes and red blood cell contaminants by discontinuous density gradient centrifugation⁶.

Protein synthesis was measured as described in the legend to Fig. 1. The first increase in protein synthesis of MLR appeared as a distinct increase in the rate of ^3H -leucine incorporation beginning as early as 15 min after lymphocytes were mixed in culture. This increase lasted for about 4 h and then declined to a rate more characteristic of unstimulated lymphocytes. The second phase, probably more intimately related to DNA synthesis, appeared about 12 h after the initial contact of allogeneic lymphocytes in culture. This phase seems to correspond to the stimulation of protein synthesis reported in MLR of guinea pigs and mice²⁻⁴.

The first phase of stimulation constituted the basis for the test. Lymphocytes were separated from their medium by filtration and cellular proteins and the proteins released

Table 2 Correlation of stimulation ratio in rapid and standard MLC

Lymphocytes in MLC	Stimulation ratio	
	DNA synthesis	Protein synthesis
A × Z	3.7	2.7
B × Z	4.0	3.2
C × Z	6.2	4.8
D × Z	9.6	10.0
E × Z	18.5	15.6

Correlation of protein synthesis in rapid MLC with DNA synthesis in 5 d MLC. Triplicate cultures were prepared described in Table 1, and stimulation ratios were calculated as follows c.p.m. of A × Z/1/2 c.p.m. of A + 1/2 c.p.m. of Z and so on. Lymphocytes A, B, C, D and E showed similar degrees of stimulation of DNA and protein synthesis by cell Z in two-way reactions.

into the medium by the lymphocytes were isolated. The patterns of leucine incorporation into these proteins after electrophoresis on polyacrylamide gel⁷⁻⁹ were compared with those of isogeneic lymphocytes in culture (Fig. 2). The results indicate that a major portion of the increase of radioactivity in MLC is associated with the proteins excreted into the medium. The pattern of ^3H -leucine incorporation into proteins of allogeneic lymphocytes changes within 1 h of initial contact between these cells. The appearance of new and high peaks of leucine uptake suggests the synthesis of new proteins in stimulated cells.

To confirm the participation of both cell types in the

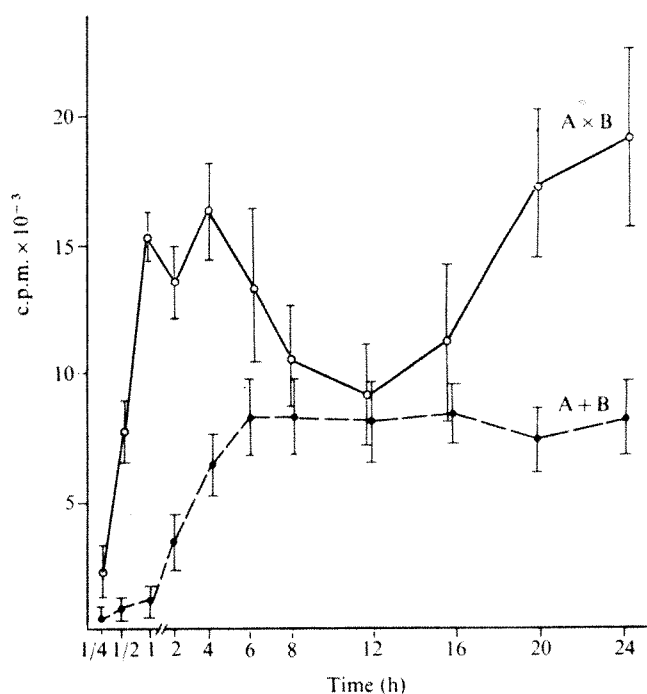


Fig. 1 Biphasic protein synthesis in MLR: protein synthesis was measured by uptake of ^3H -leucine into acid-precipitable proteins. Allogeneic lymphocytes A and B (2×10^6 cells per ml) were incubated in a medium with low concentrations of leucine ($1 \mu\text{mol l}^{-1}$) by themselves or mixed (A:B=1) at 37°C for up to 24 h. Ten cultures of each type (A, B and A × B) were collected at each time point. For the first three time points (15, 30 and 60 min), $0.001 \mu\text{mol ml}^{-1}$ of ^3H -leucine was added to each culture at the plating time and cultures were collected after 15, 30 and 60 min of incubation. Other cultures were collected at 2-4 h intervals 1 h after the addition of $0.001 \mu\text{mol ml}^{-1}$ of ^3H -leucine. The total acid-precipitable protein of the medium and cells of each culture was collected on Millipore filters ($0.2 \mu\text{m}$ pore size), dried and counted in a scintillation counter. ○, Mean c.p.m. of allogeneic MLC (A × B); ●, 1/2 mean c.p.m. of lymphocyte A + 1/2 mean c.p.m. of lymphocyte B (A + B). The bars indicate ± 1 s.d.

first phase of protein synthesis, one cell type was pretreated with emetine¹⁰⁻¹², which irreversibly inhibits cell protein synthesis without affecting viability. This prevented stimulation of protein and DNA synthesis in allogeneic lymphocytes in MLC (Table 1), strongly suggesting that active protein synthesis in both the stimulating and responding cells is necessary for MLR. This reaction, at least in the early phase of MLC, is a two way response.

In our leucine-free medium, the addition of a minimum of $0.001 \mu\text{mol ml}^{-1}$ of labelled leucine was required for an adequate response (Fig. 3). Different degrees of uptake of ^3H -leucine occurred when the leucine concentration was varied. Detection of stimulation of protein synthesis in a given pair of lymphocytes was optimal with $0.001 \mu\text{mol l}^{-1}$ of leucine (Fig. 3).

We have compared the stimulation ratio (c.p.m. of MLC per c.p.m. of individual allogeneic lymphocytes in culture)

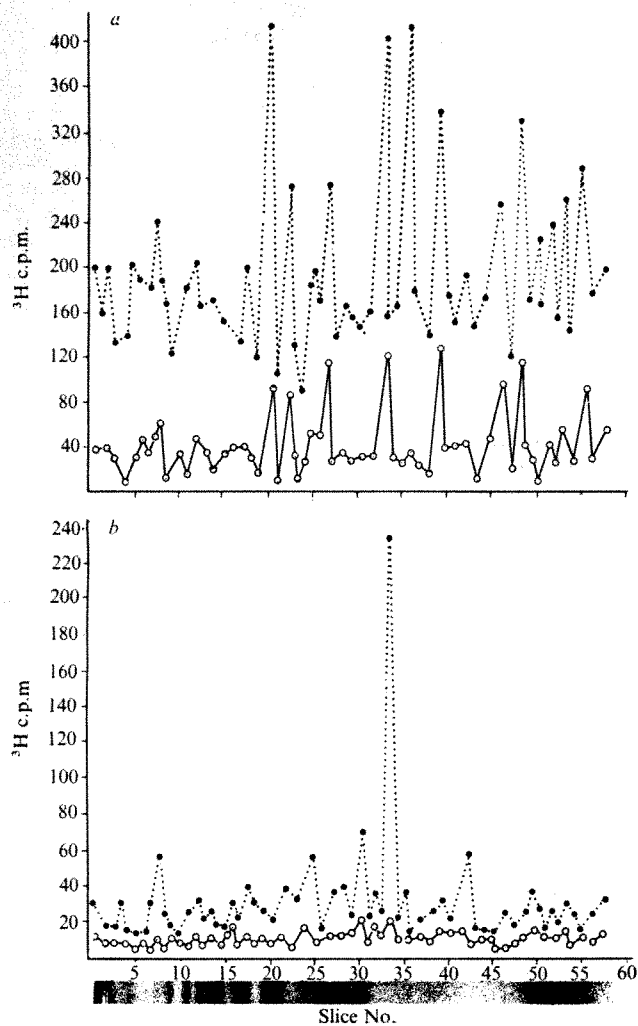


Fig. 2 Electrophoretic patterns of ^3H -leucine-labelled proteins of stimulated (●) and unstimulated (○) lymphocytes. Lymphocyte cultures were prepared as described in Fig. 1: $0.001 \mu\text{mol ml}^{-1}$ of ^3H -leucine was added to each culture at the time of plating and incubated for 1 h. Lymphocytes were separated from their culture medium by filtration through a Millipore filter ($0.45 \mu\text{m}$ pore size). Cells were suspended in 0.4 ml of 0.05 M Tris-HCl, $\text{pH } 7.4$, and mixed with 0.8 ml of 2% sodium dodecyl sulphate (SDS) solution containing 6 M urea and 25% mercaptoethanol and dissociated by heating at 100°C . Similarly, 0.4 ml of medium was mixed with 0.8 ml of 2% SDS containing 6 M urea and 25% mercaptoethanol and dissociated by heating at 100°C . Aliquots (0.1 ml) of each denatured solution were applied to 7.5% acrylamide gel columns prepared according to Russell and Skehel⁷⁻⁹ and electrophoresed for 8 h at 3 mA per gel. Gels were washed in 7% acetic acid and sliced 1 mm thick, each slice was solubilised in 0.1 ml of NCS (Nuclear Chicago) and counted in a scintillation counter after addition of counting fluid. *a*, Electrophoresis of proteins released into medium: ●, c.p.m. of stimulated lymphocytes ($A \times B$); ○, c.p.m. of unstimulated lymphocytes ($A + B$); *b*, electrophoresis of cellular proteins: ●, c.p.m. of stimulated lymphocytes ($A \times B$); ○, c.p.m. of unstimulated lymphocytes ($A + B$).

Table 3 Correlation of Rapid MLC with graft rejection

Number of cases	Rejection	Stimulation ratio	
		Range	Mean
4	0	1.2–1.8	1.65
8	1+	2.1–3.8	2.60
6	2+	3.4–4.8	4.05
3	3+	6.7–10.4	8.10
17	4+	3.8–24.8	11.60

Correlation of stimulation ratio in rapid MLC with graft rejection. Thirty-eight recipients of cadaveric kidneys were selected and transplanted on the basis of negative cross match. The stimulation ratios in rapid MLC were measured as described. Rejection was classified as follows: 0, no rejection—normal function (serum creatinin less than $1.5 \text{ mg}\%$); 1+, one rejection crisis, reversed—normal function; 2+, two rejection crises, reversed—less than normal function (serum creatinin more than $1.5 \text{ mg}\%$); 3+, two or more rejection crises, but kidney lost after 3 months; 4+, uncontrollable rejection requiring graft nephrectomy.

(A, B, AB or O) were mixed with lymphocytes of each recipient in the corresponding panel for the rapid MLC test. Thus a gradient of stimulation ratios was obtained, and the donor-recipient pair with the lowest stimulation ratios was selected. Stimulation ratios among a panel of recipients tested against lymphocytes of a cadaveric donor ranged between 2 and 30.

The rapid MLC test allows rapid selection of a suitable donor-recipient pair from a recipient panel based on the MLR. For the selection of donor-recipient pairs on this basis, however, the panel would have to contain a large pool of recipients to allow for the exclusion of those donor-recipient pairs that have a low stimulation ratio of protein synthesis but a positive cross-match. Our preliminary study

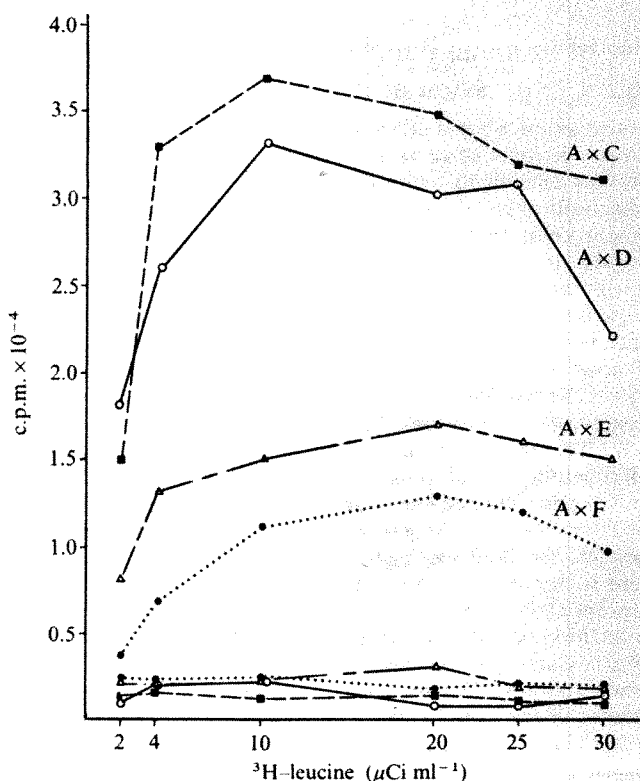


Fig. 3 The effects of variation of leucine concentration on detection of MLR. Allogeneic lymphocytes C, D, E and F were prepared as described in the text and suspended in leucine-free medium (2×10^6 cells per ml) in triplicate. They were incubated by themselves or mixed with lymphocytes A (2×10^6 cells per ml) for 1 h in the presence of variable amounts of ^3H -leucine. Radioactivity of incorporated leucine was measured as described for Fig. 1. Mean c.p.m. of stimulated lymphocytes are shown as $A \times C$, $A \times D$ and so on. The lower curves represent $1/2$ (mean c.p.m. A + mean c.p.m. B) and so on.

of protein synthesis in rapid MLC with the stimulation ratio of DNA synthesis in 5-d MLC of 500 related and 100 unrelated donor-recipient pairs. The stimulation of DNA synthesis in all cases correlated well with the increase in the rate of protein synthesis in rapid MLC (Table 2).

This method was used to pair prospective kidney transplant recipients and cadaveric donors. Frozen cells* from recipients were prepared and stored at -80°C in four panels according to their blood types (A, B, AB or O). Lymphocytes from cadaveric donors of a given blood type

in 10 cadaveric transplants suggested that the degree of survival of the renal transplant is inversely related to the magnitude of the stimulation ratio in rapid MLC¹³. Present data (Table 3) confirms our previous findings.

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- ¹ Bach, M. L., Solliday, S., and Stumbuk, M., in *Histocompatibility testing* (edit. by Terasaki, P.), 643-653 (Williams and Wilkins, Baltimore, 1970).
- ² Schechter, B., Rosenberg, S. A., Levy, R., Ficker, S., and Terry, W. D., *Cell. Immun.*, **6**, 261-272 (1973).
- ³ Rosenberg, S. A., and Levy, R., *J. Immun.*, 1080-1087 (1972).
- ⁴ Levy, R., and Rosenberg, S. A., *J. Immun.*, 1073-1079 (1972).
- ⁵ Parsa, I., Marsh, W. H., and Fitzgerald, P. J., *Exp Cell Res.*, **59**, 171-175 (1970).
- ⁶ Boyum, A., *Scand. J. clin. Lab. Invest.*, **21**, 9-26 (1968).
- ⁷ Russell, W. C., and Skehel, J. J., *J. gen. Virol.*, **15**, 45-57 (1972).
- ⁸ Ornstein, L., *Ann. N. Y. Acad. Sci.*, **121**, 321-349 (1964).
- ⁹ Davis, B. J., *Ann. N. Y. Acad. Sci.*, **121**, 404-427 (1964).
- ¹⁰ Grollman, A. P., and Huang, M. T., *Fedn Proc.*, **32**, 1673-1678 (1973).
- ¹¹ Grollman, A. P., *J. biol. Chem.*, **243**, 4089-4094 (1968).
- ¹² Grollman, A. P., *Proc. natn. Acad. Sci., U.S.A.*, **56**, 1867-1874 (1966).
- ¹³ Butt, K., Parsa, I., and Kountz, S. L., *Surgical Forum*, **24**, 272 (1973).

Use of immune lymphocytes to detect expression of herpetic genome

HERPES simplex virus DNA and RNA have been demonstrated in one specimen of cervical carcinoma¹ but these experiments are very difficult to reproduce, possibly because sophisticated hybridisation techniques must be applied to large amounts of tumour cells. In hamster fibroblasts transformed² by herpes simplex virus 2 (HSV-2), viral RNA sequences have been detected³, but not viral DNA; 60% of these transformed cells reacted with a herpesvirus antiserum, using immunofluorescence techniques applied to unfixed cells⁴, but the background of positive cells was 10%, in control cells not treated with HSV-2. To confirm or complement hybridisation and immunofluorescence techniques, alternative methods may be useful.

We have tried to transfer the nucleic acids of a transformed cell line to normal cells, and to check whether functional viral nucleic acid had been transferred, by assaying the recipient cells for herpetic antigen. For the assay, we used immune lymphocytes since we found that the donor transformed cells were susceptible to specific toxic action of lymphocytes from appropriately immunised animals (lymphocytotoxicity test); in addition, killed transformed cells could be used as antigens to stimulate lymphocytes from immune animals (one way mixed cultures). We shall first describe the antigenic characteristics of the transformed cells before reporting the attempts to transfer these properties to normal cells, by means of nucleic acid transfer.

To provide immune lymphocytes for the cytotoxicity test, 4 to 6-week-old hamsters were inoculated subcutaneously with 2×10^6 cells of the 333 line transformed by HSV-2. Tumours appeared 1-2 week later, in 80-100% of the inoculated animals. Four weeks after inoculation, hamsters were killed and a lymphocyte suspension was prepared from the spleen. Toxicity of these lymphocytes was tested⁵ on cells prelabelled with ⁵¹Cr; Table 1 shows that lymphocytes from tumour-bearing animals were the most toxic for the homologous HSV-2 oncogenic cell line but were also active on HSV-2 infected

cells. The effect was low or absent on a normal hamster cell line, as well as on an adenovirus type 12 oncogenic cell line (NilTr.). Conversely, hamsters inoculated intraperitoneally with ultraviolet-irradiated purified HSV-2 virions possessed lymphocytes which were the most toxic for HSV-2 infected cells, but also released ⁵¹Cr from HSV-2 transformed cells and from HSV-1 infected cells, and had no effect on normal hamster cells.

These experiments showed that 333 transformed cells can be used as herpetic antigen targets; to investigate whether they could be used as an antigenic stimulus, we combined two published methods: one showed that mixed lymphocyte cultures can be miniaturised and performed with whole heparinised blood, without purification of the lymphocytes⁶, and the other applied the mixed lymphocyte reaction to the detection of tumour antigens⁷. We inoculated hamsters subcutaneously with 2×10^6 cells from the 333 line and killed some of them at various times after inoculation; their heparinised blood was cultivated for 5 d in the presence of mitomycin C-treated 333 cells. Tritiated thymidine was added on the fourth day, and its incorporation counted on the next day. The index of stimulation was considered positive when lymphocytes incorporated at least three times more thymidine after cultivation in the presence of the antigen than after culture in nutrient medium alone. Immune lymphocytes

Table 1 Toxicity of immune lymphocytes for various target cells

Lymphocytes from hamsters inoculated with	⁵¹ Cr release after action on cells Transformed with HSV-2	Adeno-virus 12	Infected with HSV-2	HSV-1	Normal (C13)
Transformed HSV-2 cells (333)	64*		12 19	0 8	0
Irradiated HSV-2 virions	25	0	3		
	35		51	23	0

* Specific ⁵¹Cr release (%).

Lymphocyte suspensions were prepared from spleens of hamsters which either bore a subcutaneous tumour resulting from inoculation of 333 cells 4 weeks previously or had been immunised by two intraperitoneal injections of 5×10^6 ultraviolet irradiated HSV-2 plaque-forming units. 10^6 lymphocytes were added to cups containing monolayers of 10^4 target cells, grown for 20 h after labelling with ⁵¹Cr; 18 h after lymphocyte addition, radioactivity released into 100 μ l of the supernatant was counted.

showed the highest index of stimulation when collected 1 week after inoculation of the antigen; the index decreased below significant values when the tumour appeared, a confirmation of other observations⁷. Table 2 shows the results obtained with lymphocytes collected one week after inoculation of the antigens. Lymphocytes from hamsters immunised with 2×10^6 HSV-2 transformed cells, or with ultraviolet-irradiated HSV-2 virions, were stimulated specifically by the transformed cells. Hamsters inoculated with 2×10^6 HeLa cells, or with 10^6 - 10^8 normal cells aspirated from the cervix of healthy women, did not react; however, one hamster showed a weak but significant reaction with the 333 cells after inoculation with about 10^4 cells from a secondary culture of a biopsy of human carcinoma of the cervix. The specificity and reproducibility of the latter result must be confirmed. It has been shown recently⁸ that lymphocytes from women with cervical cancer were cytotoxic to HeLa cells, indicating the existence of an antigen common to these two types of cells; our results do not favour the hypothesis that this antigen is herpetic.

Having demonstrated that immune lymphocytes can detect herpes antigens in cells, we then tried to transfer the expression of these antigens to other cells by means of a nucleic acid transfer method used to rescue the SV 40 genome from transformed cells⁹; we chose this method because it was shown to be more efficient than cell fusion¹⁰. Hep-2 cultures were grown

Table 2 Mixed culture of immune lymphocytes with various mitomycin C-treated cells

Blood from hamsters inoculated with	³ H-Thymidine incorporation in the presence of cells	Transformed with HSV-2 (333)	Infected with HSV-2	Normal (C 13)
333 cells	36*			8
Irradiated HSV-2 virions	25		54	0
Human cervical carcinoma culture	4.3			1
Normal cervical cells	0.88 ± 0.48†		1.1	
HeLa cells	2.6		2.7	0.5

* Index of stimulation: amount of ³H-thymidine incorporated in the presence of stimulating cells divided by the amount incorporated after culture in nutrient medium alone.

† Mean ± s.e. of six tests performed after inoculation with 10⁵–10⁶ cervical cells from six women.

One week after a single injection of the antigens, hamster blood was collected with 10 U ml⁻¹ of heparin without preservative; aliquots of 0.1 ml of blood were added to 2 ml nutrient medium with 5% inactivated foetal calf serum, containing 4 × 10³ stimulating cells pre-treated for 30 min at 37° C with 60 µg ml⁻¹ of mitomycin C. After 4 d culture, 1 µCi ³H-thymidine ml⁻¹ was added and 24 h later the amount of label incorporated into acid-insoluble material was counted.

for 24 h after treatment with nucleic acids from 333 or Hep-2 cells, or from HSV-2 virions; they were then either labelled with ⁵¹Cr and used as targets for spleen lymphocytes from hamsters immunised with HSV-2 grown in Hep-2 cells; or they were treated with mitomycin C and used as stimulating antigens in mixed cultures with heparinised blood from the same immunised hamsters. In both tests, lymphocytes immune to HSV-2 reacted with Hep-2 cells treated with nucleic acids from the transformed cells, but not with untreated Hep-2 cells (Table 3). The immune lymphocytes also reacted with Hep-2 cells infected with either HSV-2 virions or HSV-2 DNA; the latter result is not unexpected since the experiment was done under the conditions used to demonstrate that HSV DNA is infectious^{12–14}. After treatment with nucleic acids from the transformed 333 cells, there was a zone phenomenon, since Hep-2 cells treated with 0.1 µg nucleic acids were more responsive than those treated with 5 µg. A nucleic acid transfer experiment was then repeated, using hamster C 13 cells as recipients; the appearance of herpetic antigens in these cells

Table 3 Reaction of lymphocytes immune to HSV-2 with Hep-2 cells grown after treatment with various nucleic acids (NA).

Hep-2 cells grown with	Target cells for lymphocytotoxicity	Used as Stimulating cells in mixed culture
Exp. 1		
PBS	0*	
Hep-2 NA 5 µg	0	
0.1 µg	0	
333 NA 5 µg	6	
0.1 µg	9	
HSV-2 NA 5 µg	69	
0.1 µg	10	
HSV-2 virions	52	
Exp. 2		
PBS	0	1†
333 NA 5 µg	2	16
0.1 µg	32	55.5
HSV-2 NA 5 µg	29	51
0.1 µg	3	6.5

* % ⁵¹Cr release from Hep-2 cells.

† Stimulation index of thymidine incorporation into lymphocytes from hamsters immunised with ultraviolet HSV-2.

After extraction¹¹ from 333 and Hep-2 cells and from HSV-2 virions, nucleic acid solutions at 5 and 0.1 µg ml⁻¹ were prepared and mixed with CaCl₂ at final concentration of 125 mM (ref. 12) before inoculation of 0.5 ml nucleic acid solution to two Petri dishes containing 10⁶ cells each. After 20 min at room temperature, each dish received 5 ml of nutrient medium and the cells were grown for 24 h at 37° C. They were then washed, suspended, and ⁵¹Cr labelled for lymphocytotoxicity test (see Table 1) or treated with mitomycin C for the mixed culture test (see Table 2).

could also be demonstrated (results not shown).

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- Frenkel, N., Roizman, B., Cassai, E., and Nahmias, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3784–3789 (1972).
- Duff, R., and Rapp, F., *Nature new Biol.*, **233**, 48–50 (1971).
- Collard, W., Thornton, H., and Green, M., *Nature new Biol.*, **243**, 264–266 (1973).
- Rapp, F., and Duff, R., *Cancer Res.*, **33**, 1527–1534 (1973).
- Brunner, K. T., Mariel, J. C., Cerottini, J. C., and Chapins, B., *Immunology*, **14**, 181–196 (1968).
- Stockman, G. D., Munford, D. M., and Wilbur, J. R., *Int. Archs Allergy*, **43**, 759–765 (1972).
- Senik, A., Gossard, E., Plata, F., and Levy, J. P., *Int. J. Cancer*, **12**, 233–241 (1973).
- Saksela, E., and Meyer, B., *J. natn. Cancer Inst.*, **51**, 1095–1102 (1973).
- Boyd, V. A. L., and Butel, J. S., *J. Virol.*, **10**, 399–409 (1972).
- Melnick, J. L., and Butel, J. S., in *Viral Replication and Cancer: Proc. 2nd Duran-Reynals Symposium*, 243–254 (Labor., Barcelona, 1973).
- Van der Eb, A. J., Van Kesteren, L. W., and Van Bruggen, E. F. J., *Biochim. biophys. Acta*, **182**, 530–541 (1969).
- Graham, F. L., and Van der Eb, A. J., *Virology*, **52**, 456–467 (1973).
- Graham, F. L., Veldhuisen, G., and Wilkie, N. M., *Nature new Biol.*, **245**, 265–266 (1973).
- Sheldrick, P., Laithier, M., Lando, D., and Ryhiner, M. L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3621–3625 (1973).

Athymic (nude) mice express gene for myxovirus resistance

DISAPPOINTINGLY little is known about the mechanisms of natural, genetically determined resistance to infectious agents¹. Even in the simplest situation, where a single, dominant gene is responsible for resistance to a group of closely related viruses, no satisfactory explanation of the phenomenon has been offered. A case in point is the resistance towards the lethal action of a number of myxoviruses, mainly influenza A, exhibited by the inbred mouse strain A2G and attributed to the presence of the dominant gene *Mx* (refs 2–5). Serial virus titrations in A2G and comparable susceptible mice showed that resistance must depend on an event occurring early in the infectious process, since by day 2 after challenge virus titres were significantly lower in resistant animals². It was conceivable that an early triggering of the immune system might exert such an effect. If this were the case, one would expect the expression of the resistance gene to be impaired in mice showing profound disturbances of their immunological apparatus. Nude mice homozygous for the gene *nu* (refs 6–9) show such a disturbance, mainly of the T cell system. We have therefore introduced the gene *Mx* into *nu/nu* mice, and we have investigated the progeny from appropriate crosses for the phenotypic expression of resistance to neurotropic influenza virus.

Nude males (genotype *nu/nu* on a predominantly BALB/c genetic background, from G.L. Bomholtgard, Ry, Denmark) were mated with A2G females (*Mx/Mx*, bred locally). The F₁ generation was normal with respect to coat and thymus development, and was resistant to intracerebral challenge with 100 LD₅₀ (as estimated in susceptible mice) of neurotropic influenza A_v virus, strain NWS (ref. 3). Brother-sister matings

Table 1 Resistance of nude F_2 mice* to neurotropic influenza A virus

Experiment No.	No. challenged	Resistant†	Susceptible‡
1	9	8	1
2	11	8	3
3	11	8	3
Total	31	24	7

* 75% expected to be *Mx* carriers.† Survived intracerebral challenge with 100 LD₅₀ of stock NWS virus for 14 d or longer.

‡ Died of viral infection within 5–7 d.

among F_1 mice yielded an F_2 generation, of which approximately 25% were phenotypically nude. Assuming independent segregation of the genes *nu* and *Mx*, 75% of these nude F_2 should carry the gene *Mx* either in homozygous or heterozygous form. At the age of 3 weeks, nude F_2 together with comparable numbers of non-nude littermates, and with control resistant (*Mx*+) and control susceptible mice for monitoring the challenge virus, were inoculated intracerebrally with 100 LD₅₀ of NWS. All susceptible control animals succumbed to viral infection, whereas all control resistant *Mx*+/+ mice survived. Of both nude and non-nude F_2 mice approximately 75% survived. The results of the nude group are shown in Table 1; the observed seven deaths among 31 challenged mice are close to the expected 25%.

It is thus apparent that the nude phenotype did not preclude expression of virus resistance. The numbers observed also

antigen of influenza A virus. Tests on delayed hypersensitivity were not performed.

Expression of resistance in *Mx*-bearing mice seems therefore independent not only of a functional T-cell system, but also of the orderly development of HAI antibodies, usually regarded as the protective antibody in influenza virus infection. These findings agree with the observations that resistance of A2G mice could not be broken by various immunosuppressive regimens, including whole body X irradiation and neonatal thymectomy (P. A. Klein, personal communication, and unpublished work).

We conclude that the inborn resistance of mice carrying the dominant gene *Mx* does not require an intact immunological machinery for its expression. Both cellular and humoral immunity may be less important in recovery from influenza virus infection than hitherto suspected.

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- 1 Allison, A. C., *Arch. Virusforsch.*, **17**, 280–294 (1965).
- 2 Lindenmann, J., Lane, C. A., and Hobson, D., *J. Immun.*, **90**, 942–951 (1963).
- 3 Lindenmann, J., *Proc. Soc. exp. Biol. Med.*, **116**, 506–509 (1964).
- 4 Lindenmann, J., and Klein, P. A., *Arch. Virusforsch.*, **19**, 1–12 (1966).
- 5 Rusanova, N. A., and Soloviev, V. D., *Vop. Virus.*, **11**, 398–402 (1966).
- 6 Pantelouris, E. M., *Nature*, **217**, 370–371 (1968).
- 7 De Sousa, M. A. B., Parrott, D. M. V., and Pantelouris, E. M., *Clin. exp. Immun.*, **4**, 637–644 (1969).
- 8 Kindred, B., *Eur. J. Immun.*, **1**, 59–61 (1971).
- 9 Wortis, H. H., *Clin. exp. Immun.*, **8**, 305–317 (1971).
- 10 Pantelouris, E. M., and Hair, J., *J. Embryol. exp. Morph.*, **24**, 615–623 (1970).
- 11 Wortis, H. H., Nehlsen, S., and Owen, J. J., *J. exp. Med.*, **134**, 681–692 (1971).
- 12 Virelizier, J.-L., Postlethwaite, R., Schild, G. C., and Allison, A. C. (in the press).

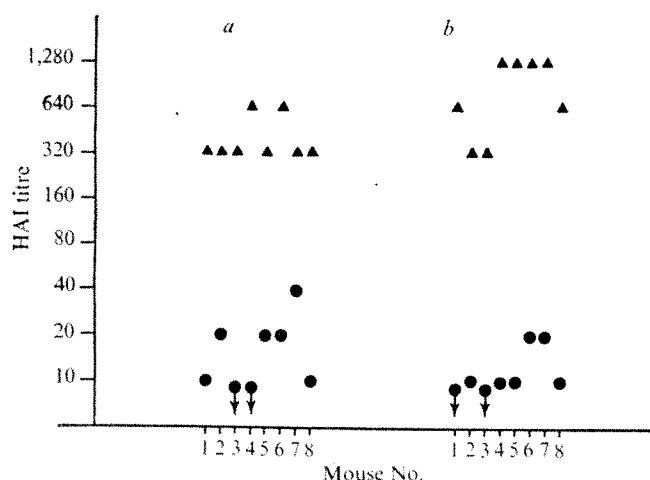


Fig. 1 Serum antibody titres in myxovirus-resistant athymic and control mice surviving 100 LD₅₀ of influenza A NWS. ●, Congenitally athymic (nude) mice; ▲, non-nude littermates. a, Serum taken on day 14; b, serum taken on day 21.

suggest that the genes *Mx* and *nu* are not closely linked. To make sure that those mice which were assumed to be nude on the basis of their coat development also had abnormal thymuses, a few survivors were examined histologically. We found the typical picture of thymic dysplasia with cystic and tubular structures among irregularly arranged epithelial-like cells surrounded by connective tissue. Spleen and lymph nodes were found depleted of lymphoid cells in the thymus-dependent areas^{9,10}. To measure functional impairment of the T cell system in nude, *Mx*-bearing mice, use was made of the fact that formation of haemagglutination-inhibiting (HAI) antibodies is a T cell dependent function¹². Two and three weeks after virus challenge, individual surviving nude and non-nude littermates were bled and the titres of HAI were determined. Figure 1 shows the results: nude mice surviving virus challenge had significantly lower antibody titres than similar non-nude littermates. The same difference was also revealed with antibodies to the complement-fixing nucleoprotein

Location of nuclear proteins on the chromosomes of newt oocytes

In eukaryote cells chromosomal proteins are responsible for the organisational state of the DNA and the control of genetic expression, therefore knowledge of their location is an essential prerequisite for understanding chromosome function. Cytological localisation of chromosomal proteins is feasible using cells with giant chromosomes such as the oocytes of the newt *Triturus cristatus carnifex* where there are clearly defined regions active in RNA transcription (the loops) and regions containing most (>95%) of the DNA in a condensed state (the chromomeres)¹. There are two main classes of protein associated with chromosomes: histones, which are generally considered to be complexed with the sugar-phosphate backbone of the DNA²; and nonbasic proteins which are constituents of metabolically active chromatin³.

We extracted the nonbasic proteins from ribonucleoprotein (RNP) particles which are released from the chromosomes into the nucleoplasm⁴. These nonbasic proteins have been shown previously⁵ to comprise a heterogeneous size distribution of polypeptides. Here the polypeptides were separated on a preparative scale by column chromatography and injected into rabbits to stimulate antibody production. Histone protein was extracted from newt liver chromatin and was also used to produce antibodies. By treating chromosome preparations with

fluorescein-conjugated antibodies the chromosomal sites of the various proteins were determined.

Nuclear RNP particles were isolated from homogenates of newt oocytes as described previously^{4,5}. The final step in purification was the collection of the peak fraction from a 30–50% (w/v) sucrose gradient. At all stages in isolation, solutions contained 5 mM 2-mercaptoethanol to prevent protein aggregation⁶. The RNP was solubilised in SDS-buffer (0.1% sodium dodecyl sulphate; 10 mM Tris-HCl, pH 7.4; 10 mM 2-mercaptoethanol; 50 mM NaCl) and dialysed for 4 h against

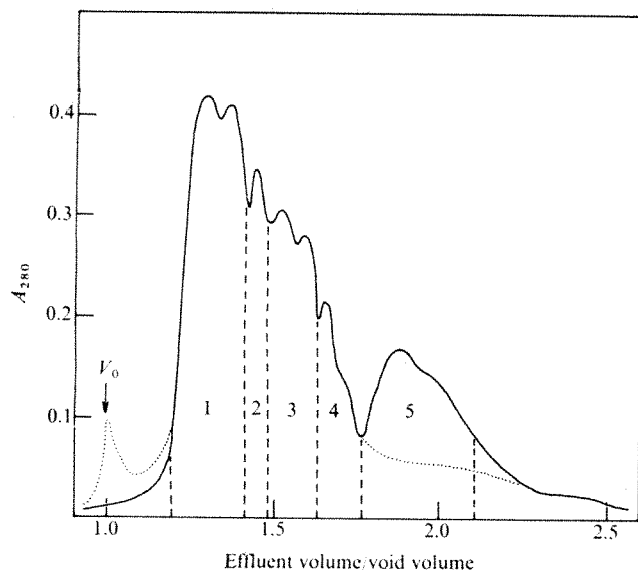


Fig. 1 Separation of nuclear RNP proteins by column chromatography. An 0.5 ml solution of SDS-solubilised protein was applied to a 56 cm column of Sephadex G-200 Superfine gel and eluted with SDS-buffer. The eluted material was divided into five fractions as shown on the A_{280} profile. At the void volume of the column (V_0) there was occasionally elution of contaminating RNA. The peak assigned as fraction 5 was not present in all preparations (dotted line).

this buffer. The solubilised RNP complex was centrifuged at 95,000g for 16 h to remove RNA by pelleting. To separate the RNP proteins on a molecular weight basis a 0.5 ml sample was eluted through a column of Sephadex G-200 gel with SDS-buffer. The elution profile of absorbance at 280 nm is shown in Fig. 1. Resolution of peaks was not as good as was obtained by SDS-acrylamide gel electrophoresis but large amounts of protein could easily be separated. The eluted protein was divided into five fractions each of which was concentrated to 0.5 ml by pressure dialysis. The complexity of protein content in each

fraction was analysed by SDS-acrylamide electrophoresis. These were compared with electropherograms of total RNP protein and of total histone (Fig. 2). Of the 13 main bands in the total RNP extract, between two and four bands were contained in each of the five gel-filtration fractions and distributed between these fractions in groups of decreasing molecular weight (see also Table 1). The separation of the proteins by gel filtration was judged to be satisfactory.

As antigen sources, 0.5 ml samples of total RNP protein, fractionated RNP proteins and total histone were each incorporated into multiple emulsions⁷ and injected into different rabbits. Similar samples were injected into different rabbits on three separate occasions to eliminate any anomalous effect due to a particular animal. After a minimum of 60 d the rabbits were bled and the sera were prepared as described previously⁸.

The various antisera were reacted with chromosomes as follows. Lampbrush chromosomes were prepared from newt oocyte nuclei⁹ and firmly attached to the surfaces of coverslips by centrifugation through Tris-buffered saline⁴, at pH 7.8, to obtain maximum transmission of fluorescence. The chromosome preparations were treated with various dilutions of antisera in buffered saline for 30 min, washed in buffer for 1 h, placed in fluorescein-conjugated sheep anti-rabbit antibody solution (Wellcome Reagents Ltd) at a dilution of 1:100 and again washed

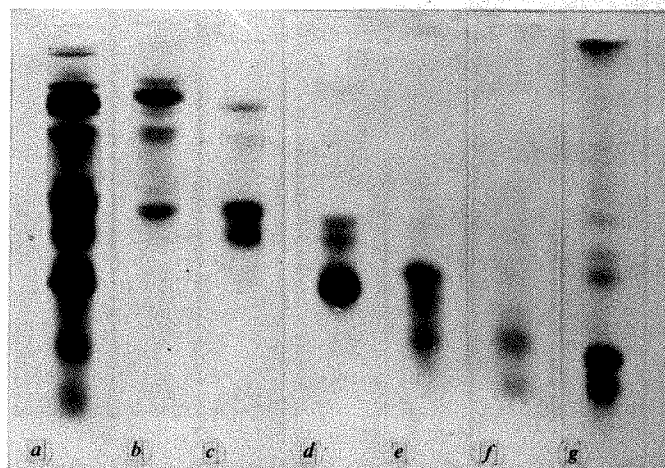


Fig. 2 SDS-acrylamide gel electrophoresis of solubilised proteins. *a*, Total nuclear RNP protein; *b–f*, fractions 1–5 derived by gel filtration (Fig. 1); *g*, newt liver histones (kindly supplied by Dr D. B. Malcolm). 20 μ l samples were applied to 7% polyacrylamide gels containing 0.1% SDS and 0.1 M phosphate buffer, pH 7.1. Electrophoresis was at 10 mA per tube until the buffer front had migrated 6–7 cm. The gels were fixed and stained with Coomassie blue.

Table 1 Characteristics of antisera produced against chromosomal proteins

Antiserum produced against	Molecular weights of main components used as antigen	Titre (greatest dilution giving a fluorescent reaction)	Specificity of reaction with lampbrush chromosomes
Total nonbasic protein	155,000–17,000 (13 bands)	1 : 2,500	With all loops and with chromomeres to a lesser extent
Fraction 1	135,000 125,000 90,000 50,000	1 : 1,750	With all loops
Fraction 2	65,000 50,000	1 : 1,750	With all loops
Fraction 3	65,000 50,000 35,000	1 : 2,500	With only 10 pairs of loops
Fraction 4	35,000 20,000	1 : 1,750	With all loops and with chromomeres to a lesser extent
Fraction 5	20,000 17,000	1 : 1,500	Mainly with chromomeres
Total histone	20,000 16,000	1 : 2,500	With chromomeres
Normal serum	—	1 : 500	With all structures

in buffer. The chromosomes were located by phase contrast microscopy and then the fluorescent reaction was observed in a beam of ultraviolet light using the appropriate exciter and barrier filters.

Antiserum produced against the extract of nonbasic proteins reacted specifically with the chromosomes and more intensely with the loops than with the chromomeres. The antiserum produced against histone reacted primarily with the chromomeres (Fig. 3d). Control preparations using normal rabbit serum reacted nonspecifically with all structures, nucleoli as well as chromosomes, but only at a relatively high serum concentration. The greatest serum dilution at which a fluorescent reaction could be obtained (see Table 1) was routinely used to avoid cross reaction and nonspecific effects. There was some reaction with the loops with anti-histone serum but this is most likely to result from contamination of histone extract with some nonbasic protein. In fact a few minor bands of high molecular weight protein could be seen on the electropherogram (Fig. 2). Low concentrations of histones may well be present in the loops but the technique used here is probably not sensitive enough to detect these.

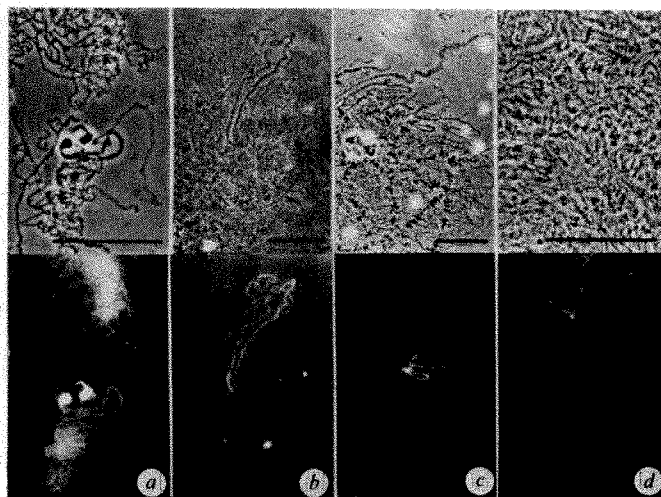


Fig. 3 Phase contrast and fluorescence photomicrographs showing location and specificity of reaction of fluorescein-conjugated antibodies with lampbrush chromosomes. *a*, Antiserum produced against RNP-protein fraction 2 at a concentration of 1:1,000; *b* and *c*, antiserum against fraction 3 at a concentration of 1:1,250; *d*, antiserum against histone at a concentration of 1:1,000. The photomicrographs were taken on Ilford FP4 (1 s exposure for phase; 2.5 min exposure for fluorescence) and developed in Paterson's Acutol. The bars represent 10 μ m.

Of the protein fractions derived from solubilised RNP fractions 1, 2 and 4 stimulated antibodies which reacted specifically with all the chromosome loops (Fig. 3a), that is, one or more of the proteins present in these fractions were common to the RNP matrix of all loops. As a result of the distribution of different polypeptides between these fractions (see Fig. 1 and Table 1) at least two different proteins are common to all loops. Antibodies produced against fraction 4 showed some cross reaction with the chromomeres. Furthermore, antibodies against fraction 5 reacted specifically with the chromomeric axis. Fraction 5 gave a very similar banding pattern on SDS-acrylamide gels to that of histone (Fig. 2). Because this fraction was not present in all RNP protein extracts (Fig. 1) fraction 5 was judged to be contaminating histone probably as a result of excessive homogenisation of the oocytes.

The most interesting and unexpected result was given by antiserum produced against fraction 3. This antiserum reacted with the proteins associated with only certain loops—approximately 10 loop pairs in each chromosome complement. These loops did not have any peculiar morphology and could not be

identified before the fluorescent reaction (Fig. 3b and c). As far as can be discerned the same loops give this specific reaction in all chromosome preparations and are randomly distributed. Because the whole length of spatially isolated loops gives a specific reaction this is conclusive evidence of the integrity of the loop structure, that is, the lampbrush loop is a functional unit. Evidence that the loop is a unit of transcription will be reported later (J.S., unpublished work). The protein which is the main component of fraction 3 and which probably elicits the specific loop reaction has a molecular weight of approximately 35,000.

Thus histones are present primarily in the regions of condensed DNA (chromomeres) in lampbrush chromosomes (previously shown to contain basic protein by staining¹⁰ while RNP-derived nonbasic proteins are restricted to the loops. The implications of the high protein content of nuclear RNP remains unknown. Preliminary evidence suggests that various types of enzymic activity are present, specifically the ability to polymerise ribonucleotide triphosphates and to cleave large nuclear RNA into smaller fragments. Homoribonucleotide polymerases¹¹ and endonuclease¹² have already been reported to be present in mammalian RNP particles. Obviously enzymes concerned with the processing of primary transcript RNA can only account for a very small amount of protein which may even not be visible in gel electropherograms. A secondary RNP formed during oocyte maturation has been isolated and has been shown to have a similar protein constitution to the RNP described here. Results pertaining to the protein content of the different RNP products at various stages in the processing of RNA transcripts will be reported later.

We thank Dr D. B. Malcolm for supplying the histones and Professor H. G. Callan for critically reading the manuscript.

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- ¹ Callan, H. G., *Int. Rev. Cytol.*, **15**, 1-34 (1963).
- ² Shih, T. Y., and Bonner, J., *J. molec. Biol.*, **48**, 469-487 (1970).
- ³ Paul, J., and Gilmour, R. S., *J. molec. Biol.*, **34**, 305-316 (1968).
- ⁴ Sommerville, J., *J. molec. Biol.*, **78**, 487-503 (1973).
- ⁵ Sommerville, J., and Hill, R. J., *Nature new Biol.*, **245**, 104-106 (1973).
- ⁶ Hill, R. J., Maundrell, K., and Callan, H. G., *Nature new Biol.*, **242**, 20-22 (1973).
- ⁷ Herbert, W. J., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.) 1207 (Blackwell, Oxford, 1967).
- ⁸ Sommerville, J., in *Protein Biosynthesis in Non-Bacterial Systems* (edit. by Last, J. A., and Laskin, A. I.), 215 (Dekker, New York, 1972).
- ⁹ Callan, H. G., and Lloyd, L., *Phil. Trans. R. Soc. Lond. B*, **243**, 135-219 (1960).
- ¹⁰ Macgregor, H. C., and Callan, H. G., *Q. Jl. microsc. Sci.*, **103**, 173-203 (1962).
- ¹¹ Niessing, J., and Sekeris, C. E., *Nature new Biol.*, **243**, 9-12 (1973).
- ¹² Niessing, J., and Sekeris, C. E., *Biochim. biophys. Acta*, **209**, 484-492 (1970).

Erratum

In the article "Deterioration of high school students' attitudes to physics" by P. L. Gardner (*Nature*, **250**, 465; 1974), the following corrections should be made to the 3rd paragraph: line 5, for 4 yr read 2 yr; lines 10-11 should read "in coeducational State high schools in moderately affluent suburban areas of Melbourne."

matters arising

Geomorphological dating of cave openings in South Africa

In a recent article in *Nature*¹ Partridge dates South African hominid sites with remarkable precision by relating them to retreat of cyclic nickpoints. The soundness of his analysis and results depends on the validity of his geomorphological framework, the date of 20 Myr assigned to the inception of his cyclic nickpoints and his method.

The framework is well known and provides for the existence of identifiable remnants of 'Gondwana', 'post-Gondwana', 'African' and later cyclic surfaces of former continental extent². The dating and correlation of erosion surfaces over wide areas of Africa with quite different climatic and tectonic histories rest, however, on shaky grounds³. Moreover, results of recent work connected with the South African oil search on land and offshore contradict the framework in many material respects. Lack of space makes it impossible for me to elaborate here, but, as an example, the Paleogene, during which the African surface on land reputedly stood so low that the rivers were sediment starved⁴, saw the most rapid deposition off the Natal coast from the mid-Cretaceous onwards.

The dating of 20 Myr for the inception of the 'Post African 1' cyclic nickpoint, based on transgressive sediments above an unconformity at Uloa, Natal⁵, is taken as factual. The correlation of transgressive coastal sequences with incision on land is debatable, as it presupposes rather special conditions; but if we accept that the sediments above the unconformity could record the first incision of the 'African' surface, the figure may have to be doubled: in the J (c)-1 well, drilled 24 km off Stanger, the unconformity and 'Pecten Bed' are clearly identifiable, but the age is early Oligocene⁶. Relationships at Uloa, on which so much has been based, are representative of one stand of a slowly migrating shoreline.

Briefly, Partridge's method involves the calculation of mean rates of nickpoint recession in respect of each site, which are then plotted in relation to the midpoints of the segments affected by incision. He assumes that nickpoint migration was subject to linear decline culminating in zero at the stream sources, and apparently reads off the rates of migration at the hominid sites from the graph in Fig. 2. He admits

that the assumption is erroneous, but maintains that the errors of the linear interpolation technique are greatly reduced due to the close proximity of the sites to the headwaters. He thus makes an assumption that seems to be basic to his argument and calculations, admits that it is erroneous, but brushes away any possible reservations by implying that migration rates near the sites were so low that they would have been little affected by what happened downstream anyway.

Recognition of small nickpoints so far upstream as expressions of an ancient coastal event cannot be an easy exercise in a region with numerous nickpoints clearly attributable to differential rock resistance, warping, and so forth. Moreover, a method based on nickpoint recession cannot be applicable to a major basin such as the Orange, where great differences in gradient and channel characteristics reflect the extreme variations in lithology. The present regime illustrates the influence of climate: below Prieska the flow probably decreases at normal times downstream, and nickpoint recession rates would presumably not have decreased upstream under comparable past climates. My gravest criticism, however, is that the implications of differential nickpoint recession up a main stream and its tributaries are ignored. Thus the Orange, the Vaal, the Harts and a small tributary are lumped together, although they are clearly not amenable to treatment as a single unit; this procedure presupposes that recession rates are mainly dependent on distances of watersheds and nickpoints from the main drainage outlet to the ocean, and not on conditions along the stream courses themselves.

Partridge's methods and basic assumptions are of doubtful scientific validity, and his datings can only serve to confuse and mislead South African anthropologists.

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¹ Partridge, T. C., *Nature*, **246**, 75-79 (1973).

² King, L. C., *The Morphology of the Earth* (Oliver and Boyd, Edinburgh and London, 1962).

³ De Swardt, A. M. J., and Trendall, A. F., *Oversea Geol. Miner. Resour.*, **10** (3), 241 (1969).

⁴ King, L. C., *The Natal Monocline* (University of Natal, Durban, 1972).

⁵ King, L. C., *Trans. geol. Soc. S.Afr.*, **73** (3), 151 (1970).

⁶ Du Toit, S. R., and Leith, M. J., *Trans. geol. Soc. S.Afr.* (in the press).

DR PARTRIDGE REPLIES: Dr de Swardt's comments, although relevant to the technique and results reported in my article, reflect fundamental misconceptions as well as lack of familiarity with recent geomorphological research in South Africa. It would therefore be unprofitable to take issue with him point for point, though I should like to refute certain statements of his which have direct bearing on my argument.

(1) I claimed no "remarkable precision" for my estimates for the date of first cave opening at the various hominid sites. My results were presented cautiously and with deference to limitations in accuracy imposed by availability of data for the construction of the nickpoint migration graphs, and the resulting possibility of small scale fluctuations between observation points.

(2) The geomorphological framework which de Swardt calls to question is firmly based on scientific observation and measurement, and does not rely on the unsupported views of any one worker. In particular, Professor L. C. King has never claimed that all of the cyclic surfaces referred to by de Swardt were of former continental extent. This is a totally unreasonable proposition, and was certainly not applied in the context of my work. Indeed, the study of such surfaces in southern Africa has revealed that some are represented over limited areas, others have been partially planed and most have been subject to post-formational warping. All these factors have been subjected to detailed analysis (see references in my original article), and the results have been taken into account in the application of my technique.

(3) There is no contradiction of the geomorphological framework which I applied in the records of offshore oil prospecting cited by de Swardt. Various workers, including King, have recorded sedimentation of the continental shelf, dating from mid-Cretaceous to early Tertiary times¹⁻³, which can

be referred to onshore incision in the African erosion cycle. These offshore results merely add to the overwhelming body of evidence in support of the framework which de Swardt wishes to discredit.

(4) In assessing the date of inception of the Post-African I erosion cycle, the age of littoral sediments immediately overlying the Post-African I planation surface must be conclusive. Where such sediments have been preserved, not only at Uloa, but along considerable stretches of the South African coast between East London and Saldanha Bay, their age has proved to be Miocene³. Simple palaeontological principles do not permit that the Oligocene "Pecten Bed" identified 24 km offshore during drilling operations is the same biostratigraphical unit as that of Uloa, since both the shark and Pecten fauna at the latter locality have been confidently placed within the Miocene on the basis of reliable evidence^{4,5}. The 20 Myr date for the inception of the Post-African I incision can therefore be accepted with confidence. Relationships along considerable stretches of the coast do not indicate a slowly migrating shoreline, but several phases of transgression during the Tertiary^{3,6}.

(5) A small margin of error in my assessment of nickpoint migration rates cannot be avoided, as the linear assumption provides for a mean representation, which cannot readily be refined, as the affects of some variables are impossible to measure. This is a potential source of some inaccuracy, but can by no stretch of argument be regarded as fatal to the technique. The absolute range of possible inaccuracies must, of necessity, decrease towards the watershed origin. This range is considered to be sufficiently small to permit the technique to be used to derive general orders of age and a relative sequence of dates for cave opening—all that I originally claimed for my results.

(6) Numerous erosional nickpoints in South Africa can be recognised through careful fieldwork. Their cyclic associations are not difficult to determine through the application of careful morphometric and statistical analyses such as formed the basis for my article. Erosional nickpoints are clearly distinguishable from those of lithological or local tectonic origin.

(7) Decline in nickpoint migration rates upstream is by no means exclusively a function of diminishing discharge. Owing to large scale Plio-Pleistocene upwarping of the South African coastal margins, many rivers show substantially steeper gradients along their lower courses than in higher reaches; for example, between the Orange River mouth and Prieska,

the mean channel gradient is 0.9 m km⁻¹; between Prieska and Buxton on the Harts River the gradient is 0.4 m km⁻¹.

(8) Variations in nickpoint migration rates between trunk and tributary channels are, indeed, taken into account in my analysis, since my measurements are related to the actual channel segments concerned. Varying conditions along such segments have naturally affected the present positions of the nickpoints which have migrated along them, upon which my graphs are based. The limited inaccuracies which may result from small-scale fluctuations between observation points have been considered.

(9) The validity of my method and assumptions is further confirmed by two age estimates based on entirely different techniques. My estimate of 4.8 Myr for the 61 m terrace of the Vaal River at Windsorton⁷, based on the same methods used to estimate dates of first cave opening, is generally confirmed by the presence at this locality of elephant remains which existed some 4 Myr ago in the East African potassium-argon chronology⁸. Moreover, Butzer⁹, in a lengthy analysis to be published in December, has independently arrived at a maximum age estimate almost identical to my own for the Taung hominid deposit.

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¹ Maud, R. R., *Z. Geomorphol.*, **7**, 155 (1968).

² King, L. C., *The Natal Monocline* (University of Natal, Durban, 1972).

³ Siesser, W. G., *Trans. geol. Soc. S. Afr.*, **75**, 177 (1972).

⁴ King, L. C., *Geol. Mag.*, **106** (2), 206 (1969).

⁵ King, L. C., *Trans. geol. Soc. S. Afr.*, **74**, 241 (1971).

⁶ King, L. C., *Z. Geomorphol.*, **16**, 239 (1972).

⁷ Partridge, T. C., and Brink, A. B. A., *S. Afr. geogr. J.*, **49**, 21 (1967).

⁸ Maglio, V. J., *Nature*, **225**, 328 (1970).

⁹ Butzer, K. W., *Curr. Anthropol.* (in the press).

Ascorbic acid and nitrosamine

SIR—Edgar¹ has proposed that ascorbic acid might inhibit the carcinogenic action of nitrosamines and other carcinogens that may act by alkylation. The rationale was that ascorbate might be alkylated *in vivo* before the carcinogens can react with cell macromolecules. The experimental basis for Edgar's thesis was that statement by Kamm *et al.*² that ascorbate inhibited the liver necrosis induced by dimethyl-

nitrosamine (DMN). This statement, however, was presented without experimental details and was subsequently withdrawn³.

The main concern of the study by Kamm *et al.* was the inhibition by ascorbate of the liver toxicity induced by oral administration of aminopyrine plus nitrite. This study followed our report in 1972 suggesting, on the basis of *in vitro* experiments, that ascorbate might be used to block *in vivo* formation of N-nitroso compounds from nitrosatable chemicals (for example, drugs), since ascorbate efficiently reduces nitrate⁴. Subsequently, the report of Kamm *et al.*² appeared. Greenblatt⁵ found similar results in mice to those of Kamm *et al.*, but stated that ascorbate did not affect DMN toxicity. We found that ascorbate prevented liver damage from gavage of dimethylamine plus nitrate to rats and, from experiments presented in detail, that ascorbate did not significantly affect the production by DMN of liver necrosis and elevated serum transaminase levels⁶. Ascorbate did not affect transplacental carcinogenesis in rats by ethylnitrosourea, but inhibited carcinogenesis by ethylurea plus nitrite⁷.

We are concerned that our original suggestion should not be extended without basis to the hypothesis that ascorbate might have a much wider inhibitory action on carcinogens. The interesting suggestion of Edgar is not supported by the results reviewed here, which were mostly made public after Edgar's paper was submitted.

Yours faithfully,

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¹ Edgar, J. A., *Nature*, **248**, 136-137 (1974).

² Kamm, J. J., Dashman, T., Conney, A. H., and Burns, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 747-749 (1973).

³ Kamm, J. J., Dashman, T., Conney, A. H., and Burns, J. J., in *N-Nitroso Compounds in the Environment* (edit. by Bogovski, P., Walker, E. A., and Davis, W.) (International Agency Research Against Cancer, Lyon, in the press).

⁴ Mirvish, S. S., Wallcave, L., Eagen, M., and Shubik, P., *Science*, **177**, 65-68 (1972).

⁵ Greenblatt, M., *J. natn. Cancer Inst.*, **50**, 1055-1056 (1973).

⁶ Cardesa, A., Mirvish, S. S., Haven, G. T., and Shubik, P., *Proc. Soc. exp. biol. Med.*, **145**, 124-128 (1974).

⁷ Ivankovic, S., Preussmann, R., Schmähl, D., and Zeller, J., in *N-Nitroso Compounds in the Environment* (edit. by Bogovski, P., Walker, E. A., and Davis, W.) (International Agency Research Against Cancer, Lyon, in the press).

reviews

Immunoglobulins

The Antigens. Vol. 1. Edited by Michael Sela. Pp. xiii+573. (Academic: New York and London, January 1974.) \$31; £14.90.

THE appearance of a multivolume work about any topic is a sign that the subject has now become part of the body of accepted scientific knowledge. A period of rapid progress is coming to an end and there is a sufficient volume of fact and theory to form the basis of undergraduate courses as well as to be an essential part of the background of all scientists working in related fields. In biochemistry, proteins were the first subject to receive this accolade some twenty years ago followed quickly by enzymes, nucleic acids and many others. The aim was to present research workers, teachers and advanced students with a rather full account of the field and on the whole they have served a useful purpose, though of necessity they have been a year or two out of date by the time they reached the libraries. Now it is the turn of immunochemistry, or as some would have it, molecular immunology. How does it match up?

The first glance is disconcerting as volume 1 of *The Antigens* contains four chapters on antibodies, one general chapter on protein evolution and only two on antigens. Volume 2, of which the contents are listed, will have three chapters, each on antibodies and antigens. This is perhaps not important, as any potential reader will be well aware that it is hardly possible to write of antigens without extensive reference to antibodies and indeed much of the progress in knowledge of antibody structure, genetics and synthesis has come from study of their antigenic properties, but it would have been helpful if the title had given a better guide to the contents.

The antigens chosen for discussion in this volume are nucleic acids by Dr David Stöller and enzymes by Dr Ruth Arnon. The former are difficult to handle as injection of purified nucleic acids into an animal of different species rarely gives rise to a significant immune response and specific antibodies are found only when protein-nucleic acid complexes are used; that is the nucleic acids are behaving as large molecular weight haptens. Nevertheless, in recent years satisfactory antisera have been obtained (and have been found to

occur spontaneously in pathological conditions) and have been used effectively in investigations of the structure and function of nucleic acids in cell and subcellular fractions and a useful summary of the work is given here. Enzymes are much more easy to handle as antigens and Dr Arnon has led much of the work directed to using specific antisera to gain information about their structure and catalytic activity. This has proved a valuable adjunct to the very extensive studies of enzymes by protein chemists, X-ray crystallographers, enzymologists and all the others and, over an increasing range, has complemented and confirmed knowledge of these key molecules.

Immunoglobulins are the major subject of this book, about a quarter of which is given to their structure, and rather less space given in turn to the immunoglobulin allotypes, phylogeny of immunoglobulin and the chemistry and biology of immunoglobulin E. Dr Gall has given a good account of the chemical structure which is now well understood, but it is unfortunate that the article was written just too early to include the recent work of the X-ray crystallographers which is clarifying the nature of the combining site and other topics which he discusses. Allotypes have long had a fascination for some of us, particularly the *a* locus allotypes of the rabbit which remain the odd man out in most of the work on these allelic variants of the immunoglobulins. These investigations have contributed greatly to knowledge of their genetic origin and the conviction remains that when their behaviour is fully understood it will give a much clearer understanding of the source of the multiplicity of form of immunoglobulins. This chapter by Dr Rose Mage and colleagues has got all the facts and some of the speculation well presented. Dr Ishizaka has given perhaps the most personal account in his chapter on IgE, the minor immunoglobulin class responsible for immediate type hypersensitivity and apparently of major importance in the immune defence of the host against a variety of parasites. Of obvious clinical importance, this is a field where the myeloma proteins, for a long time the only source of large amounts of homogeneous immunoglobulin, have been of exceptional importance.

A general chapter on protein evolution by Dr Norman Arnheim serves as an introduction to the phylogeny of

immunoglobulins by Drs Kubo, Zimmerman and Grey. This is another way to try and understand the genetic origin of the complexity of immunoglobulin structure and, although the contributions from this approach have not so far been of major importance, it is well worthwhile from this point of view as well as for its intrinsic interest in biology. The transition from invertebrates to vertebrates seems to have been the most important point in the development of a specific immune response and much of the work described centres on the immunoglobulins of the lower vertebrates. No evidence has, however, been found so far for the original gene product of 11-12,000 molecular weight from which the immunoglobulins of present vertebrates are believed to have been derived by gene duplication.

The chapters are reasonably balanced in content, but their arrangement seems arbitrary. Together with the second volume a fair coverage is given of the more biochemical aspects of immunology, though there are surprising omissions such as the absence of any discussion of immunoglobulin biosynthesis. Perhaps with the title of 'The Antigens' this is not surprising, but with more than half the space given to immunoglobulins, biosynthesis would seem a stronger candidate for inclusion than, say, the individual classes of immunoglobulin. The editor's difficulties are apparent and it is perhaps questionable whether these multivolume works are the most effective way of summarising present information. The alternative of an advanced textbook giving a briefer but more integrated survey and supplemented, for the enthusiast, by the annual volumes of reviews on specialised topics has much to commend it. There is no doubt room for both and this volume and its successor should be a useful source of reference for some time to come.

R. R. PORTER

Betas and muons

Beta Decay and Muon Capture. By Masato Morita. Pp. xi+361. (Benjamin: Reading, Massachusetts and London, March 1974.) \$19.50.

THIS is a curious book, though it will probably be very useful to a specialised audience. It has grown from a course of postgraduate lectures given at the University of Osaka. Judging by the

notation and the balance of the material, the course was written in the early sixties and has not changed its shape since then. The first five chapters, which occupy half of the main text, give an introduction to weak interactions. There is a great deal of historical detail on the Fermi and Gamow-Teller theories. Parity non-conservation is treated at length, as if it were a brand new phenomenon, before a long and circumstantial chapter on the V-A theory. The weak interactions of elementary particles are given a reasonable amount of space, but with an alarming vagueness. This is certainly not the place to read about the Cabibbo theory, for instance. Its results are mentioned, but after a page and a half of introduction the author announces that "we do not have space to introduce the SU3 computation". Surely he could introduce Clebsch-Gordan coefficients, by analogy with SU2, to justify the precise numerical predictions of the theory which he tabulates.

The second half of the main text is devoted to the chief business of the book, a review of beta decay and muon capture as tools for nuclear structure physics. The presentation is pedestrian, but the details of individual experiments are given at unusual length. Although most of the reference lists stop in about 1969, there is a chapter which deals with more recent developments, such as the investigation of second class currents in the transitions of mirror nuclei.

Because of the detail in the later chapters, and because of the patiently long-winded explanations of many technical points, this book should be very useful to experimental students of nuclear physics. But they should come to it after a simple course on the weak interactions of elementary particles, based for instance on some of the excellent reports published by CERN, Geneva (Cabibbo and Veltman: CERN 65-30; and J. S. Bell: CERN 72-4).

D. J. MILLER

Crust of the Earth

Structural Geomorphology. By J. Tricart. Translated by S. H. Beaver and E. Derbyshire. Pp. xiii+305. (Geographies for Advanced Study.) (Longman: London, April 1974.) £4.75.

PROFESSOR TRICART'S book, published in France in 1968, has been admirably translated by two geographers from the University of Keele. It is intended not only for students taking a first degree in geography but also for specialists in the "cognate disciplines" of geology, pedology, ecology and planning. The

book is built round the premise, stated on the first page of the introduction, that the surface features of the lithosphere have been moulded by the interaction of internal and external forces at work in the Earth's crust and the author recognises a close relationship between characteristic landforms and large-scale crustal structures. The first chapter deals with the distinctions between continents and oceans, the second with geosynclines and fold belts, the third with platform areas and the final chapter with faults and volcanoes. Stress is laid on the fact that landscapes evolve over long periods.

This scheme of treatment, together with Professor Tricart's excellent aphorism (page 22) that "nature herself is a unified whole" raises the hope that the book, by bringing geological and geographical thinking to bear on a subject involving both disciplines, may illuminate the whole field of geomorphology. Where details are concerned, the book does indeed provide many clear and well illustrated examples of the relationship between structure and landform.

Further reading, however, raises serious doubts about the geological treatment. The author's views of crustal evolution have much in common with those of Soviet geologists who discount the possibility that large-scale horizontal displacements of continents have taken place during the past few hundred million years. This view is seldom expressed today in English or American works and a reasoned exposition of it might have been welcome. But it is disconcerting to find no discussion of the evidence concerning seafloor spreading (the mid-Atlantic ridge is referred to only once, on page 36, as a structure "somewhat similar to an island arc") and even more so to find on page 45 the statement that "despite the precision of the techniques, no measurements yet made have shown the slightest signs of change in the relative positions of Europe and North America, which should have occurred if the two continents are drifting apart".

This statement, made without any qualification, is the more surprising in that classic palaeomagnetic studies by Runcorn, Tarling and others are cited in the bibliography. Errors of fact are not uncommon: for example, the Moho, though defined as the base of the crust on page 41, is referred to on the next page as a discontinuity within the crust: the *schistes lustrés* are described as a flysch facies. More important, perhaps, from a student's point of view is the lack of a stratigraphical table or any other data from which he can discover the relative ages of the geological systems mentioned, the absence of any account of landforms associated

with depositional processes, and the cursory treatment meted out to rift valleys. Since the geological aspect is fundamental to the author's approach, I feel that defects of this kind undermine the whole structure of the book.

J. WATSON

Excited molecules

Excited States. Vol. 1 Edited by Edward C. Lim. Pp. xii+347. (Academic: New York and London, January 1974.) \$24.50; £11.75.

INTEREST in the structure of electronically excited species in the gas phase has been growing apace in the last two or three decades. A discussion of the Faraday Society in 1963, on just that subject, was an undoubted success. Somewhat earlier (1955) had come a text by Laidler on *The Chemical Kinetics of Excited States*, and Reid's book (1957), *Excited States in Chemistry and Biology*, was a stimulating early work.

The present volume, it might be argued, is not one but six. Certainly, its six chapters differ considerably in subject matter. The first, by G. Wile Robinson, deals with radiationless transitions, whereas the remaining five deal with topics that involve emission or absorption of radiation. Robinson's chapter is much the most readable of the six. The second chapter, by M. A. El-Sayed, contains a comprehensive review of phosphorescence microwave double resonance spectroscopy (PMDR). It seems from this article that there will be a considerable increase in the number of abbreviations that infest the literature.

The third chapter, by Robin M. Hochstrasser and Paras N. Prasad, deals with optical spectra and relaxation in molecular solids. It outlines various aspects of the electronic spectra of solids (molecular crystals and mineral crystals) and contains detailed discussion on phonon interactions that have not previously been treated in detail. The fourth chapter, by Wolfgang Liplay, is an excellent account of the determination of dipole moments and polarisabilities of molecules in excited electronic states. The fifth chapter, by C. J. Selisker, O. S. Khalil and S. P. McGlynn, treats the luminescence of polar aromatic molecules; and the sixth, by A. J. Duben, L. Goodman and M. Koyanagi, deals with interstate interactions in aromatic aldehydes and ketones.

The book is very well produced. I found perhaps half-a-dozen printer's errors, none of them of great import. Further volumes are planned.

A. D. WALSH

Fact and language

Linguistics and Information Science. By Karen Sparck Jones and Martin Kay. Pp. xii+244. (Library and Information Science Series.) (Academic: New York and London, January 1974.) \$14.50; £6.80.

THIS book is a state-of-the-art survey on the use of linguistic theories and techniques in information retrieval. The emphasis is on automated methods; that is, on linguistic theories that have been (or could be) used to develop computational methods of analysing language, and on their use in automated information retrieval systems. The survey concentrates on the literature of 1965–1970.

The authors first take a bird's-eye view of activity in the separate fields of information retrieval and linguistics, and then consider the particular aspects of information retrieval which have an apparent linguistic content. The meat of the book comes in two chapters on syntax and semantics respectively, where a detailed analysis of the present state of linguistic theory is followed by a discussion of how this theory has been, or might be, applied to information retrieval. There is a further section on fact retrieval.

The authors lean fairly heavily on an analysis of the retrieval process which identifies three stages: informal interpretation of the document, formal representation of this interpretation, and manipulation of the representation in searching. One can imagine other views of the process (indeed, they mention another)—not necessarily better ones, but ones which emphasise other aspects. In some instances the authors are restricted by their particular approach. For example, they point out quite rightly that most comparative experiments that have been carried out with index descriptions using syntax seem to show that this syntax is not particularly useful in retrieval. But they do not consider one possibility: that the fact that the indexer (man or machine) has to formulate the description in a syntactically coherent form may influence his choice of terms, and hence may affect retrieval performance even if the terms are searched in a postcoordinate manner, ignoring syntax.

But this argument serves to reinforce one of the main conclusions of the book: that there is a lack of suitable theories of information retrieval which would indicate where linguistic theories might be of use. Overall, the conclusions are somewhat negative: both fields have to develop further before the one is likely to be a major contributor to the other. Indeed, there is some doubt that this will ever happen; but the authors argue

persuasively that there are still plenty of possibilities.

The book performs its function as a survey very well, with a surprisingly good international coverage. It is coherent and well thought out, and also well written. The production is decidedly cheap-looking (yellow paper, a number of misprints, badly aligned type and uneven type density). But in content the book is a valuable contribution to the literature, which should help to bring order to a somewhat fragmented field. S. E. ROBERTSON

Why plans go awry

Planning and Budgeting in Poor Countries. By Naomi Caiden and Aaron Wildavsky. Pp. xvii+371. (Comparative Studies in Behavioural Science.) (Wiley: New York and London, March 1974.) £8.40.

A MAIN theme of this book had been anticipated by Bert Brecht in the *Threepenny Opera*. Freely translated, the song goes something like this:

Well, go and make a plan!

Seek where matrices lurk

Then go and make a second plan,

Neither of them will work.

Or, in the words of the Nepalese planner, summarising the experience of his country: "Try one form of organization; no result. Try something else; no result. The result is always the same" (Page 216).

The two authors of this book are political scientists who have covered more than 80 poor countries with questionnaires and interviews in order to examine government planning and budgeting and to recommend reforms. Their work reflects the disenchantment with planning.

Some of the criticisms of plans are ambitious, abstract, irrelevant and unrealistic miss the point. The purpose of formal models is to trace the indirect effects of alternative policies. Things often turn out differently in the second and third round from the intentions of the first. First-round socialism may turn into third-round monopoly capitalism. Particularly but not only in poor countries good causes tend to be turned into rackets. The reason why planning has failed, where it has failed, is not that it uses abstract models and traces interdependence between variables, but that it has sometimes been pseudo-planning, confined to a ceremonial superstructure, without being geared to where the action is.

The authors argue for the central importance of the annual budget. Though it is obviously true that no plan can be implemented unless it is integrated into the annual budget, there is a danger of mistaking a nec-

essary, though minor, condition for the strategic one. Budgets are, at best, annual public expenditure plans. A focus on fiscal magnitudes, though essential for proper public accounting, obscures and evades the real activities. Links between fiscal (or even financial) expenditures and results are tenuous, especially in underdeveloped countries. There are no fixed coefficients between money expenditure and land reform, population policy, incomes policy, education, public health, nutrition. Foreign exchange budgeting, manpower budgeting, raw material budgeting are just as important as fiscal budgeting and even they do not exhaust the range of necessary policies. Proper public accounting is necessary to ensure, negatively, that public money is not spent extravagantly or corruptly, but it cannot ensure, positively, that it is spent according to social priorities and that the necessary complementary actions are taken. Budgeting is to planning what bookkeeping is to business management: without it, management is impossible; but with the best book-keeper in the world, a firm can go bankrupt. These points are not enough stressed by the authors, who see the plan essentially as a many-year public capital budget.

Much is made of the need for redundancy. In a piece of exquisite jargon, the authors write: "Broadly speaking, we can regard social poverty as a lack of functional redundancy" (Page 49). But there is a vast difference between reserves (which serve a purpose) and redundancies. A more analytical and quantitative approach would have made the distinction clear. It is now well known that in poor societies, not only unskilled labour, but also capital and technically trained professional manpower are redundant: but, alas, they are not reserves.

The authors make a large number of entirely fair and commonsensical criticisms of planning. "If we were asked to design a mechanism for decisions to maximise every known disability and minimise any possible advantage of poor countries, we could hardly do better than comprehensive, multi-sectoral planning" (Page 293). The need for unavailable information, for political stability, for consistent aims are cited as unattainable conditions. But perhaps the most serious criticism of planning is omitted, viz. that its very success, measured by coherence and consistency, becomes an obstacle to adaptation and innovation. Plans introduce an additional rigidity into societies already inflexible. Plans, for this reason, in spite of their declared intentions, are elements strengthening conservatism.

The conclusion is not, however, reliance on *laissez-faire* and the free play

of market forces. The authors rightly point to the need for a combination of contingency planning, continuous budgeting and rolling planning, so that there can be adequate and speedy responses and adaptations to unforeseen events, both favourable and unfavourable.

The authors treat planners and planning as part of the social and political environment which they are supposed to plan. Planning the planners is not an invitation to an infinite regress but a reminder that there must be continuous mutual adaptation between plan objectives and social constraints.

PAUL STREETEN

Steroid receptors

Steroid-Cell Interactions. By R. J. B. King and W. I. P. Mainwaring. Pp. 440. (Butterworth: London, February 1974.) £10.

JENSEN and Jacobsen showed in 1962 that tissues such as the uterus and vagina, which are responsive to oestrogens, will retain administered oestradiol to a greater extent than non-responsive tissues and that this retention was due to the presence in the responsive tissues of specific receptor proteins. Since then the receptor hypothesis has been extended to many other hormones and the interaction between hormone and receptor is considered to be one of the links in the chain of events by which the hormone exerts its biological action. This concept has thus become of interest to molecular biologists as well as endocrinologists. The increased interest in this topic over the past five to six years has led to its discussion in numerous reviews and symposia; so what are the advantages of reading this monograph over consulting the reviews? Undoubtedly in the chapters reviewing the interaction of the various steroid hormones with the cell receptors there is more detail than found in most of the reviews. By far the main advantage, however, is the account of both the theoretical and practical background to the topic contained in the first two chapters on "Physicochemical Considerations of Steroid-Receptor Interactions" and "Methods Used to Study Steroid-Tissue Receptor Interactions" and the chapter giving a readable account of the molecular biological aspects of steroid-receptor interaction.

The title is slightly misleading since the monograph is mainly concerned with interactions involving receptors. Both androgens and oestrogens affect tissues or metabolic processes in which receptors have not yet been shown to be involved and this might suggest that there are other kinds of steroid-cell interactions. Many examples are

quoted by the authors. Androgens are anabolic but androgen receptors in skeletal muscle are difficult to identify nor has 5 α -reductase activity been demonstrated in the muscle of many species. Androgens stimulate both RNA and protein synthesis in liver but androgen receptors have not been identified in this tissue although it seems to contain oestrogen receptors. Normal breast tissue, which is influenced by oestrogens, accumulates oestradiol but does not seem to contain oestrogen receptors whereas breast tumours do. Little attention is paid to the interaction between the hormones in the tissues (uterus for example seems to contain an androgen receptor in addition to oestrogen and progesterone receptors), nor is the effect of other modifying influences on the hormones considered. This might be particularly important regarding events in the pituitary and brain. Conversion of testosterone to dihydrotestosterone seems not to be necessary for all biological activities of testosterone. Indeed some biological effects of testosterone seem to be produced by its conversion to oestrogen whereas dihydrotestosterone is not aromatised in this way. Until more knowledge is available it might be better to draw a distinction between androgen-sensitive tissues and androgen-dependent ones.

The authors point out the remarkable similarity of the different receptor proteins from different tissues. These similarities are deduced from current physicochemical data and it will be of interest to see whether they are upheld on further examination. Evidence that hormone binding correlates with hormone activity is reviewed. In respect of oestrogens and progesterone the evidence is quite good even though knowledge is limited. For androgens the situation is complicated by the extensive metabolism undergone by testosterone in the target organs and the possibility that the various metabolites may have different biological activities.

The monograph is well arranged and the newcomer to the field will have no difficulty in finding what he wants. In an attempt to bridge the gap between the writing of the book and its publication a summary of current literature is included. Whereas some of the chapters contain general summaries which are useful, others do not. I feel that the brief chapter on clinical and immunological aspects of steroid binding is superfluous to the main theme of the book. It will be a handy reference book to all investigators in this field and because of the amount of time it will save, it should be greatly appreciated by research students starting work in the area.

K. FOTHERBY

Spoonful of saccharin

Sensory Processes: The New Psychophysics. By Lawrence E. Marks. Pp. x+334. (Academic: New York and London, February 1974.) \$17.50; £8.25.

PEOPLE who use saccharin to sweeten their tea may have noticed a surprising thing: halving the concentration of sugar in a solution reduces its sweetness far more than halving an equally sweet (although much weaker) concentration of saccharin. This is one of many examples that demonstrate differing relationships between sensory magnitude and physical stimulus. How does one measure sweetness, brightness, pitch, odour? It has been shown, particularly in the pioneering work of the late S. S. Stevens, that asking subjects to assign numbers to sensation strength leads to a power function with an exponent dependent upon the stimulus and sensation considered. Lawrence Marks draws a clear distinction between this, the "new" psychophysics, and sensory physics, the "old" psychophysics, in which the observer is simply a detector of threshold, masked threshold, or null point, with measured quantities all in the physical domain. He describes and attempts to interrelate the various psychophysical procedures such as fractionation, category rating, and magnitude estimation and discusses the influences of extraneous factors. The senses are each considered under the headings of sensitivity, temporal and spatial factors, and qualitative aspects. Although one detects a certain antipathy towards the "old" psychophysics it is a carefully reasoned and comprehensive account and shows great concern for validation of the approach. Unfortunately his rather detached attitude coupled with the large number of references makes difficult reading in parts and a certain amount of repetition is inherent in the organisation he has adopted.

Although the new psychophysics gives insights into sensory processes not obtainable in any other way the field has a certain contrived air to it. We do not generally use our senses, or numbers, in this way. We normally use our sensory systems to perceive objects and relationships in the outside world. The idea that perceptions are built up from elementary sensations has been superseded by the concept of perception as an active, generative, process. We frequently see more than our sense organs convey because of past experience and expectations which can be triggered by a few salient features of sensory data. In this wider, more complex field of perception, detection could be of greater importance than sensory magnitude.

J. P. WILSON

Bouncing molecules

Chemical Applications of Molecular Beam Scattering. By M. A. Fluendy and K. P. Lawley. Pp. xi+400. (Studies in Chemical Physics.) (Chapman and Hall: London; distributed in the USA by Halsted Press, 1973.) £8.

THIS book is to be welcomed as the first non-specialist account of a challenging new field of chemical physics: the direct investigation of binary collision processes by the molecular beam technique. The authors discuss the design, performance and interpretation of the experiments, and also give an outline discussion of the background theory. The coverage reflects the present development of the field with major emphasis on elastic scattering and reactive processes.

The strongest chapters are those which deal with the technical design of the apparatus and with the analysis of elastic scattering experiments. Of these the sections of molecular beam sources, energy and state selection and detection and measurement will be of interest to a wide range of experimentalists. Readers with a more theoretical bias will be attracted by the clear account of quantal interference phenomena, seen not as abstract effects, but as sources of additional information in establishing the relation between the elastic scattering cross-sections and the intermolecular force field. The discussion of reactive scattering is equally complete in relation to present understanding, but the intrusion of mathematical detail makes it less easy to follow a clearly defined line of argument. Nevertheless there are few such comprehensive introductions in the literature. I only wish that the bibliography, which covers the important experimental work published before May 1972, could have been extended to include more recent studies on non-alkali atom systems, and that the tables could have contained a fuller resumé of available experimental information.

The level is that of the graduate student, since few undergraduates will have the necessary mathematical background. As such the book will be a welcome addition to the shelves of every serious chemical physics library.

M. S. CHILD

A cool look at doom

Man's Responsibility for Nature: Ecological Problems and Western Traditions. By John Passmore. Pp. x+213. (Duckworth: London, April 1974.) £5.95 cloth; £1.95 paper.

THE so-called 'environmental crisis' has been treated by scientists, economists and theologians, at levels ranging from

clinical objectivity to crude polemics; and it has been exploited by journalists and television producers. It is now the turn of philosophers; Professor Passmore's book is doubtless the first of a shelf-load of philosophical reflections on doom. It sets a very high standard in erudition, accuracy and clarity. The book opens with a masterly summary of the history of man's attitude to nature. Passmore is slightly apologetic about including this; he assures any anti-historical readers that they can, if they wish, skip it and go straight on to the second part of the book, dealing with ecological problems. In fact the historical essay is the best part of the book, and the only part with anything new to say. It describes vividly the Judaeo-Christian attitude to man, which divides him from other living things and makes him unique by investing him with a soul. This anthropocentric tradition has been blamed for man's exploitation of nature and it has prompted some writers to suggest that Western societies will not show proper concern for the environment unless they adopt a new ethic or a new religion, perhaps borrowed from the East.

Passmore argues powerfully and persuasively against this attitude, and especially against the implication that a scientific and rational attitude to nature cannot be reconciled with concern for the environment. The business of science, he says, is to turn mysteries into problems; only then can one set about trying to solve them. It was Descartes and Bacon, not the author of *Genesis*, who propagated the view that man can do as he pleases to nature with impunity. But there is no guidance to be found in the views of some contemporary theologians either; Passmore rejects Bishop Montefiore's idea of stewardship and Teilhard de Chardin's idea of cooperation with nature. He has no use for the arcadian musings of *Blueprint for Survival*. He realises that intellectuals like himself would be the first people to suffer if society were to discard some of the conveniences of modern technological society, such as air travel and motor cars.

From his historical summary Passmore turns to the contemporary ecological problems, which he distinguishes from "problems in ecology": the latter are scientific; the former are social. He covers familiar ground, reminding his readers (though it is superfluous for anyone likely to read this book) that ecological problems have three components: scientific, economic and ethical; and he brings the reader to the familiar conclusion: that the solution to ecological problems must be politically feasible. It is at this point that

the reflections of a philosopher might be expected to illuminate the issues. Unfortunately (but not surprisingly) Passmore's lucid analysis, like that of so many other writers, comes to a stop. We know that some ecological problems (such as pollution) can be solved within the framework of Western democratic institutions. We suspect that others (such as the dedication to ever-increasing material consumption) cannot. Before political decisions can be made an ethical question has to be answered: What duty, if any, do we have to posterity? If we cannot make sacrifices to ameliorate the human condition in the third world, which we have seen, is it likely that we shall make sacrifices to ameliorate the human condition of our great-grandchildren, whom most of us will never see?

Passmore seems to have a vague and tentative belief that this ethical question is soluble by rational thought in the framework of Western democratic institutions; perhaps by acting with concern for our offspring and expecting that they will act with equal concern for their offspring. It is a very modest conclusion to a very sophisticated essay. But Passmore is right to be modest. Heroics and utopias are the opiate of environmentalists. We can hope to proceed only through what William Blake called "Minute Particulars".

E. ASHBY

Defects in oxides

Oxide Semiconductors. By Z. M. Jarzebski. Translated from the Polish by B. Grzybowski-Swierkosz. Translation edited by Brian Randall Pamplin. Pp. xi+285. (Pergamon: Oxford and New York; Wydawnictwa Naukowo-Techniczne; Warsaw, January 1974.) £6.

THIS is a translation of a treatise by a member of the Warsaw Research Centre for crystals and its title is misleadingly broad. It is written by an oxide physicist for oxide physicists and concentrates on the transport of carriers in these materials as influenced by defects. It certainly does not attempt to teach, although an early chapter, on preparation techniques for oxides, provides a useful survey of one aspect of oxide technology. The main interest of the author is the effect on the conductivity of oxides produced by heating them at various pressures of oxygen; the last half of the book is a survey of such effects in about ten metal oxides. The data on carrier transport are exhaustively reviewed but the physical mechanisms involved in this transport are referred to in a rather passing manner and certainly not treated at any depth.

The physics of the structure of defects in the oxides is avoided in a

manner which might almost be called studied, as if the author held that it was quite enough to note the entrance and exit of an electron or hole from the oxide without worrying very much about what happened to it while in this medium, except to note an activation energy for the process. This extends even to the chapters on zinc oxide, aluminium oxide and silicon dioxide, for which, in fact, a rich literature on defect structure exists. Other subjects which one would expect to be treated in a book of this title are carrier trapping (no mention at all); band and hopping conduction models (glancing references only); the role of the d orbitals in defect and band structure in the transition metals (orbitals mentioned only occasionally in passing); and the applications of oxide semiconductors (not even a bibliographical reference given). A subject which is treated well in the explanatory chapters is the law of mass action applied to interactions between oxide and oxygen at high temperatures, in which defects are treated as an active participant in the chemical reaction.

There seems somehow to be a gulf between the kind of defect science described here and the world of 'frozen-in' defects typical of radiation damage and mechanical deformation; the latter science uses a wide range of spectroscopic and scattering measurements to build structural and wave-mechanical pictures of defects, while the former relies largely on measurements of the transport of electrons or atoms in the solid. This book, while it can be read without difficulty, perhaps even with profit, by one of the 'frozen-in' community, does not bridge that gulf. Moreover, those who wish to use this book as a quick summary of the field should note that the references do not, except for a few cases, extend past 1970.

A. G. HOLMES-SIEDLE

The chemical industry

The Chemical Economy: A Guide to the Technology and Economics of the Chemical Industry. By B. G. Reuben and M. L. Burstall. Pp. xix+530. (Longman: London, February 1974.) £6.95.

THAT chemistry courses must become more relevant has been the anguished cry of students and a source of frustration for many teachers of that subject for the past decade. Drs Burstall and Reuben have demonstrated what sort of effort is required if a beginning is to be made. They have written a long book crammed full of information directly related to their title. The book is well organised and the data in it are clearly presented so that prospective

readers are guided step by step through the complexities of chemical technology without undue fatigue. The primary objective of the book is to answer the question "How do we put chemistry to work for us?". As a second objective they intend to do something, in the concrete, to improve the spirit of university-industry cooperation. By providing such a lively and contemporary account of the ways in which chemical technology is used by the industry, the authors have achieved both of them. The book is aimed at an audience of first year university students having an A-level qualification in chemistry but no previous training in economics is presupposed.

This work is not an introductory course in chemistry juxtaposed with an elementary introduction to economics. Quite the contrary. The greater part of the book discusses a wide range of chemical reactions and processes but sight is never lost of the socioeconomic setting in which they function. By describing petroleum, natural gas, heavy organic chemicals, polymers, soaps and detergents, dyes and pigments, pharmaceuticals and heavy chemicals in relation to the firms, predominantly those in the United Kingdom, which utilise them, one is led, albeit in a piecemeal manner, to build up a picture of the chemical industry. A further section attempts to set the UK chemical industry into the world-wide complex of chemical industries. A disappointing feature of the presentation—though it would be difficult to remedy—concerns the historical description of the factors that have led up to the application of a particular process. These descriptions tend to be rather too brief and, consequently, may give the impression that chemical technology develops largely from discoveries in chemistry which appear, somewhat fortuitously, a little while before they are actually needed. None the less for students, who presumably know very little about this sector of industrial activity, it is perhaps a reasonable compromise to ensure that at least a little history is included with the technical and economic factors in the description of the industry.

The book also explores, in a descriptive manner, the economics of the firm. The aspects of microeconomics which are discussed include the decision of management to invest money in a new process and a very interesting section entitled "How do you know you are making money?". In a further chapter the authors discuss some of the problems of scaling up a laboratory experiment to industrial production. This leads to a discussion of the problems of disposing of the wastes from these processes and thence to some of the problems associated with pollution by in-

dustrial effluents.

The authors have produced a book which is doubly useful. It will certainly be useful for students of the growing number of broad-based science courses who are demanding that their curricula be relevant both to the society they live in and the sorts of jobs they may expect. For them it will provide more than a set of introductory materials because the authors have so organised the book that by following up the many references and footnotes, the student will be able to approach more closely the core of many contemporary technical and social problems. Also for the academic who is being asked to prepare more socially relevant course material this book should provide a useful guideline.

MICHAEL GIBBONS

Computer techniques

Operating Systems. By D. C. Tsichritzis and Philip A. Bernstein. Pp. xviii+298. (Computer Science and Applied Mathematics: a Series of Monographs and Textbooks.) (Academic: New York and London, March 1974.) \$13.50; £6.36.

OPERATING systems research seems to have become a popular line with computer scientists, perhaps from a desire to put their own house in order before dealing with more general techniques. Most books about it fall into a middle range, assuming basic knowledge and providing abstracts of the more subtle techniques described in journals.

The authors of this book are clearly competent in their subject, so it is unfortunate that the book is not more readable. The volume is best described as loosely written, and the authors themselves felt compelled to apologise (page 121) for the incoherence of the first five chapters. In particular, the frequent cross references embedded in the text are particularly difficult to follow. Footnotes would have been much easier to scan, and would have helped maintain a continuity within the main text. The entire work carries an air of editorial neglect, and falls into the trap of collaborative work, repetition. Although the book starts at a low level, it would be very difficult for a student to follow.

Apart from the problems at the end of each chapter, and the admirable bibliography, the most worthwhile chapters cover design and implementation principles, dealing with problems of project management plus a collection of the authors' solutions. The book could be worth buying for these chapters alone; in fact the width of scope leaves me wondering why they did not publish three well organised volumes, instead of trying to cram the entire subject into 300 pages.

JEFFREY GRIBBIN

"It's like this . . ."

An exhibition of 500 years of pictures for science at Nottingham Castle, until September 15th.

Not so much an exhibition of pictures 'for science' as an exhibition of illustrations designed to make apparent that which is not immediately so, and with a rather heavy emphasis on anatomy and mechanics at the expense of 'pure' science notions. The devices which illustrators have employed in order to reveal the unseen realities of man's world, range from the simple stripping of skin from muscle and skeleton, to the cross-sectional representation beloved of Victorian steam engineers, on to the scanning electron microscope, whose shaded and textured images have so captured the lay reader's curiosity as to merit page spreads in the Sunday supplements on purely aesthetic grounds. Alas, no 200,000 times magnified study of the human sweat gland in sight here, but the bristle of a caterpillar does service to amaze the casual visitor.

The aim of the exhibition is not so much to illustrate the history of science as to make familiar some of the more visually striking works done for it. According to the organisers the visual interest of the pictures is firstly in the information which they were made to convey, but in the best of them this is raised to a 'celebration of understanding'—a rather attenuated concept to keep in mind when browsing over Robinson's engine working gear or Davis's Fire Escape Machine, regardless of the sturdy commonsense that these undertakings embodied. The impression one gathers from the narrative explaining the Fire Escape Machine is rather one of a mind awakening from slumber, and peering unsteadily at the ordering of things, than of a *bona fide* celebration of understanding.

"On the alarm of fire," John Davis remarked, circa 1810, "I would have the machine brought out directly, as I consider it an improvident method when a house has been on fire some time to have to search about for the keys of the church yard or some other obscure place, to bring the fire ladders . . ."

The celebratory aspect of the science illustrator's work is most plainly evident in the anatomical woodcuts and engravings of the centuries preceding the invention of photography. From the mediaeval autopsy studies of Mondino de' Luzzi, who dissected corpses in cases of suspected foul play, to Joseph Maclise's plates from the Victorian *Surgical Anatomy*, there is a distinct sense that apart from providing maps and tools for surgeons, the artists were revelling in the insights which their probing of cadavers had revealed. The

unravelling arrangement by Govaert Bidloo and Gerard de Lairese of the ligaments whose contractions control the movement of the human hand, for example, is presented with an objectivity which might equally have been applied to a study of a marionette's limb. And thereby the point is made: that the workings of human machinery are as much a function of mechanical and chemical laws as that of everything else in the universe—not an entirely uncontroversial proposition half way through the seventeenth century.

Apart from the insights which raise many of these studies to the level of fine art, one is aware of a scrupulous technical achievement in their execution and their conventions. The study on this week's cover, by Albinus and Wandelaar, is one of six plates derived from the *Tabulae Skeleti et Musculorum Corporis Humani* of 1747, a brilliantly engraved series, employing devastatingly complex perspective techniques devised by a professor of physics to ensure accuracy and uniformity. Albinus' own account of the process offers an understanding of the niceties involved:

"Not a single figure" wrote Albinus, "has been drawn free hand. All have been measured and brought down to scale, either from an indeterminate distance, as the architects do, a method which has been followed in most cases, or from a distance of 40 feet through diopeters which correct to an indeterminate distance in such cases as, for instance, the pictures of skeletons, upon which finally, as upon a ground plan, the muscles have been drawn in. The tiny bones of the ear the artist measured with a very small and perfect compass." A system of nets in front of the subject allowed the artist to relate details to the whole in scale when he approached from his 40 feet vantage point to draw details. Most important of all, he drew nothing "he had not first thoroughly understood".

The portion dealing with modern science pictures, called 'Images of the Invisible', loses a little through familiarity: everybody knows from Arthur Mee's encyclopaedia what the drop of milk looks like when splashing into a saucer, and the bullet when hitting the shutter trip-wire. And to a lesser extent the zebra striped schlieren photographs of pressure waves caused by a body moving in a density stratified fluid, and field ion micrographs have lost their power to surprise anybody but the most unobservant man on the Clapham omnibus. The best loved exhibits in the show are camera obscuras which afford views of the city, a reflex camera obscura, which affords one the opportunity of drawing one's travelling companion, and a machine which affords sound the change to be seen by using its varying frequencies to agitate the

surface of a pool of oil. All in all, a worthwhile undertaking which succeeds on its own modest terms.

JOHN HALL

Verdict on DDT

DDT has been accused of killing birds and fish, causing cancer, poisoning babies at the breast and disrupting the ecosystem of the oceans. Tuesday's Documentary, "The Rise and Fall of DDT," found the insecticide guilty on the first charge but innocent of all others except the last where a verdict of 'not proven' was reached. In mitigation it was shown beyond all reasonable doubt that DDT has saved the lives of millions of people throughout the world who would otherwise have died from malaria, typhus and other insect-borne diseases. With commendable objectivity the programme did not recommend a sentence.

The case of dichlorodiphenyltrichloroethane (DDT) is certainly a curious one and the producers of the documentary exploited this to their advantage. Although it is now a symbol of all things wrong with intensive farming, DDT was shown to begin life as a revolutionary breakthrough in pest control.

Old newsreel film skillfully illustrated DDT's first really big success in the Second World War. Shortly after the liberation of Naples, it was used there to forestall an epidemic of typhus—the first time that this had been done on a large scale. DDT was also shown to have played a vital part in the campaign against the Japanese. But the recipe for DDT is very simple and soon after the war it was being produced in bulk by various companies. This led to indiscriminate use; indeed DDT became so popular that it was made into an American cocktail—a 'Micky Slim'.

The documentary traced the beginnings of DDT's downfall to 1949 and the use of a similar compound, DDD, against mosquitos in Clear Lake, California. By 1954 the population of a rare grebe on the lake had dropped alarmingly and DDD was blamed. Studies on DDT in other environments followed and lawsuits naming the chemical began to be won. The change in public opinion was so great that even when President Nixon's environmental Protection Agency exonerated DDT, its findings were overruled and the insecticide banned from the United States.

Following this largely historical introduction, Tuesday's Documentary brought forward a long series of scientific witnesses to testify to the damage, or otherwise, that DDT inflicts on the environment. Throughout this argument, the programme success-

fully maintained its impartial attitude, described by one of its producers as "beleagured neutrality".

It is just as well it did for a good deal of the evidence is contradictory. DDT was said to circulate in the air and soil, ultimately contaminating the sea but Dr George Harvey of Woods Hole Oceanographic Institute, Massachusetts, described his failure to find it there. When DDT was seen to distend the livers of mice it was said to lower their tolerance to some chemicals like carbon tetrachloride but actually increase their resistance to others such as aflatoxin. Almost all the evidence in the programme pointed to the sublethal effect of DDT but there was little opposition to the charges that

it was responsible for deaths due to eggshell thinness in birds and increased mortality in fish eggs.

Out of 23 factories which once produced DDT in the United States only one, Montrose Chemicals, survives. Its workers are continuously exposed to a high level of DDT and if there is any truth in the charge that DDT is carcinogenic then it should be found here. But the programme showed that these workers actually have a lower incidence of cancer than normal. On the dangers to human beings perhaps the most pertinent comment came from Professor James Busvine of the London School of Hygiene and Tropical Medicine who felt that DDT has never been proved to cause the death of a single

person but has undoubtedly saved up to ten million lives.

Just before the end of the film the increasing resistance of many insects to DDT was briefly mentioned. Nature itself seems to be passing the final judgment on DDT as more and more pests become immune to its effects. As the future of many underdeveloped countries depends on effective pest control and the use of DDT and other insecticides is becoming increasingly unsatisfactory (even leading to an increase of the pest in some cases), insect resistance is surely important enough to warrant more than this cursory treatment in an otherwise informative and balanced production.

JOHN WILSON

Announcements

Appointments

Anthony Kelly has been appointed Vice-Chancellor of the **University of Surrey**.

A. J. Leadbetter has been appointed to the chair of physical chemistry at the **University of Exeter**.

M. A. Sleight has been appointed to the chair of biology at the **University of Bristol**.

International meetings

September 19–20, **551st Meeting of the Biochemical Society**, University of St Andrews (The Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

September 24, **The Use of Molten Salts in Surface Treatment**, Leatherhead (Dr A. J. B. Cutler, C.E.R.L., Kelvin Avenue, Leatherhead, Surrey, UK).

September 24–25, **552nd Meeting of the Biochemical Society**, University College, Galway (The Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

September 25–26, **Symposium on the Chemistry of Liquid Metals**, University of Leicester (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

October 3, **Symposium on Micro Organisms in Food**, University of Leeds (Mr A. J. Crowther, The Metal Box Co. Ltd., 24 Fennel Street, Manchester M4 3FH, UK).

October 6–9, **6th Lunteren Lectures on Molecular Genetics**, Netherlands (Dr W. P. M. Hoekstra, Department of Microbiology, State University of Utrecht, Padualaan 8, Utrecht, The Netherlands).

October 7–12, **22nd International Meeting of Transportations and Communications**, Genoa (Segretaria Generale I.I.C., Villa Piaggio, Via Pertinace, 16125 Genova, Italy).

October 10–12, **Genetic Engineering: Danger or Hope**, Davos (Rudolf Brun, Gottlieb Duttweiler Institute for Economic and Social Studies, CH-8803 Rüschlikon, Switzerland).

October 24, **Inaugural Meeting of the Hydrogeological Group of the Geological Society of London**, London (Dr J. D. Mather, Geological Society of London, Burlington House, London W1V 0JU).

Errata

In the article "Memory in enzyme membranes" by A. Naparstek *et al.* (*Nature*, **249**, 490; 1974) the legend to Fig. 1 should read . . . internal pH as a function of decreasing (a) and increasing (b), the external pH values.

In the article "Bioassay of a *Drosophila* pheromone influencing sexual selection" by J. E. Leonard, L. Ehrman and M. Schorsch (*Nature*, **250**, 261; 1974) the blocks for Figs 1 and 2 were reversed.

Reports and Publications

Great Britain

Science Research Council. The Work of the Rutherford Laboratory in 1973. Pp. 215 (Chilton, Didcot: Rutherford Laboratory, 1974). [246]
Standing Stones of Maeshowe of Stenness. By Magnus

Spence. Pp. 25. (London: Research into Lost Knowledge Organization, Mrs. Janette Jackson, 36 College Court, Queen Caroline Street, Hammersmith, 1974.) [246]

CIRCA—International Centre for Co-operation in Agricultural Research. Intensive Agriculture and the Environment. (North Western European Region Symposium, School of Agriculture, The University, Newcastle-upon-Tyne, 19–21st September 1973.) Pp. iv + 123. (Dublin: An Foras Taluntais, 1974.) £2. [266]

National Radiological Protection Board, NRPB-R17: The Determination of Plutonium in Urine by Ultrafiltration. By G. N. Stradling, D. S. Popplewell and G. J. Ham. Pp. 12. NRPB-R18: Measurement of Activity of Surfaces Contaminated by Electron-capture Nuclides. By W. J. Iles and D. F. White. Pp. 43. (Harwell, Didcot: National Radiological Protection Board, 1973 and 1974.) [266]

Oundle School Natural History Society Annual Report for 1973. Pp. 56. (Oundle, Peterborough: Oundle School Natural History Society, 1974.) [266]

Rothamsted Experimental Station. Report for 1973. Part 1: Pp. 412. Part 2: Pp. 276. (Lawes Agricultural Trust.) (Harpenden, Herts: Rothamsted Experimental Station, 1974.) £3 the two parts. [266]

Other countries

National Research Council of Canada, NRC Associate Committee on Scientific Criteria for Environmental Quality. Picloram: The Effects of Its Use as a Herbicide on Environmental Quality. (Subcommittee Report No. 1.) Pp. 128. (Ottawa: Publications, NRC, 1974.) [216]

National Institutes of Health, Bethesda, Md. Annual Report of International Activities, Fiscal Year 1973. Pp. vi + 100. (DHEW Publication No. (NIH) 74-374.) (Bethesda, Md.: National Institutes of Health, 1974. For sale by US Government Printing Office.) \$1.35. [216]

Journal of Cutaneous Pathology, Vol. 1 No. 1, February, 1974. Edited by Leopoldo F. Montes. Pp. 71. Subscriptions: Vol. 1 (6 issues) D.kr. 220.00 plus postage D.kr. 14.00 (US \$38.60, £15.40, DM109.50) subject to exchange rate fluctuation. (Copenhagen, Denmark: Munksgaard, 1974.) [216]

Smithsonian Contributions to Zoology, No. 154: *Ataenius*, *Aphotaenius*, and *Pseudataenius* of the United States and Canada (Coleoptera: Scarabaeidae: Aphodiinae). By Oscar L. Cartwright. Pp. iv + 106. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.85. [216]

Smithsonian Contributions to Zoology, No. 169: Studies of Neotropical Caddisflies, XVIII: New Species of Rhyacophilidae and Glossosomatidae (Trichoptera). By Oliver S. Flint, Jr. Pp. iv + 30. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 80 cents. [216]

Environmental Conservation, Vol. 1 No. 1, 1974. Edited by Nicholas Polunin. Pp. 80. Subscriptions: Vol. 1 (Quarterly) SFRs 120 (approx. US \$41) (Lausanne, Switzerland: The Foundation for Environmental Conservation, 1974.) [216]

Smithsonian Contributions to Paleobiology, No. 20: Ultrastructural Studies on Graptolites. 1: The Periderm and Its Derivatives in the Dendroidea and in *Mastigograptus*. By Adam Urbanek and Kenneth M. Towe. Pp. iii + 48. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.35. [216]

Smithsonian Contributions to Zoology, No. 171: Spider Mites from Northwestern and North Central Mexico (Acarina: Tetranychidae). By D. M. Tuttle, E. W. Baker and M. Abbatiello. Pp. 18. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 60 cents. [216]

Classified Advertisements

CONDITIONS. All advertisements will only be accepted on the condition that the advertiser warrants that the advertisements do not in any way contravene the provisions of the Trade Descriptions Act 1968 and the Race Relations Act 1968. The Publisher also reserves the right to refuse, amend, withdraw or otherwise deal with all advertisements submitted to him at his absolute discretion and without explanation. All advertisements must comply with the British Code of Advertising Practice.

The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes.

Semi-displayed £3.60 per 10 mm. Minimum £7.20, each additional 2 mm 72p. Full page £230.00. Half page across £125.00. 30p is charged for the re-direction of replies to advertisements with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-242 6264. Telegrams: Textualist, London, W.C.2.

APPOINTMENTS VACANT**UNIVERSITY COLLEGE GALWAY
JUNIOR LECTURESHIP IN BOTANY**

Applications are invited for the above post. Salary scale £2,478 by 99 (10) to £3,468 p.a. plus Family Allowances. Applications will be particularly welcome from candidates with a specialised knowledge of Irish vegetational history.

The closing date for receipt of applications is **September 12, 1974**. Prior to application, further information should be obtained from the Registrar of the College. (738)

**The Marie Curie
Memorial Foundation
THE CHART, OXTEAD RH8 0TL**

1. **RESEARCH ASSISTANT FOR THE METABOLIC UNIT** with a degree in Nutrition or Biochemistry and an interest in clinical research in cancer. Experience of drug metabolism would be an advantage.
2. **RESEARCH ASSISTANT FOR THE BIOLOGICAL CHEMISTRY UNIT** with Grad. R.I.C., or B.Sc. (Hons) in chemistry, with an interest in organic chemistry. Current projects involve the design, synthesis and evaluation of new anti-tumour agents and metabolism of anti-cancer drugs.
3. **RESEARCH ASSISTANT FOR THE IMMUNOCHEMISTRY UNIT** with a degree in biochemistry to investigate the role of acid hydrolases in tumour invasion and metastatic spread. Experience in immunochemical and biochemical analytical techniques would be an advantage.

Salaries are based on the Whitley Council Scale with superannuation. Successful applicants may register for a higher degree after an initial probationary period. Applications including the names of two referees and indicating the post sought should be sent to the Secretary at the above address. (802)

**UNIVERSITY OF GLASGOW
DENTAL HOSPITAL AND SCHOOL
RESEARCH ASSISTANTSHIP IN
DENTAL BIOCHEMISTRY**

Applications are invited for a **RESEARCH ASSISTANTSHIP IN DENTAL BIOCHEMISTRY**, tenable for a period of up to four years from October 1974.

Candidates should have an Honours Degree in Biochemistry, Microbiology or an allied subject. There will be an opportunity to apply to register for a higher degree.

The work will be mainly concerned with the synthesis of glucans by extracellular bacterial enzymes.

Salary scale £1,500 by £75 to £1,725. F.S.S.U. Applications, including curriculum vitae and names of two referees, should be sent to Dr J. A. Beceley, University of Glasgow Dental School, 378 Sauchiehall Street, Glasgow G2 3JZ, from whom further information may be obtained.
In reply please quote Ref. No. 3527M. (792)

THE UNIVERSITY OF ASTON IN BIRMINGHAM**Department of Pharmacy****RESEARCH IN MEDICINAL AND
PHARMACEUTICAL CHEMISTRY**

As part of the Department's expanding research activities, the following posts are available:

RESEARCH FELLOW IN HETEROCYCLIC CHEMISTRY
(Ref. 984/6)

The candidate will be expected to investigate the chemical and/or biological properties of novel Nitrogen Heterocyclic Systems of potential biological importance, and to undertake a limited amount of teaching at M.Sc. level in an area of Medicinal Chemistry.

The Fellowship is tenable for up to 2 years 9 months. The commencing salary will be within the range £2,118 to £2,412 per annum with effect from October 1, 1974. The post is superannuable.

RESEARCH ASSISTANT IN TOXICOLOGICAL ANALYSIS
(Ref. 985/6)

The successful candidate will work on problems concerned with the assessment of levels of environmental pollutants in placental tissues, and will join an interdisciplinary group from the University and the Departments of Human Genetics and Pathology at Birmingham Maternity Hospital. Preference will be given to candidates having some experience of atomic absorption spectrometry, polarography, gas chromatography, neutron activation analysis, or other appropriate instrumental technique.

The post is tenable for up to 3 years and the starting salary will, with effect from October 1, 1974, be within the range £1,395 to £1,659 per annum on a scale rising to £2,058 per annum.

Requests for further details of these two posts and application forms (which should be returned not later than September 6) should be sent, preferably on a postcard, quoting the reference number, to the Staff Officer, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET.

RESEARCH STUDENTSHIPS

Applications are invited from suitably qualified graduates to undertake research in the following areas:

Heterocyclic medicinal chemistry

Characterisation of biologically active compounds in coal-tar fractions

X-ray crystallographic and other instrumental methods for the analysis of biologically active materials

Grants are based on S.R.C. regulations and may be augmented by demonstrating duties. Candidates would be expected to register for Higher Degrees of the University.

Further details of these studentships may be obtained from Dr M. F. G. Stevens, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET. Returnable by September 6, 1974.

(844)

**CENTRAL PUBLIC HEALTH
LABORATORY**

Honours Science Graduate required by the Standards Laboratory for Serological reagents for work with viral diagnostic reagents.

Applications with full details of age, experience and qualifications to the Personnel Officer, Central Public Health Laboratory, Colindale Avenue, London, NW9 5HT. (749)

**THE GRASSLAND RESEARCH
INSTITUTE**

HURLEY, MAIDENHEAD, BERKSHIRE

A vacancy exists for a **SENIOR SCIENTIFIC OFFICER/PRINCIPAL SCIENTIFIC OFFICER** to study the principles underlying the utilisation of grass by grazing cattle, with emphasis on sward and animal factors influencing the productivity of dairy cows.

Minimum qualifications: 1st or 2nd class honours degree in an appropriate subject and relevant post-graduate experience.

Salary: Senior Scientific Officer £3,157 to £4,441 p.a.; Principal Scientific Officer £4,227 to £5,550 p.a. There is a non-contributory superannuation scheme.

Applications to the Secretary by September 14, 1974 with curriculum vitae and the names of three referees and quoting 6/C/8. (823)

**THE UNIVERSITY OF SHEFFIELD
W. E. S. TURNER CHAIR OF
GLASS TECHNOLOGY**

Applications are invited for the **W. E. S. TURNER CHAIR OF GLASS TECHNOLOGY** which will become vacant in September 1975 on the retirement of Professor R. W. Douglas. Salary in the range approved for professorial appointments with superannuation provision. Further particulars may be obtained from the Registrar and Secretary, The University, Sheffield S10 2TN to whom applications (one copy only) should be sent by September 21, 1974. Please quote reference R116/G. (808)

(RE-ADVERTISEMENT)**THE UNIVERSITY OF MANCHESTER
LECTURER IN ZOOLOGY**

Applications invited for this post, for which an interest in neurobiology would be an advantage. Salary scale p.a.: £2,118 to £4,896. F.S.S.U. Initial salary not above £2,580 p.a. Particulars and application forms (returnable by September 20) from the Registrar, The University, Manchester M13 9PL. Quote ref: 186/74/N. (813)

BOC

PHD POLYMER CHEMIST

Research Project

BOC are sponsoring a research project in polymer chemistry at the University of Aberdeen Department of Chemistry, Physical Chemistry Section to commence Autumn 1974. The project is concerned with the separation of gases by permeation through polymer membranes and hollow fibres.

BOC will employ the person appointed, initially on a 2-year secondment as Honorary Fellow to the University. Upon completion of this period career development alternatives might include continuation of this project in the employ of either BOC or the University, or other scientific posts within BOC or the University.

Candidates, aged between 24 and 28, should have recently gained (or be about to gain) a doctorate in Organic or Physical Chemistry, and be particularly interested in undertaking applied research for industry. Salary negotiable; first class pension scheme and terms of employment; removal expenses if appropriate.

Please apply to:

Dr D. W. Kirkley,
New Venture Secretariat,
British Oxygen Company Limited,
Hammersmith House,
London W6 9DX.

(812)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

Applications are invited from suitably qualified persons for the following posts:-

- LABORATORY TECHNICIAN—BIOCHEMISTRY**
Grade 4. £1,848 to £2,163 per annum.
To assist research involving chemical synthesis and evaluation of novel compounds and to assist with undergraduate practical classes. Ref: 274/T/NA.
- LABORATORY TECHNICIAN—BIOCHEMISTRY/MICROBIOLOGY**
Grade 4. £1,848 to £2,163 per annum.
To be responsible for technical support to medical and science undergraduate Biochemistry/Microbiology classes and provide some support for related research. Ref: 275/T/NA.
- LABORATORY TECHNICIAN—PHARMACOLOGY**
Grade 3. £1,650 to £1,920 or Grade 4. £1,848 to £2,163 per annum, according to qualifications and experience.
To provide technical support for Science undergraduate Pharmacology/Mammalian Physiology classes and research. Ref: 276/T/NA.
- PHOTOGRAPHIC/REPROGRAPHIC TECHNICIAN**
Grade 3. £1,650 to £1,920 or Grade 4. £1,848 to £2,163 per annum.
To provide a departmental photographic service, to produce slides, and to prepare diagrams, graphs and illustrations. Ref: 277/T/NA.
- LABORATORY TECHNICIAN—RADIOISOTOPIC SERVICES**
Grade 3. £1,650 to £1,920 per annum.
To be primarily responsible for radioisotopic facilities in the Analytical Services Unit. Experience of instrumentation and/or chemistry/biochemistry. Ref: 279/T/NA.
- ELECTRON MICROSCOPY TECHNICIAN**
Grade 2B. £1,524 to £1,794 per annum.
To join a unit providing Electron microscopy services. Some experience of E.M. work and/or histology. Ref: 281/T/NA.

Cost of living allowance currently £2.40 per week payable in addition to salary shown.

Posts 1-4: Related experience, an appropriate qualification (minimum O.N.C. or equivalent) and enthusiasm are required for these established appointments. Graduates in appropriate disciplines are welcome to apply for posts 1-3.

Posts 5-6: Minimum qualification for these posts O.N.C. or equivalent in appropriate subjects.

Applications giving details of age, qualifications and experience and the names of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH, quoting appropriate reference number. (730)

"METALS ABSTRACTS"

The international abstracting service for metallurgy offers permanent positions as SENIOR EDITORIAL ASSISTANTS. The work consists of editing, indexing, and checking abstracts for publication. A science degree, preferably in metallurgy, physics, or chemistry, is necessary and a working knowledge of a foreign language would be an advantage.

Applications, stating age, education, qualifications, and experience, to Dr T. Graff, Metals Abstracts, The Metals Society, 1 Carlton House Terrace, London SW1Y 5DB. (829)

HOUGHTON POULTRY RESEARCH STATION

HOUGHTON, HUNTINGDON, CAMBS.

ELECTRON MICROSCOPIST LEUKOSIS EXPERIMENTAL UNIT

Applications are invited from veterinary graduates with experience in electron microscopy, and preferably with a higher degree, for a research post with a group studying avian tumours and their causative viruses, with particular emphasis on Marek's disease, to obtain an understanding of pathology at the ultrastructural level and its relation to Marek's disease and other diseases of poultry. The successful candidate would be in charge of a small electron microscope unit. Some collaborative work with other departments would be required.

The appointment will be made in the V.R.O. (£2,616 by 7 to £3,312) or S.V.R.O. (£3,312 by 7 to £4,004) grades, salaries (currently under review) depending upon qualifications and experience. There is a non-contributory superannuation scheme.

Further particulars and application form from the Secretary to whom completed application form should be returned by September 20, 1974. (822)

UNIVERSITY OF SUSSEX SCHOOL OF BIOLOGICAL SCIENCES

Postdoctoral Research Assistants

required for project on:-

- The biochemical basis of plasma membrane modifications associated with cell transformation.** Experience with membrane enzymes, enzyme isolation and purification of glycolipid biochemistry would be an advantage. The project is supported by the Cancer Research Campaign, it is for one year in the first instance and is renewable. Salary will be on University Lecturer scale up to £3,400 a year according to experience and qualifications.
- The changes in the biochemistry of the cell nucleus induced by chemical carcinogens.** Experience with enzymes or with chromatin would be an advantage. The project is supported by the Cancer Research Campaign, it is for one year in the first instance and is renewable. Salary will be on University Lecturer scale up to £3,000 a year, according to experience and qualifications.

Applications for both posts with the names of three referees to Dr S. Shall, School of Biological Sciences, University of Sussex, Brighton BN1 9QG. (805)

QUEEN MARY COLLEGE
University of London
CHEMISTRY DEPARTMENT

Applications are invited for an S.R.C. supported Postdoctoral Research Assistant, tenable for 1 year from October 1, for work in collaboration with Drs F. A. Hart and G. P. Moss on Fundamental aspects of lanthanide n.m.r. shift reagents. Experience in preparative coordination chemistry and/or organic chemistry in addition to n.m.r. spectroscopic experience required. Salary within range £2,118 to £2,247 p.a. plus £213 London Allowance. Applications in writing (giving qualifications, previous scientific experience and names and addresses of two referees) to The Registrar (N), Queen Mary College, Mile End Road, London E1 4NS. (821)

UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES

Applications are invited for the post of DEMONSTRATOR. This post is tenable for up to three years. Candidates are expected to give assistance in the preparation and demonstrating of undergraduate practicals, and in tutorial work, and to carry out research in aspects of soil science related to a major departmental research programme on minimum cultivation methods.

Candidates should have a good Honours Degree, and preferably some research experience, with training or experience in soil science (physics or soil chemistry).

The salary is in the range of £1,290 to £1,971 (under review). Successful applicants may work for a higher degree during their tenure of the post. Application forms and further particulars from the Registrar, University of Leeds Leeds LS2 9JT (please quote reference 41/26/D). Closing date August 31, 1974. (806)

UNIVERSITY OF EXETER
DEPARTMENT OF BIOLOGICAL SCIENCES
POSTDOCTORAL RESEARCH
ASSISTANT

Applications are invited for a Postdoctoral Research Assistant to work with Dr H. Stebbings on "Cytoplasmic microtubules and their rôle in cellular and intracellular movements". The research will involve ultrastructural, biochemical and physiological studies on a number of microtubule systems, and preference will be given to applicants who have been working in this field. Previous experience of electron microscopy is essential.

The appointment is for two years from either October 1, 1974 or such date thereafter as may be mutually agreed, with an initial salary of £2,118 p.a. plus F.S.S.U.

Applications, together with a curriculum vitae giving details of age, qualifications and experience, and the names of two referees should be sent by September 13, 1974 to The Secretary of the University, Northcote House, The Queen's Drive, Exeter EX4 4QJ. Please quote reference 1/12/7081. (817)

university of wales
**university
college of
swansea**

Research Assistant

Applications are invited for the vacancy of Research Assistant in the Department of Botany and Microbiology. Applicants should have a degree in a biological subject, preferably in genetics and the successful applicant will work with Professor P. J. Syrett on genetical aspects of the control of nitrate assimilation in *Chlamydomonas*.

The appointment, which will be for one year in the first instance, will be £1,410 per annum.

Further particulars and application forms may be obtained from the Registrar/Secretary, University College of Swansea, Singleton Park, Swansea SA2 8PP to whom they should be returned by **Monday, September 9, 1974.** (814)

**NORTHAMPTONSHIRE
AREA HEALTH AUTHORITY**

Northampton General Hospital

Applications are invited for the post of

**Basic Grade
Biochemist**

(post probationary) in busy and well-equipped Clinical Chemistry Department. Previous experience in immuno-logy and radioimmunoassay desirable but not essential.

Applications with details of age, qualifications and experience, together with names and addresses of two referees to Dr G. P. Fraser (telephone Northampton 34700 Ext. 514) from whom further particulars may be obtained.

(810)

**DEPARTMENT OF
AGRICULTURE**

Applications are invited for a permanent and pensionable post in the Crop and Animal Husbandry Division.

The successful applicant will undertake work on pig production with special reference to increased efficiency and may be required to undertake teaching duties in the Faculty of Agriculture, The Queen's University, Belfast.

The appointment may be at Senior Scientific Officer, Higher Scientific Officer or Scientific Officer level.

S.S.O. Over 25 and under 32 years of age with a 1st or 2nd Class Honours Degree in Agriculture and at least 4 years post-graduate experience preferably in relation to pig production.

H.S.O. Under 30 years of age with an Honours Degree as above and at least 2 years relevant post-graduate experience.

S.O. Under 27 years of age with an Honours Degree as above.

Salary Scales: S.S.O. £3,157 to £4,441; H.S.O. £2,461 to £3,371; S.O. £1,592 to £2,675.

Grading and salary will be related to qualifications and experience and a cost of living supplement is also payable.

Please write or telephone for an application form and further information, quoting Ref. SB 227/74/N to Civil Service Commission, Clarendon House, Adelaide Street, Belfast BT2 8ND (telephone 0232-44300, ext. 26).

Completed forms must be returned to arrive not later than September 17, 1974.



**NORTHERN IRELAND
CIVIL SERVICE**

(828)

**The Lister Institute of Preventive Medicine
(University of London) Elstree, Herts**

requires a

BACTERIOLOGIST

to undertake investigative and developmental work on bacterial vaccines and therapeutic sera. This post is one of responsibility and offers plenty of scope for interesting and rewarding research; it is suitable for a worker with a doctoral degree or of comparable seniority. Salary in accordance with experience and qualifications. Superannuation under F.S.S.U.

Applications to:

**Secretary, Lister Institute of Preventive Medicine,
Elstree, Hertfordshire.**

(859)

**UNIVERSITY OF SINGAPORE
PHARMACOLOGY**

Applications are invited for teaching appointments in the Department of Pharmacology. Candidates should have an interest in teaching and research in Medical Pharmacology. Gross monthly emoluments in the range from S\$1,310 to S\$ 4,190 approx., the initial amount depending on the candidates's qualifications and experience and the level of appointment offered. The gross emoluments comprise basic salary and the National Wages Council wage allowances. In addition, the University pays a 13th month annual allowance of one month's basic salary in December of each year; and contributes to the staff member's provident fund at 15% of basic salary and allowances. Leave, medical, housing and other benefits are also available. Candidates should write to: The Registrar, University of Singapore, Singapore, giving curriculum vitae (bio-data), with full personal particulars, and also the names and addresses of three referees.

Exchange rate, approx. Stg. £1 = S\$5.85.

(835)

**UNIVERSITY OF BRISTOL
DEPARTMENT OF PATHOLOGY**

A vacancy exists for a RESEARCH TECHNICIAN GRADE 5, having H.N.C. or equivalent qualifications plus several years relevant experience, to take charge of an electron microscope unit within the Department. Experience in cell biology is desirable. The work will involve also the use of sophisticated electronic and optical equipment in connection with studies on brain damage, investigations on tissue culture and a general supervisory responsibility for the running of a small laboratory.

This 'funded' post, which is tenable from November 1, 1974 at the School of Veterinary Science, Langford, carries a salary related to U.T.S.S. scale Grade 5, £2,007 by £75 to £2,382 per annum. The present grant, which is renewable, runs to December 1977 initially.

Applications in writing, giving the names and addresses of two referees, to Professor I. A. Silver, Department of Pathology, Medical School, University Walk, Bristol BS8 1TD. (820)

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
DEPARTMENT OF BIOCHEMISTRY
AND CHEMISTRY**

Applications are invited for the post of RESEARCH TECHNICIAN to work on biological membranes and cell fusion in a project supported by the Medical Research Council. The post is for one year in the first instance, renewable to July 31, 1976. Initial salary, depending on age and experience, from £1,692 per annum plus £126 London Weighting allowance and Threshold Payments.

Please apply as soon as possible, giving a curriculum vitae and the names of two referees, to Professor J. A. Lucy, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. (816)

**UNIVERSITY OF EXETER
DEMONSTRATORSHIP IN
PHYSICAL CHEMISTRY**

Applications are invited for a Demonstratorship in Physical Chemistry, tenable from October 1, 1974 for two years. Applicants should have a Ph.D. degree or equivalent research experience.

Salary within the range £1,941 to £2,247 per annum with initial placement in accordance with qualifications and experience. The post is superannuable.

Further particulars may be obtained from: The Secretary of the University, Northcote House, The Queen's Drive, Exeter EX4 4QJ, to whom applications (six copies; overseas applicants one copy), together with the names of two referees, should be sent by September 15, 1974. Please quote Reference No. 1/12/3093 in all correspondence. (815)

BBC RADIO SCIENCE PRODUCER

to produce "Science Now", "Scientifically Speaking" and similar programmes.

Thorough knowledge of developments in science, scientific curiosity and editorial judgement essential. Science degree and journalistic experience desirable. Based Broadcasting House.

Salary £2,967 p.a. (may be higher if qualifications exceptional) rising by annual increments of £135/156 to £4,023 p.a. maximum, plus cost of living threshold payments currently £10.42 p.m. month and £195 p.a. non-day working allowance. Salary revision due October 1, 1974.

Write or telephone now for application form (enclosing addressed foolscap envelope and quoting reference 74.G.451N) to Appointments Department, BBC, London W1A 1AA. Tel. 01-580 4468 Ext. 4619. (824)

**NORFOLK AREA HEALTH
AUTHORITY**

**GRADUATE
AUDIOLOGIST**

Required to provide a service for the conduct of audiological and vestibular investigations for adults and children at the new Regional Audiology Centre which is being established at the Jenny Lind Hospital, and to undertake combined clinics with E.N.T. surgeons. Although based at the Regional Centre, the Audiologist will also visit other hospitals in the East Anglian region to undertake clinics.

Training of Physiological Measurement Technicians in Audiology for the Region will be his or her responsibility; in addition, the Audiologist will undertake or assist in research projects in Audiology and related fields.

Appropriate qualifications would be B.Sc. or equivalent in Biology, Physiology, Engineering or Mathematics; with a higher degree in Audiology.

The appointment will be on the Scientific Officer Grade, with one year's seniority, with promotion thereafter to Senior Scientific Officer. Commencing salary will depend on qualifications and experience; a graduate with a 1st or 2nd class Honours degree would receive a commencing salary of £1,875 p.a., and for a graduate with a 3rd class Honours degree the commencing salary would be £1,593 p.a.

A Job Description and application form is available from the Area Personnel Officer, Norfolk Area Health Authority, 102/104 Prince of Wales Road, Norwich. An informal visit to the Regional Audiology Centre can be arranged prior to interview.

Closing date for receipt of applications is September 11, 1974.

(811)

Immediate vacancy for a fully qualified

TECHNICIAN

for research work on rheumatoid arthritis utilising immunological and tissue-culture techniques. Previous experience preferable but not essential. Apply in writing, stating age and giving details of education, qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. D.M.3. (837)

**THE INSTITUTE
OF ORTHOPAEDICS**
(University of London)

Royal National Orthopaedic Hospital
234 Great Portland Street,
London W1N 6AD

Applications from graduates in either
medicine or science are invited for the
whole time post of

RESEARCH ASSISTANT

in the

Department of Morbid Anatomy

The appointment is for one year in the
first instance, and is renewable annually.
Salary on the appropriate scale for lecturer
(£3,135 to £4,041 Clinical; £2,118 to £3,462
Non-Medical) according to qualifications and
experience. Superannuation under F.S.S.U.

The post will provide opportunity for
research in the general field of bone structure
and bone pathology, under the supervision
of Prof. H. A. Sissons. For a medically-qualified
pathologist it will also provide training and
experience in orthopaedic pathology. Further
information can be obtained from Prof. H. A. Sissons.

Applications, together with the names of
two referees, should reach the Secretary, at
the above address, by September 30 1974.
(798)

CHEMICAL PATHOLOGIST

An opportunity exists for a Chemical Pathologist within
the Human Pharmacology Department at Brockham Park.
The successful applicant will be responsible for a small
group of technical staff conducting the clinical chemistry
and haematology services for this department.

This challenging new position will be of interest to an
applicant with 2/3 years post graduate experience in
Chemical Pathology.

A good starting salary will be offered and reviewed
regularly. Beecham Pharmaceuticals, Research Division is
part of the Beecham Group and is situated in a pleasant
area of Surrey; within easy reach of London.

The successful applicant will qualify for the Groups
generous bonus scheme and non-contributory pension and
life assurance scheme.

If you are interested in this
vacancy please contact the
Site Personnel Officer,
Beecham Pharmaceuticals,
Research Division, Brookham
Park, Betchworth, Surrey RH3 7AJ,
or ring Betchworth 3202 ext. 55
for an application form.

(861)

**UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES**

Applications are invited from graduates with
appropriate qualifications in agronomy for the
post of **RESEARCH OFFICER** in the Department
of Plant Sciences, Leeds University. The appointment
is for one year, with possible renewal for a
further two years, and is associated with a major
research project financed by the Agricultural Research
Council on direct drilling and reduced
cultivation. The person appointed will take charge
of field experiments and assist with the co-ordination
of the experimental requirements of a
small research group. Salary £1,929 by £129 to
£2,058 by £165 to £2,223 (at present under revision).
Application forms and further details from the
Registrar, University of Leeds LS2 9JT (please
quote reference number 4125).

Closing date for applications August 31, 1974.
(807)

**ROTHAMSTED EXPERIMENTAL
STATION**

**HARPENDEN, HERTS. AL5 2JQ
ASSISTANT STATISTICIANS**

to analyse and interpret data from agricultural and
other biological experiments using the Station's
computer. Degree, H.N.C. or equivalent qualification
in mathematics or statistics. Some knowledge
of agriculture or biology an advantage.

Appointment in grade of Scientific Officer
(£1,592 to £2,675). Starting salary according to
qualifications and experience. Superannuation with
a contribution of 14% for family benefits.

Apply in writing to the Secretary giving names
and addresses of two referees and quoting reference
236 by September 15, 1974.
(826)

**UNIVERSITY OF LEEDS
DEPARTMENT OF PLANT SCIENCES**

Applications are invited from graduates
with appropriate qualifications for the post
of **RESEARCH OFFICER** in the Department
of Plant Sciences, Leeds University. The appointment
is for one year, with possible renewal for a further two years,
and is associated with a major research
project financed by the Agricultural Research
Council on direct drilling and reduced
cultivation. The person appointed
will take charge of field experiments and
assist with the co-ordination of the experimental
requirements of a small research
group. Salary £1,929 by £129 to £2,058 by
£165 to £2,223 (at present under revision).
Application forms and further details from the
Registrar, University of Leeds LS2 9JT
(please quote reference number 41/25/D).

Closing date for applications August 31,
1974.
(807)

Opportunity Overseas

Ghana

Agronomist

To work with local research agronomists at the Crops Research
Institute, Kumasi on a range of crops.

Candidates should have a degree in agriculture or allied science
with experience of agronomic research in tropical countries.
Appointment 18 months in first instance. Salary in scale £4,550
to £6,500 p.a. plus a tax-free allowance in scale £760 to £1,765 p.a.

Other benefits include free family passages, paid leave, children's
education allowances and free accommodation and medical
attention. Applicants should normally be citizens of and
permanently resident in the United Kingdom.

For full details and an application form please apply giving
age and brief details of qualifications and experience to:

Appointments Officer

Ministry of Overseas Development

Room E301 Eland House
Stag Place London SE1E 5DH

(868)



Microbiologist

John Wyeth & Bro. Ltd., an associated Company of Wyeth International of Philadelphia, U.S.A. produce a wide range of Pharmaceutical and infant food products at their modern factory at Havant, Hampshire.

A vacancy exists for a recently qualified graduate in Microbiology who will be required to join a small team involved in the Microbiological Quality Control of pharmaceutical nutritional products.

The company offers a non-contributory pension and life assurance scheme. Assistance with relocation expenses will be considered.

Please apply to:

Mr C. E. Henry,
Staff Personnel Manager,
John Wyeth & Brother Limited,
Huntercombe Lane South,
Taplow, Nr. Maidenhead,
Berks.

or Telephone Slough 28311.

(836)



AGRICULTURAL RESEARCH COUNCIL INSTITUTE FOR RESEARCH ON ANIMAL DISEASES DEPARTMENT OF BIOCHEMISTRY

Compton, Newbury, Berkshire

Applications are invited for a postgraduate studentship available for a suitably qualified candidate to study the nature of the scrapie agent using biochemical and virological techniques. The studentship is funded by the Multiple Sclerosis Society and the stipend will be approximately £1,200 per annum for three years. Opportunities exist to register for a higher degree at the University of Reading, with which the Institute is an associated institution. Hostel accommodation is available in a modern building.

A full curriculum vitae and the names of two referees should be submitted to the Secretary as soon as possible, quoting the reference number 159. (827)

AREA LABORATORY KING EDWARD VII HOSPITAL WINDSOR

Senior Technician, Technician and a Junior Technician required for Microbiology Department. Laboratory provides a comprehensive service for approximately 1,000 beds and for out-patient clinics and general practitioners in the Windsor, Ascot and Maidenhead areas. Expansion of services anticipated. Hospital close to public transport termini and has its own swimming pool.

Applications to Senior Pathologist. Enquiries to Senior Chief Technician. Tel: Windsor 61424. (830)

UNIVERSITY OF GLASGOW DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY RESEARCH ASSISTANT

Applications are invited from Biologists/Biochemists for the post of Research Assistant in the above Department. The post is grant-aided, tenable for a period of three years from October 1, 1974. The project is to study steroid production by human ovarian cells.

Applications will be considered from either Honours Graduates or Postdoctoral Scientists. Salary will be within the range £1,600 to £2,412 per annum according to age, qualifications and experience.

Further particulars may be obtained from Dr J. R. T. Coutts, Department of Obstetrics and Gynaecology, Royal Maternity Hospital, Glasgow, with whom applications should be lodged by August 30, 1974.

In reply please quote Ref. No. 3530M. (832)

UNIVERSITY OF PITTSBURGH CRYSTALLOGRAPHY DEPARTMENT

Applications are invited for a faculty appointment. Preference will be given to applicants with widely recognised achievements in research pertaining to diffraction and its use for the determination of structure at the electronic, atomic or molecular level. The appointment will be at a rank commensurate with the experience of the successful applicant. Applications should be sent to:

Professor Bryan M. Craven
Crystallography Department
University of Pittsburgh
Pittsburgh, Pa. 15260 U.S.A.

(AN EQUAL OPPORTUNITY EMPLOYER) (834)

UNIVERSITY OF SYDNEY LECTURESHIP IN GEOGRAPHY (three year fixed term appointment)

The Department of Geography requires a lecturer for senior classes in economic geography, and for advising postgraduate students. Candidates should have research and teaching experience, which must include expertise in quantitative techniques. Preference will be given to applicants who can contribute to the Department's interests in resource management and urban and regional planning. Applicants should have a Ph.D. or equivalent qualification. It is hoped that the successful applicant will be able to take up duties early in 1975, but later dates may be negotiated.

The lectureship advertised is for three years in the first instance.

Salary range: \$A9,002 to \$12,352 p.a.

Applications, including curriculum vitae, list of publications, and names of three referees by September 30, 1974 to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information available. Further information also from Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. (845)

Bass Charrington Graduate Microbiologist

This interesting and challenging position involves quality control duties at our laboratories at Cape Hill Brewery, Birmingham.

The successful applicant will have an Honours Degree in either Microbiology or a biological subject with substantial Micro-biological content.

We offer an attractive salary, commensurate with the importance we attach to this position, together with the usual fringe benefits associated with a large and progressive organisation.

Apply giving full details to:—

Personnel Manager,
Mitchells & Butlers Limited,
P.O. Box 27,
Cape Hill Brewery,
Birmingham B16 0PQ.

(841)



Senior Physicist

required in the Department of Clinical Physics and Bioengineering. Duties will be to engage in Clinical Radiotherapy Physics, Treatment Planning, Radiation Dosimetry and Radiation Protection. Some teaching in D.S.R. Courses.

Applicants must have a good honours degree in Physics. Clinical experience is essential. Salary scale £2,964 to £3,843 plus £126 London Weighting plus current threshold payment. Application forms obtainable from Personnel Department, Guy's Hospital, London SE1. Tel. 01-407 3662 Ext. 68.

(850)

AGRICULTURAL RESEARCH COUNCIL

LETCOMBE LABORATORY SCIENTIFIC OFFICER

required to fill a vacancy in the Field Studies Department to assist in work on the long term effects of reduced cultivation and direct drilling on soil conditions and crop growth.

Minimum qualifications: Pass degree, H.N.C. or the equivalent in an agricultural or biological science.

Starting salary £1,592 to £1,931 according to qualifications and experience, on scale to £2,675. There is a non-contributory Superannuation Scheme.

Application forms and further particulars may be obtained from the Secretary, Agricultural Research Council, Letcombe Laboratory, Wantage OX12 9JT. The closing date for application is September 20, 1974. (848)

UNIVERSITY OF LONDON KING'S COLLEGE

POSTDOCTORAL ASSISTANTSHIP

Applications are invited for a postdoctoral assistantship in the Department of Pharmacology, for research on neurotransmitter synthesis and turnover. The post is tenable for two years, starting salary according to age and experience, from £2,118 p.a. plus £213 p.a. London Weighting. A knowledge of enzyme purification, immunological, or neurochemical procedures an advantage.

Applications, as soon as possible, to Dr A. K. Prince, Department of Pharmacology (N), King's College, Strand, London WC2R 2LS. (838)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PHARMACOLOGY

Applications are invited for two POSTDOCTORAL ASSISTANTSHIPS for work on the mechanism of calcium transport in sarcoplasmic reticulum, or the coupling of hormone receptors to adenylyl cyclase activity, in collaboration with Dr J. C. Metcalfe and Dr G. B. Warren.

Candidates should have research experience in enzymology or physical biochemistry, preferably applied to membranes. The posts are supported by the S.R.C. and the M.R.C. and are tenable for up to three years from October 1974 or later. Salary range £2,223 to £2,553 p.a. (under review) (with F.S.S.U.).

Applications, including curriculum vitae and the names of two referees should be sent to Dr J. C. Metcalfe, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD, England. (819)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY

CHEMICAL ENGINEERING DEPARTMENT SPECIAL RESEARCH ASSISTANT

A vacancy exists for a Research Assistant to work on a Science Research Council research contract to study the condensation of vapours of immiscible liquids. The contract is for a period of three years. The work of the research assistant will entail laboratory studies of condensation, study of the influence of important physical and surface properties, and the operation of a pilot plant experimental rig.

The salary will be within the range £2,383 to £2,746 plus F.S.S.U. contributions. Applications are invited from persons with suitable qualifications or experience in chemistry, physics or chemical/mechanical engineering. Applications should be sent as soon as possible to Dr G. A. Davies, Department of Chemical Engineering U.M.I.S.T., P.O. Box 88, Manchester M60 1QD. (851)

SOUTHAMPTON UNIVERSITY MEDICAL SCHOOL BASIC GRADE BIOCHEMIST

Basic grade biochemist required to work on lipid project in the University Departments of Child Health and Chemical Pathology. The study involves using techniques for lipoprotein electrophoresis, ultracentrifugation and analysis of triglycerides and phospholipids. The successful applicant would be based in the Department of Chemical Pathology and work in close liaison with both clinical and biochemical staff. Applications and enquiries to Personnel Department, Southampton Health District, 119 Tremona Road, Southampton. (843)

UNIVERSITY OF STRATHCLYDE

DEPARTMENT OF CIVIL ENGINEERING

POSTDOCTORAL RESEARCH ASSISTANTSHIP IN WATER POLLUTION MONITORING

A Postdoctoral Research Assistantship, sponsored by the S.R.C., is available in the Department of Civil Engineering. The research programme involves development of a continuous electroanalytical method for monitoring organic pollutants in effluents. Applicants should have experience of electrochemical and analytical techniques.

The appointment is initially tenable for two years on the salary scale £2,118 per annum rising to £2,247 per annum with F.S.S.U. benefits.

Applications, including curriculum vitae and the names of two referees (quoting R30/74) should be sent to Dr C. L. Page, Department of Civil Engineering, University of Strathclyde, Colville Building, 48 North Portland Street, Glasgow G1 1XN. (874)

Graduate Biochemist/Plant Physiologist

Shell Research Limited, Sittingbourne, Kent have a vacancy for a young graduate biochemist or physiologist in the Biochemistry and Plant Physiology Division of their Milstead Laboratory of Chemical Enzymology. You will be concerned with detailed studies of the regulation of plant metabolic processes and of the enzymes involved. There will be extensive use of radio isotope techniques. You will be expected to have knowledge of and experience in a variety of biochemical techniques. You should have a good Honours degree in Biochemistry or Plant Physiology, and preferably a PhD. Chemistry post-graduates will also be considered if their studies have involved a substantial amount of plant biochemistry and enzymology. The level of appointment will depend upon your background and experience; the salary will be competitive. Please write or telephone for an application form to Shell Research Limited, Recruitment Division (N), PNEL/34, Shell Centre, London SE1 7NA. Telephone 01-934 2948.



AUSTRALIA

New South Wales Department of Agriculture ENTOMOLOGIST

Agricultural Research Station,
Narrabri

Applications are invited for the above position.

Salary :

\$A7,287 per annum range \$A10,953 per annum but Entomologists with post-graduate experience and/or qualifications eligible for payment of salaries up to \$A12,619 per annum. Further progression to higher salaries is available subject to meeting certain specified conditions.

Qualifications :

Degree in Agricultural Science of equivalent but post-graduate qualifications and experience highly desirable.

Duties :

Investigation of insect pests of economic importance to the cotton industry in New South Wales with particular regard to developing suitable integrated pest control measures.

Location :

The successful applicant will be initially located in the Entomology Branch, Biological and Chemical Research Institute, Rydalmere, Sydney, for a short period before subsequent location at the Narrabri Agricultural Research Station.

Subject to certain conditions the successful applicant will be eligible for:—

Payment of fares to Sydney

Financial assistance towards cost of removal expenses

Financial assistance
towards cost of initial accommodation expenses

For further information and application form telephone or write to the Recruitment Section, New South Wales Government Offices, 66 Strand, London WC2N 5L $\frac{1}{2}$ (Tel: 01-839 6651 Extension 194) where applications close on FRIDAY, 27th SEPTEMBER, 1974. When telephoning or writing please quote reference 44/570.

(884)

ANTARCTIC EXPEDITION

requires an OBSERVATORY PHYSICIST to serve at British stations in Antarctica. The tour of duty covers two Antarctic winters involving an absence from the United Kingdom of about 2 $\frac{1}{2}$ years. The successful applicant will be required to work on his field data for up to a year after his return.

Qualifications: Preferably a First or Upper Second Class Honours degree but an ordinary degree with experience or appropriate H.N.D. will be considered.

Starting salary from £1,793 per annum rising in scale to £2,889 per annum. Low income tax, free messing, bonus for satisfactory service.

Applicants must be single and aged 22 to 30.

If you are interested in seeing a largely unknown, remote and fascinating part of the world, please write to:

The Establishments Officer,
British Antarctic Survey,
30 Gillingham Street,
London SW1V 1HY.
Tel: 01-834 3687.

(869)

UNIVERSITY COLLEGE DUBLIN APPOINTMENT IN AGRICULTURAL CHEMISTRY

Applications are invited for a teaching post either as:

- (a) COLLEGE LECTURER, or
- (b) ASSISTANT LECTURER

in the Department of Agricultural Chemistry. Candidates should be Graduates in Agricultural Chemistry, Chemistry, Biochemistry, Microbiology or Agricultural Science, with a Ph.D. and preferably with some teaching and research experience.

The current salary scales are:

College Lecturer £3,514 by £162 to £4,648
Assistant Lecturer £2,420 by £129 to £3,323.

Entry point on the relevant scale will be in accordance with qualifications and experience.

A non-contributory pension scheme and family allowances are additional to salary. An alternative contributory F.S.S.U. type scheme is also available.

Prior to application, further information (including application procedure) should be obtained from:

Mr J. P. MacHale,
Secretary and Bursar,
University College,
Belfield,
Dublin 4.
Telephone 693244 Extn. 431.

Latest date for receipt of completed application is September 16, 1974. (840)

UNIVERSITY OF GLASGOW DEPARTMENT OF BOTANY POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a Postdoctoral Research Assistantship in the Department of Botany to work on an S.R.C. sponsored research programme concerned with the hormonal regulation of nitrogen fixation in higher plants. Preference will be given to applicants with previous experience of the biochemistry and/or physiology of plant growth hormones.

The appointment will be for one year in the first instance, from October 7, 1974, and the starting salary will be not more than £2,118 per annum plus F.S.S.U. benefits.

Applications, with curriculum vitae and the names of two referees, should be sent not later than September 6, 1974, to Dr C. T. Wheeler, Department of Botany, University of Glasgow, Glasgow G12 8 QQ.

In reply please quote Ref. No. 3532M. (854)

UNIVERSITY OF THE WEST INDIES TRINIDAD

Applications are invited for (a) LECTURESHIP or ASSISTANT LECTURESHIP IN CROP PRODUCTION in the Department of Crop Science, tenable from January 1975. Applicants should possess a higher degree in Agriculture or Plant Science, and experience in teaching and research on tropical crops. Experience in management of plantation and field crops in the Caribbean area is considered desirable but not essential. Salary scales: (a) TT\$12,612 to TT\$20,316 p.a. (b) TT\$10,200 to TT\$11,232 p.a. (£1 sterling=TT\$4.8), F.S.S.U. Unfurnished accommodation at rental of 10% of salary. Thereafter 20% of salary paid in lieu of housing. Family passages; study leave. Detailed applications (6 copies), including a curriculum vitae and naming 3 referees, should be sent by airmail, as soon as possible, to the Secretary, University of the West Indies, St Augustine, Trinidad. Detailed particulars of the post will be sent to all applicants. (819)

UNIVERSITY OF QUEENSLAND AUSTRALIA

ONE LECTURER AND ONE SENIOR TUTOR IN EXPERIMENTAL PHYSICS

Applicants with experience in one of the following areas will be preferred: Beam foil spectroscopy; environmental (hydrodynamic modelling) physics; ionospheric physics; microwave and molecular physics.

Salary: Lecturer within the range \$A9,002 to \$A12,352 per annum; Senior Tutor within the range \$A7,545 to \$A9,002 per annum.

Other Benefits: Superannuation similar to F.S.S.U., housing assistance, and travelling and removal expenses. Lecturer also entitled to study leave.

Additional information and application forms are obtainable from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in London and in Brisbane on September 30, 1974. (856)

UNIVERSITY OF LONDON GRANTS FOR RESEARCH

Applications are invited from members of the University and teachers in Schools of the University for grants from the Central Research Fund to assist specific projects of research and for the provision of special materials and apparatus. Grants are not made for normal maintenance. Applications must next be received not later than September 15. Forms of application and further particulars may be obtained from the Secretary to the Central Research Fund, University of London, Senate House, London WC1E 7HU. (846)

UNIVERSITY OF DUNDEE DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

Applications are invited for a RESEARCH ASSISTANTSHIP in the Department of Therapeutics at Ninewells Hospital, Dundee.

The appointment, which will be for three years, will involve work in a research project on the effect of drugs on red blood cells and the protective effects of the enzyme systems.

The candidates should possess a good Honours Degree in Biochemistry. The initial salary will be £1,680 per annum.

Informal enquiries may be made to Dr G. R. Tudhope, Department of Therapeutics, Ninewells Hospital, Dundee DD2 1UD.

Applications, quoting Ref. EST/53/74J, together with a curriculum vitae and the names of two referees, should be sent to The Secretary, The University, Dundee DD1 4HN, by September 13, 1974. (847)

UNIVERSITY OF NEW SOUTH WALES SCHOOL OF MICROBIOLOGY LECTURER

High academic qualifications required, together with training, research and teaching experience in the general field of microbiology. Appointee will participate in a number of microbiology courses offered to science, food technology and medical students.

Salary: \$A9,002 range \$A12,352 per annum. Commencing salary according to qualifications and experience. Appointment to commence during February 1975.

Details of appointment, including superannuation, study leave and housing scheme, may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close in Australia and London on September 20, 1974. (857)

PUBLIC HEALTH LABORATORY AND DEPARTMENT OF MICROBIOLOGY CENTRAL MIDDLESEX HOSPITAL Park Royal, London NW10 7NS

VIROLOGY TECHNICIANS

required to work in a new microbiology laboratory. There are excellent facilities for routine and research work in this well equipped laboratory which includes an ultra centrifuge and electron microscope.

Applicants should possess a H.N.C. or equivalent qualification, but experience in virology is not essential. The successful applicant will be encouraged to study for the advanced examination in virology.

Whitley Council salary and conditions. Apply in writing to the Director, Dr C. E. D. Taylor. (867)

WEST BERKSHIRE HEALTH DISTRICT MEDICAL PHYSICS TECHNICIAN

for the modern Isotope Laboratory at the Royal Berkshire Hospital, Reading, for interesting work in the chemistry and physics of medical isotope techniques. Post will be on Grade V or Grade IV (£1,308 to £1,667 or £1,530 to £1,953). Day release for further training possible. (Normally O.N.C. or 2 'A' levels required.)

Reading is a pleasant University town offering easy access to London, Oxford, Windsor, Henley and attractive surrounding countryside.

Written applications with relevant details and naming 2 referees to Hospital Secretary, Royal Berkshire Hospital, London Road, Reading. (862)

UNIVERSITY OF BRISTOL LONG ASHTON RESEARCH STATION SCIENTIFIC OFFICER

required to work in the Microclimatology Section. Applicants should have some knowledge of environmental physiology, cropping systems and scientific instrumentation. Part of the work will be done in France. Qualifications: Pass Degree, H.N.C. or equivalent. Salary in scale £1,592 to £2,675 according to qualifications and experience, with non-contributory superannuation scheme.

Applications by September 25 to Secretary, Research Station, Long Ashton, Bristol BS18 9AF, who will provide further particulars of the post. (839)

POLYTECHNIC OF THE SOUTH BANK

FACULTIES OF SCIENCE AND ENGINEERING

RESEARCH ASSISTANTS

Applications are invited for posts in the following areas of work:

Ionisation of Gases.

Polymer Science (Organo-Silicon Polymers, physical and chemical properties of polymers, or polymerisation processes).

Chemistry (Peptide Chemistry or Organo-Metallic Chemistry).

Control Engineering (stochastic processes applied to the optimisation of industrial control systems).

High-voltage Engineering (mechanisms of dielectric breakdown by discharge and use of non-destructive tests for electrical insulation).

Applicants should be graduates in appropriate subjects, who wish to take a higher research degree.

Appointments are normally for three years on a salary scale currently:

£1,648.40 to £1,758.40.

Application forms and further details from the Clerk to the Council (Room 720), Polytechnic of the South Bank, Borough Road, London SE1 0AA. Tel. 01-928 8989. (865)

UNIVERSITY OF READING DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY

RESEARCH DEMONSTRATORS

required to do research for a higher degree in the following areas:

(a) with Dr B. A. Nicholson on the structure and functions of RNA Polymerase;

(b) with Professor G. M. H. Wailes on the metabolic control and endocrinology of the mammalian testis and epididymis.

Candidates should have a good Second Class Honours Degree in a relevant subject. The posts will be for three years with the possibility of extension and involve about 12 hours demonstrating per week during term time. Salary £1,122 by £54 to £1,230 p.a. plus threshold payment.

Apply with names of two academic referees to Professor G. M. H. Wailes, Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG6 2AJ, or ring Reading 85123 extn. 7675. (Ref: TN70). (864)

UNIVERSITY OF RHODESIA FACULTY OF SCIENCE

LECTURESHIP IN ANIMAL NUTRITION

Applications are invited for the post of Senior Lecturer or Lecturer in Animal Nutrition in the Department of Agriculture. Candidates should have at least an honours degree in Biochemistry or Agricultural Science, together with relevant research experience and knowledge of animal nutrition. A special knowledge of ruminant nutrition would be of substantial advantage.

Salary Scales (Approx. Stg. equivs.): Senior Lecturer: £5,484 by £219 to £7,239; Lecturer Grade I: £4,984 by £184 to £5,720; Lecturer Grade II: £3,071 by £158 to £3,545 by £175 to £3,895 by £185 to £4,635 by £174 to £4,809.

Family passages and allowance for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel allowance. Superannuation and medical aid schemes.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience and publications, and naming three referees, should be submitted by September 30, 1974, to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. Applicants from outside Southern Africa should send a copy of their application to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. (855)

THE UNIVERSITY OF MANCHESTER Institute of Science and Technology DEPARTMENT OF OPHTHALMIC OPTICS ANIMAL TECHNICIAN

(GRADE 4)
(Ref. O/128/AI)

Applications are invited for the post of Animal Technician in the Visual Sciences Laboratories of the above department.

Duties will involve the care of a variety of animals including monkeys, assistance with research involving animals and organisation of breeding programmes.

In addition some assistance with physiological demonstrations will be required.

Appropriate experience is essential and membership of the Institute of Animal Technicians is desirable.

Salary scale, according to age and experience will be £1,848 to £2,163.

Forms of application are obtainable from the Registrar, U.M.I.S.T., P.O. Box 88, Sackville Street Manchester M60 1QD, to whom completed forms should be returned by September 13, 1974. (842)

HISTOLOGY TECHNICIAN

required by private company carrying out a wide variety of diagnostic and experimental pathology. A.I.M.L.T. or similar qualifications. MICROBIOLOGIST also required to head department. Applications in writing to The Personnel Manager, Wickham Laboratories Limited, Winchester Road, Wickham, Hants PO17 5EU. (825)

SOUTH WEST THAMES REGIONAL NUCLEAR MEDICINE CENTRE, ROYAL MARSDEN HOSPITAL, DOWNS ROAD, SUTTON, SURREY

SCIENTIST

required to take part in a multi-disciplinary study of radiopharmaceuticals used to localise tumours and study their metabolism. Candidates who should be graduates in a physical or biological science will be expected to undertake some routine work within this large modern and well-equipped department. Salary is (basic scientist grade). Details of qualifications, age and experience should be sent with the names of two referees to Miss I. Mayor, Deputy Administrator, Royal Marsden Hospital, Downs Road, Sutton, Surrey. (831)

BIOCHEMIST or CELL BIOLOGIST, SWEDEN

Pharmacia Fine Chemicals AB, manufacturers of Sephadex^R and other products for biochemical separations, require a biochemist or cell biologist to work at our headquarters in Uppsala, Sweden.

The successful applicant will join our Scientific Information and Technical Services group. The activities of the group include

- ★ the preparation of all kinds of scientific information material
- ★ applications work and laboratory testing of new products
- ★ lecturing

but applicants should be prepared for extremely varied work and certain amount of travelling, mostly within Europe.

Applicants should have research experience in biochemistry or cell biology. Clear and accurate expression in the written word is essential with English as the Mother tongue.

This is an important position and carries an appropriate salary. Preferred age 25 to 30.

Write in confidence to:

Mr D. Sweetman
Pharmacia (Great Britain) Ltd
Paramount House
75 Uxbridge Road
London W5 5SS



Pharmacia
Fine Chemicals

(885)

THE POLYTECHNIC OF NORTH LONDON

Holloway, London N7 8DB

Applications are invited from graduates with a good Honours Degree for appointment as RESEARCH ASSISTANT to work in the field of Cytogenetics (with special reference to polymorphic gastropods). The successful candidate will be expected to read for a higher degree. The post will commence in January, 1975.

Salary scale: £1,544 by £55 by £1,654.

Apply in writing, stating qualifications and the names of two referees, to: Head of Department of Biology, The Polytechnic of North London, Holloway Road, London N7 8DB. (866)

RESEARCH SCIENTIST (BIOCHEMISTRY)

Ref: RES/73/7
(RE-ADVERTISED)

Qualifications: Ph.D. degree in biochemistry immunology or physiology. Experience with investigations in insect science would be an advantage. This position is open to young postdoctoral scientists who have interest in immunology, enzyme biochemistry, or immunochemistry.

Responsibilities: The successful candidate will be required to collaborate in the investigation of the tsetse salivary gland physiology and its relationship to the immunology of resident trypanosomes.

Salary: Not less than 44,000/-.

Other benefits include a ten per cent gratuity of basic salary, a house allowance and medical insurance.

Date of appointment: Immediate.

Applications, in six copies, should give the following information: General education. Professional qualification, experience, marital status, age, present salary and terms of service, and names and addresses of four referees (including a personal reference). Photostat copies of relevant transcripts, thesis abstracts and certificates should be enclosed.

Applications should be addressed to:

The Administrative Officer
ICIPE Research Centre
P.O. Box 30772,
NAIROBI, Kenya.

(863)

MONASH UNIVERSITY Melbourne, Australia DEPARTMENT OF GENETICS LECTURER

Candidates should have suitable qualifications and experience in some branch of Genetics. The Department already has appointments in microbial genetics, population genetics and cytogenetics and preference may be given to applicants who are qualified in one or another of these aspects of Genetics. The successful applicant will be expected to initiate and supervise research and conduct classes in the general area of his specialty as well as assisting in the general teaching programme of the Department.

Salary Scale: \$A9,002 to \$A12,352 per annum with superannuation based on an endowment assurance scheme the employee and employer contributing 5% and 10% respectively.

Benefits: Travelling expenses for appointee and family; removal allowance; repatriation after three years appointment if desired; temporary housing for an initial period; availability of loans for home purchase; study leave entitlement accumulates at the rate of one month's leave for each six months' service up to six years, with provision for financial assistance.

Further general information and details of application procedure are available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia, or the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Enquiries about departmental research interests and facilities to Professor B. W. Holloway in the University.

Closing Date: **September 30, 1974.**

The University reserves the right to make no appointment or to appoint by invitation. (873)

NORTH MIDDLESEX HOSPITAL CLINICAL CHEMISTRY DEPARTMENT

Applications are invited from graduates in Biochemistry, Physiology or Chemistry or a vacant post in this department.

The post will provide wide experience in routine hospital work, and will allow opportunity to develop methods in a specialist field such as lipoprotein investigations, radioimmunoassay or steroid Chemistry.

Applications or enquiries should be sent to: Dr Dangerfield, North Middlesex Hospital, Silver Street, Edmonton, London N18 1QX. (870)

M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital)

Watford Road, Harrow, Middlesex HA1 3UJ.
The Division of Psychiatry requires a BIO-CHEMICAL PHARMACOLOGIST with post-graduate experience to join a team of clinicians and biochemists working on metabolic disturbances in the functional psychoses and the mode of action of psychotropic drugs. It is intended that the candidate appointed will develop a particular interest in the metabolism of psychotropic drugs but may also collaborate in projects on mechanisms of action. An interest in developing new assay techniques would be an advantage. Conditions of service in accordance with M.R.C. regulations. Salary depending upon age and experience.

Further details and application forms may be obtained from Mrs J. Tucker-Bull.
Please quote ref. 125/1/Y5. (871)

HISTOLOGY TECHNICIAN

required for Pharmacological and Toxicological Laboratory. Experience essential in the preparation and processing of animal tissues.

Good working conditions. Pension and Assurance Scheme. Application forms from:

The Secretary, Biorex Laboratories Ltd.,
Biorex House, Canonbury Villas, London N1 2HB. (876)

UNIVERSITY OF MELBOURNE LECTURESHIP (Limited Tenure—Three Years)

in the
DEPARTMENT OF PHYSIOLOGY

This appointment is to be made to assist in the development of a neurosciences training programme within the Department of Physiology. In this programme we aim to provide a co-ordinated course of study of the functions of the brain (in physiological terms). The course commences in the third year of the science course, continues through the honours year and also provides supervision of research activities and course work for postgraduate M.Sc. and Ph.D. students. The appointee will participate in this programme together with four other staff members. A separate neurophysiology laboratory is to be used for laboratory work for undergraduate students, but postgraduate students will work and be supervised in the research laboratories of the Department.

The research facilities available in the Department for the study of brain function have recently been considerably expanded with the setting up of a sensory processes laboratory (I. Darian-Smith and K. O. Johnson). Neurophysiology and experimental psychology laboratories of this unit provide facilities for correlative studies of cutaneous sensibility and the underlying neural events as observed in human subjects and monkeys. Each laboratory has independent access to a PDP11/40 computer system. In a second research laboratory, also with computer facilities, functions of the limbic system are being studied (J. S. McKenzie) by correlating neural events with motivated behaviour.

Applicants should have teaching and research experience in neuro-science and either a Ph.D. in this field, or an equivalent qualification.

Salary: \$A9,002 to \$A12,352 (US\$13,360 to US\$18,300 as at July 26, 1974).

Commencing Date: January 1, 1975 or as soon as possible thereafter.

Further details may be obtained from Professor I. Darian-Smith, Department of Physiology in the University. Details of application procedure together with conditions of appointment may be obtained from the Registrar, University of Melbourne, Parkville, Victoria 3052, Australia, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF.

Applications close on September 20, 1974. (872)

FELLOWSHIPS AND STUDENTSHIPS

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF BIOCHEMISTRY RESEARCH STUDENTSHIP IN PHYSICAL BIOCHEMISTRY

Applications are invited from honours graduates in Chemistry and Biochemistry for studies on the physical biochemistry of enzymes. The nature of the award will be equivalent to an S.R.C. Studentship and the candidate will be able to register for a Ph.D. degree.

Applications giving curriculum vitae and the names of two referees should be sent by August 31 to Superintendent, Department of Biochemistry, Tennis Court Road, Cambridge CB2 1QW.

(X664)

HERIOT-WATT UNIVERSITY DEPARTMENT OF CHEMISTRY

Applications are invited from persons holding good honours degrees in Chemistry for an S.R.C. (C.A.S.E.) RESEARCH STUDENTSHIP in liaison with Nuclear Enterprises, Edinburgh. The studentship, tenable for three years, is available from October 1, 1974. The successful candidate will register for the degree of Ph.D. and will work under the joint supervision of Dr. I. Soutar and Dr. W. Steedman. The project is concerned with various aspects: the efficiency of plastic scintillator materials and will involve both the formulation of novel plastic scintillator systems and their evaluation using fluorescence and other techniques and will provide a broad experience in polymer chemistry research.

Applications should be sent to Dr W. Steedman, Department of Chemistry, Heriot-Watt University, Riccarton, Currie, Edinburgh EH14 4AS, from whom further information may be obtained. (860)

THE ROYAL MARSDEN HOSPITAL Fulham Road, London SW3

Applications are invited for a ROYAL MARSDEN FELLOWSHIP for training and research in Clinical Immunochimistry as related to malignant disease. Suitable for persons wishing to pursue a career in Clinical Oncology or Pathology.

Arrangements have been made for successful applicant to take the M.Sc. Course in Immunology at Birmingham starting October, 1974, and then to spend two years in the Division of Tumour Immunology of the Institute of Cancer Research at Sutton, Surrey.

Salary on Registrar Scale (£3,198 to £3,879) (Non-resident post).

Application forms are available from the Deputy House Governor at the above address (telephone 352 8171 ext. 205) to whom they should be returned by September 9, 1974. (880)

APPLIED PLANT PHYSIOLOGY CAMBRIDGE UNIVERSITY DEPARTMENT OF APPLIED BIOLOGY

invites graduates in Botany, Agricultural Botany or other relevant subject to apply for a Ministry Postgraduate Agricultural Research Studentship tenable from October 1974 to extend research on the physiological basis of varietal differences in yield in oil seed rape. Applications with full personal particulars and names and addresses of two referees should reach the Secretary, Department of Applied Biology, Downing Street, Cambridge not later than August 30, 1974. (853)

UNIVERSITY COLLEGE DUBLIN FACULTY OF AGRICULTURE POSTDOCTORAL FELLOWSHIP or RESEARCH ASSISTANTSHIP

Applications are invited for a postdoctoral fellowship or research assistantship position sponsored by the National Science Council to study aspects of nitrogen fertiliser use by crops.

Applicants should have an honours degree in agricultural science, chemistry, biochemistry or microbiology. The initial appointment, which commences on October 1, 1974, will be for one year with the probability of renewal for a further two years. Salaries will commence at £2,106 for post-doctoral fellowship and £1,395 or £1,670 depending on qualifications for research assistant.

Applications, giving details of education and experience, should be sent before August 30 to: Dr M. A. Morgan, Dept. of Agricultural Chemistry and Soil Science, University College Dublin, Faculty of Agriculture, Glasnevin, Dublin 9. (852)

UNIVERSITY OF EXETER DEPARTMENT OF CHEMISTRY

Applications are invited for an S.R.C. supported POSTDOCTORAL RESEARCH FELLOWSHIP for structural studies of nematic liquid crystals by X-ray diffraction. The appointment is for up to two years at a salary of up to £2,247 with superannuation benefits, depending on age and experience. The Research Fellow will join a group working on the structure and dynamics of disordered solids and ordered liquids by X-ray and inelastic neutron scattering techniques. Some background of scattering work, crystallography, spectroscopy or computing would be advantageous.

Applications, with the names of two referees, should be sent, as soon as possible, to The Secretary of the University, Northcote House, The Queen's Drive, Exeter, by October 1, 1974. Please quote reference 1/12/7082. The successful applicant may take up the post at any time up to January 1975. (818)

UNIVERSITY OF HULL DEPARTMENT OF PLANT BIOLOGY

Applications are invited for the post of Post-doctoral Fellow in Plant Biochemistry, to work with Professor J. Friend and Dr D. R. Threlfall on the molecular structure of potato cell walls in relation to pathogenesis. The post is financed by the A.R.C. and is tenable for up to three years from October 1, 1974, or as soon as possible thereafter.

Salary (excluding threshold payments) will be on the scale £2,226 by £114 to £2,340 by £72 to £2,412, plus F.S.S.U. benefits.

Applications (three copies) giving details of age, qualifications and experience together with the names of two referees should be sent by September 9, 1974 to the Registrar, The University of Hull, Hull HU6 7RX from whom further particulars may be obtained. (833)

Philipps-Universität Marburg/Lahn, West Germany

A temporary professorship (H2, ca. 3.100,—DM monthly) for Crystallography is available from October 1, 1974 to September 30, 1976 with the possibility of extension till September 30, 1978.

Applicants will be required to work in one of the fields of theoretical crystallography, structure determination methods or theoretical solid state research, and should be able to teach in these fields. In addition, they would be requested to give an introductory course in statistical methods for geoscientists. The holder of the professorship is a member of the Sonderforschungsbereich "Kristallstruktur und chemische Bindung".

Applicants with corresponding scientific qualifications and teaching experience are invited to send in their application (curriculum vitae, giving in particular details of their scientific career and previous teaching activity; list of publications, with a selection of important reprints) to the Dean, Fachbereich Geowissenschaften der Philipps-Universität, 355 Marburg/Lahn, Lahnberge, before September 15, 1974.

(858)

OPTICAL FIBRE COMMUNICATIONS

Communication by optical fibres is a rapidly expanding new technology which is being widely taken up by industry. The Laser research group at Southampton, comprising some 15 research workers, has been engaged in this field for some years with support from Science Research Council, industry and elsewhere. We have already announced world records for low transmission loss and high bandwidth in our fibres and wish to expand the present research team. Applications are therefore invited for a number of research fellowships, including a Pirelli Fellowship for work in collaboration with industry, at salaries linked to the scale for Lecturers in the range £2,118 to £3,462 plus threshold payments. Persons with an interest in any aspect of the subject are eligible. Applications giving details of education, experience and the names of two referees should be sent to the Deputy Secretary's Section, The University, Southampton SO9 5NH. Please quote reference No. 231/R.

(734)

UNIVERSITY OF ABERDEEN

UNIT FOR RESEARCH ON ADDICTIVE DRUGS

A S.R.C. Studentship (Co-operative Award in Science and Engineering, C.A.S.E.) will become available on October 1, 1974. It is tenable for 3 years in co-operation with the Pharmaceutical Division of Reckitt and Colman Ltd., Hull, and is open to First and Upper Second Class Honours Graduates in Pharmacology, Biochemistry or Physiology. The research project is concerned with the action of newly-synthesised morphine-like compounds on neurotransmitter release and on behavioural patterns in normal and morphine-dependent animals.

Applications (3 copies) to be submitted to Professor H. Kosterlitz, Unit for Research on Addictive Drugs, University of Aberdeen, from whom further particulars may be obtained, Marischal College, Aberdeen AB9 1AS, not later than September 6, 1974.

(789)

THE UNIVERSITY OF SHEFFIELD DEPARTMENT OF CHEMICAL ENGINEERING AND FUEL TECHNOLOGY FIRE RESEARCH

Applications are invited for the post of RESEARCH FELLOW to work with Dr D. J. Brown on BEHAVIOUR OF WOOD AND PLASTICS IN FIRES. The Fellowship, which will be available for 3 years, is financed by a grant from the Fire Research Station, Department of the Environment. Salary: on a scale up to £2,412 per annum according to qualifications and experience. Further particulars from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications (2 copies) should be sent by October 1, 1974. Please quote reference R127/G.

(809)

ROWETT RESEARCH INSTITUTE BUCKSBURN ABERDEEN

Applications are invited from Zoologists, Biochemists or others from appropriate disciplines to take up a 3-year A.R.C. STUDENTSHIP to study the amino acid requirements of insects and other organisms with potential as assay systems for food protein quality. A first or upper second class degree is required and the successful candidate will be expected to register with the University of Aberdeen for the Ph.D. degree.

For further information, those interested should apply as quickly as possible, by telephone or letter, to: The Secretary, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB. Tel: Bucksburn (0224-71) 2751.

(887)

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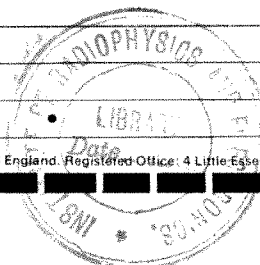
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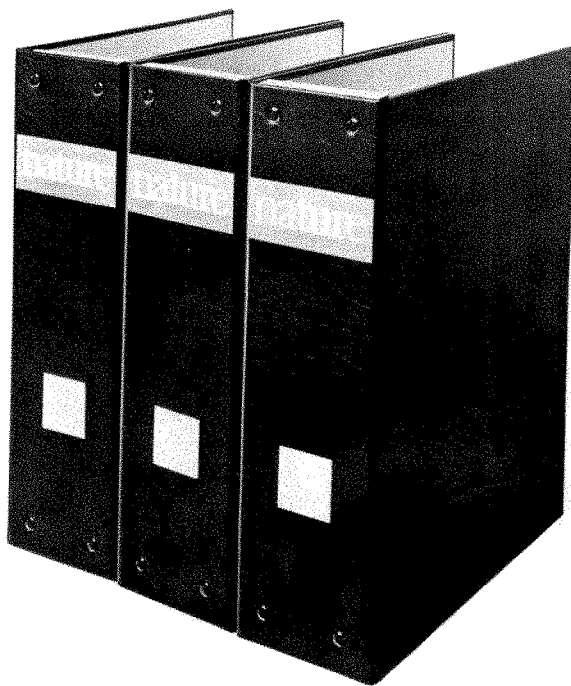
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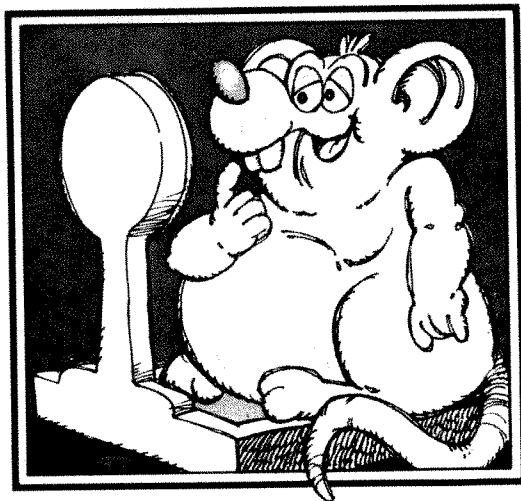
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REFERENCES

- 1) K. Gerzon, J.E. Cochran, Jr., L.A. White, R. Monahan, E.V. Krumkalns, R.E. Scroggs, and J. Mills, *J. Med. Pharm. Chem.*, **1**, 223 (1959).
- 2) K.S.V.R.V. Prasadaraao, *Chem. Abstr.*, **64**, 6431g (1966).
- 3) R.J. Rutman, F.S. Lewis, and W.D. Bloomer, *Science*, **153**, 1000 (1966).
- 4) J. Mayer, *New Engl. J. Med.*, **274**, 662 (1966).
- 5) A.F. Debons, I. Krimsky, A. From, and R.J. Cloutier, *Amer. J. Physiol.*, **219**, 1403 (1970).
- 6) R.J. Rutman, F.S. Lewis and W. Bloomer, *Trans. N.Y. Acad. Sci.*, **30**, 244 (1967).
- 7) C.C. Price, P.R. Koneru, and R. Shibakawa, *J. Med. Chem.*, **11**, 1226 (1968).
- 8) Y.N. Sinha and W.P. VanderLaan, *Proc. Soc. Exp. Biol. Med.*, **136**, 830 (1971).

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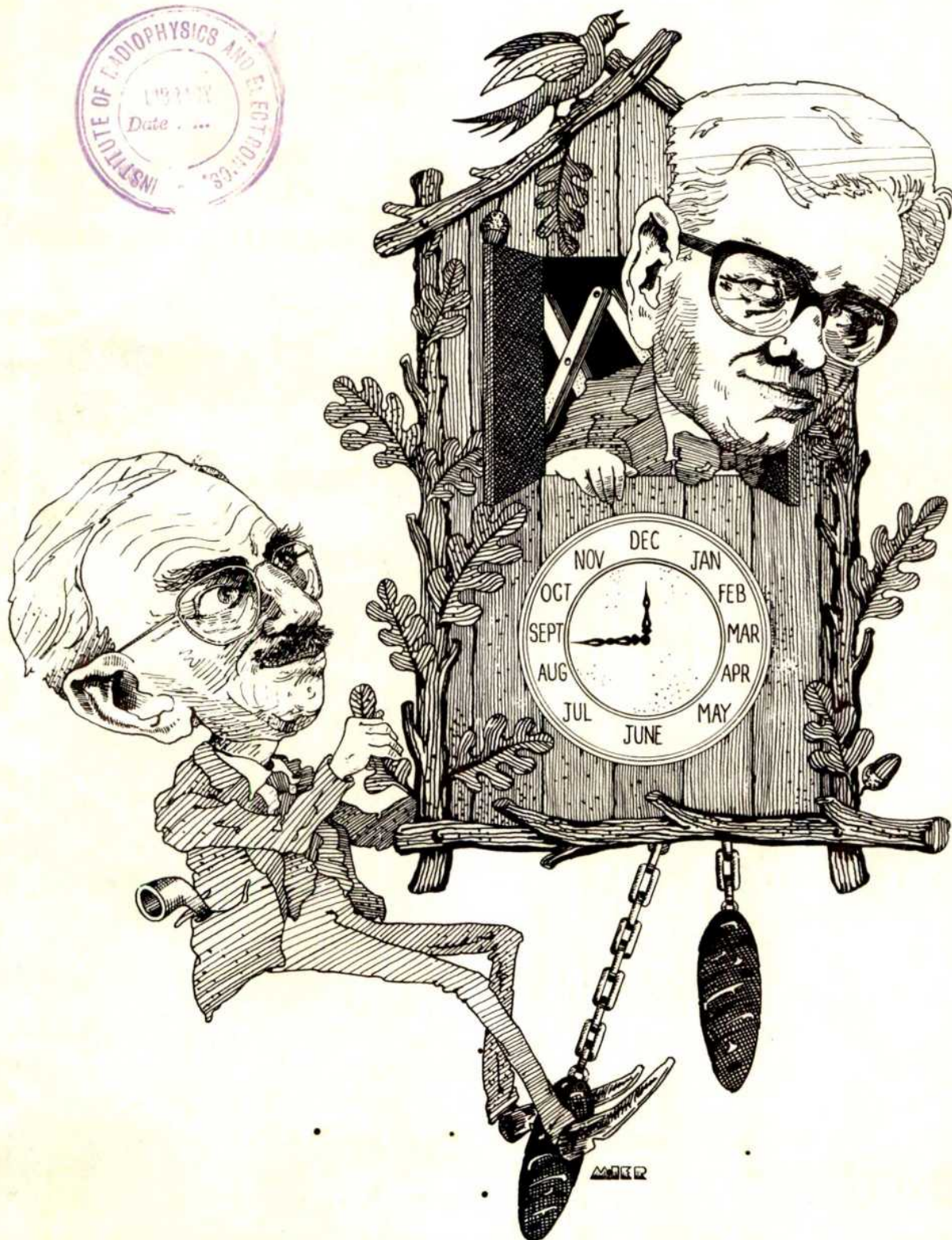
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President Kendrew; a Pyke produc-
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Abbreviations should follow the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). Symposia are often difficult to refer to and only published or soon-to-be-published volumes should be mentioned in references. Their publisher and place of publication should be clearly indicated. 'Personal communication' and 'unpublished work' should be incorporated in the text.

Artwork should be sent with the manuscript. All artwork should be marked with the author's name. Line drawings should preferably be in Indian ink on heavy cartridge paper, although other materials are acceptable; thin, shiny, folded, torn or heavily handled material should be avoided. Matt rather than glossy photographs are preferred. Figures are usually reduced to one column width. The originals should be about as wide as a page of *Nature*. Figures, particularly maps, should contain nothing but essential material. It is preferred that the original be unlabelled, but with a copy containing lettering. Labelling on photographs should if possible be avoided entirely.

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nature

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Should scientists be re-auditioned?

In general artists live by their wits and survive through being productive. If they are painters they must continue to paint and sell. If they are musical performers they must continue to keep a high standard of performance—in an orchestra that will mean a regular re-audition. It is a ruthless existence and yet one about which few of those involved in it, or even those who were once involved in it and have been left behind, feel bitter. There is often a feeling that the state should support an orchestra, an art gallery, a theatre, but rarely do individual creative or performing artists reckon that the state owes them a living. Indeed it is thought quite natural that they should move into teaching when they find their other abilities waning, without in any sense regarding teaching as an inferior profession.

What is it about scientists and the scientific profession which makes the idea of something even remotely similar amongst themselves unthinkable? In certain trades and professions it is possible to point to an accumulation of worldly goods which prevents any major changes in course—a taxi-cab, a workbench and tools, dental equipment. In others, more experience makes for greater capability as time goes on. Politicians, priests, bartenders probably feel that way. But a scientist neither owns his tools (beyond a relatively few books and journals of current use to him) nor demonstrably improves with age, unless he be in the business of cataloguing or collecting. Why then should he dig in for life, as he is very prone to?

One reason, of course, is that it is a very pleasant life, involving lots of travel, and in terms of service many scientists are near to being self-employed without the financial responsibilities that self-employment involves. Another is that the outside world seems to offer a great deal less stability, if it offers anything at all. Yet another is that scientists often delude themselves that it is creditable to go on working on the same old problems, gradually chipping them away, when the very last thing that most scientific problems need is the attention of one man, often to the exclusion of others, for thirty to forty years.

The community of older scientists must inevitably continue to grow for the foreseeable future and at some time governments, industry and academe are bound to have to ask whether the ranks of an ageing workforce can be allowed to continue to swell. This question is indeed being faced now by many institutions, world-wide, which sprang up in response to immediate post-war, defence needs. Academics can maybe justify themselves by increased teaching and administrative loads and a growing involvement in maintaining the standards of

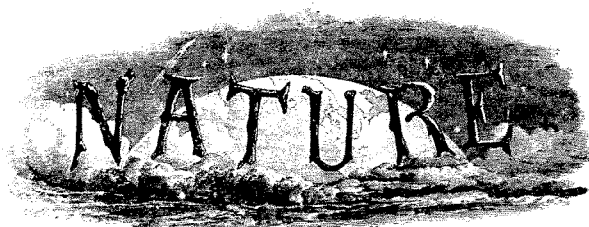
the college wine cellar. Others have no such outlets unless they are specifically plucked out of 'science' and dropped into 'administration'; and this is said to be a move that few are allowed to make.

There is a case, then, for scientists to come up with some fairly concrete proposals on the employment of older members of the profession before the government or other large scale employers step in with their own schemes for thinning the ranks. And perhaps some sort of re-auditioning system should be discussed in conjunction, of necessity, with an extensive rethinking of the career structure of scientists. It is obviously pointless to urge or even force someone out of his laboratory without any guarantee that other jobs can be found which do not involve a humiliating change.

If at the present this is not so, then it is time that scientists as a community started talking seriously about their prospects. It is only depressing that in Britain at present there is not a suitable forum for this, although we suggest elsewhere in this issue (page 743) that the British Association ought to become a gathering of thoughtful scientists. Then ultimately it should be possible to approach the government with rather specific proposals that every year a substantial number of intelligent, well trained and experienced men would be available at ages between, say, 35 and 50 for redeployment—and what could be available within the public and educational sector? The answer would, of course, be nothing, but a start would have been made in asking important questions about skilled manpower. It is impossible to believe that in reality such men are unemployable when there is an apparent shortage of intellect all around.

Many scientists would, we believe, welcome the opportunity to use formal review points in their career, perhaps every five years between 35 and 50, to make a sideways step as a means of self-regeneration. In that way the audition could almost be a self-audition. But they should not expect jobs to be waiting for them unless they have done a lot of hard lobbying first.

A hundred years ago



ON Friday evening M. Flammarion, the French astronomer, started from La Villette gas-works, Paris, in a balloon called *Lumen*, at half-past seven, with a brisk breeze from the north-west. The balloon was under the guidance of M. Jules Godard, and M. Flammarion, who was married in the beginning of August, was on board with his young wife; he wishes to spend his *lune de miel* in Italy. Such a trip was proposed in the beginning of the century to the celebrated M^{de} de Stael by the great philosopher, Saint-Simon; but the lady declined. The moon was full and bright.

From *Nature*, 10, 360, September 3, 1874.

international news

THE community of nations has gathered for the third time to develop a new order in the sea. What in 1967 started as a limited exercise to establish a regime for the seabed and the ocean floor beyond the limits of national jurisdiction, has developed into a formidable project whose objectives are to revise the whole philosophy of the law of the sea in an attempt to redress the balance between developing and developed countries. It is 15 years since the completion of the four 1958 Geneva Conventions which tried to establish rules for the use of the seas and now, after a very short interval, even their essential postulates are in question.

The old principle of the freedom of the seas which basically asserts that all States have equal rights to use the seas and which stood solid as a rock for more than 350 years is the object of serious attacks—the most dramatic change taking place is in the breadth of the coastal area over which various States claim sovereignty or jurisdiction; this may extend to 200 nautical miles (370 km) or to the outer edge of the continental rise if this feature goes beyond the 200 nautical miles. If this claim is accepted, more than 30 per cent of what today is high seas and which enjoys the freedom established in the 1958 Geneva Convention on the High Seas will come under the jurisdiction of coastal States—foreign vessels will have only the right of “innocent passage”. The other rights, including the one to conduct scientific research will disappear under this new concept.

The main battle at the Caracas Conference is being fought in the Second Committee where the right of coastal States to establish an exclusive “economic zone” or “patrimonial sea” beyond its territorial sea is under consideration. At the time of writing, about 20 days from the end of the Conference, there are the following tendencies on this matter:

(1) Territorial sea

- States which claim 12 nautical miles. The great majority of countries support this, but only if an economic zone of 200 nautical miles is accepted. This territorial sea of 12 miles, say the major maritime powers, may not impede transit through straits used for international navigation;

- States which claim 200 nautical miles; Brazil, Ecuador, Panama, Peru, Somalia, Uruguay.

(2) Economic zone or patrimonial sea

- States which claim an economic zone beyond the 12 miles’ territorial sea

Report from Caracas

extending to 200 nautical miles enjoying exclusive rights on exploration, exploitation and management of living and non-living resources; on prescribing standards for the preservation of the marine environment; and on the regulation of scientific research: Argentina, Australia, Canada, India, Kenya, Mexico, Tanzania, Venezuela and many others.

- States which accept 200 nautical miles economic zone but with certain conditions: (1) the coastal State will not necessarily have exclusive rights to living resources; that in order to ensure the full utilisation of living resources an obligation must be put on the coastal State to allow other States access to the resources which they do not utilise; (2) that the coastal State may enforce pollution standards agreed upon internationally but may not itself prescribe those standards unilaterally; (3) that the consent of a coastal State is not required to conduct scientific research once the vessels of other countries meet certain specified obligations.

(3) Definition and delimitation of continental shelf

The difference is between those States wishing to include the continental shelf in an economic zone of 200 nautical miles and those States like Argentina, Australia, Canada, which will prefer to go beyond 200 miles to the outer edge of the continental rise if this is larger than 200 miles.

(4) High seas and international seabed area

The need for a Seabed Authority is being accepted by the majority of States but they differ on the role to be assigned to such an Authority. A great number of developing States want to empower it with the management of living and non-living resources as well as the protection of the marine environment and regulation of scientific research. The great maritime countries want to endow the Seabed Authority with responsibility for the administration of the exploitation of the Seabed resources only. The system of exploitation, conditions of exploitation, machinery, and economic implications of exploitation remain to be solved.

The specific problem of marine scientific research and transfer of tech-

nology is being dealt with in Committee III. Here the discussions have concentrated on the definitions and objectives of marine scientific research, and the conduct and promotion of marine scientific research, including the right to conduct, granting of consent, participation and obligation of coastal States, and the general conditions for the conduct of marine research.

It is generally accepted that the proper management of the marine environment and its resources depends upon knowledge of the sea—however, management-orientated research is generally considered to be applied in nature; consequently the developing countries feel very strongly about the need to regulate such research, not only in the economic zone but also in the international sea. They feel less strongly about pure scientific research; but it is very difficult, if not impossible, to distinguish between pure research and economic or military research.

The general tendency (particularly within the developing countries) is to regulate scientific research in the territorial sea and economic zone. Consent can be refused in the territorial sea but in the economic zone “shall not” or “may not” be withheld if some specified conditions are met. The stronger “shall not” is proposed by Ireland and “may not” by Mexico. These conditions are very similar to those recommended by the International Council of Scientific Unions (ICSU) in its statement on freedom of scientific research and also the conditions being met within the International Decade of Ocean Exploration of the Intergovernmental Oceanographic Commission.

The conditions to be met can be summarised as follows:

- (a) Participation by developing countries in all phases of the work (from planning to the final results). This condition is directly related to the problem of development and transfer of technology, about which developing countries feel strongly.
- (b) Collected data and samples, as well as written up results, be made available as soon as possible to the coastal State.
- (c) That the research is being conducted for peaceful purposes.

The present discussions seem to indicate that there is complete agreement on the need to establish a viable framework for the conduct of marine scientific research. □

Oil platform question still confused

by Eleanor Lawrence

THE British government has finally decided that some sort of planning policy is necessary to solve the vexed question of where in Scotland to build concrete gravity-type oil production platforms. But the statement may raise more problems than it solves.

Mr Eric Varley, Secretary of State for Energy, proposes that a limited number of future sites should be taken into public ownership in order to:

- maximise their use
- avoid proliferation of sites
- ensure only the minimum amount of infrastructure (such as roads and housing) is built
- make sites available in good time for the best platform designs
- enable strict control to be exercised over the development and eventual restoration of the sites.

But Mr Varley also emphasises that normal planning controls and procedures will not be by-passed, and in this his proposals differ from those of the last government which proposed to short-circuit planning procedures but not to acquire the land.

It is difficult to see how these two requirements will marry happily. On the same day that Mr Varley made his statement, the Secretary of State for Scotland, Mr Willie Ross, announced the results of the Drumbuie enquiry. This has ended in a victory for the National Trust and the villagers amongst others, since Mowlem/Taylor Woodrow's application to build Condeep concrete gravity platforms at Drumbuie, on one of the most beautiful parts of the north-west coast of Scotland, was turned down on environmental grounds after a lengthy public enquiry.

The result of the Drumbuie enquiry has both heartened and confused the environmentalists. Mr Ross said in his statement that he was not persuaded that the possible national gains depended solely on the Condeep design which Mowlem/Taylor Woodrow wished to build. This design can only be constructed at the very deep water sites in that area. The fact that the Drumbuie land is owned by the National Trust was also an important factor in the decision, since even if permission had been granted, parliamentary approval would have been needed. But Mr Ross also said that the official Reporter for the enquiry had examined five other sites nearby and had concluded that all were, or might be, suitable. There is already an application by Howard/Doris to build gravity platforms at Loch Kishorn further up the coast; this has been approved in principle by the local authority and objections are being con-

sidered by Mr Ross at the moment.

What confuses environmentalists is that development anywhere in the area will potentially be equally as destructive as at Drumbuie, since other sites under consideration (such as on the Crowlin Islands in the strait between Skye and the mainland) are even more remote from road and rail communications and are so near Drumbuie that the same social objections can also be made. Mr Ross has refused a public enquiry at Loch Kishorn on these very grounds—that the objections are so similar that they need not be debated publicly once again.

Mr Ross has also said that his criteria for choosing a suitable site are

- that it must have access to existing facilities
- that it can draw on an existing labour source
- that it can make use of existing infrastructure such as roads and services

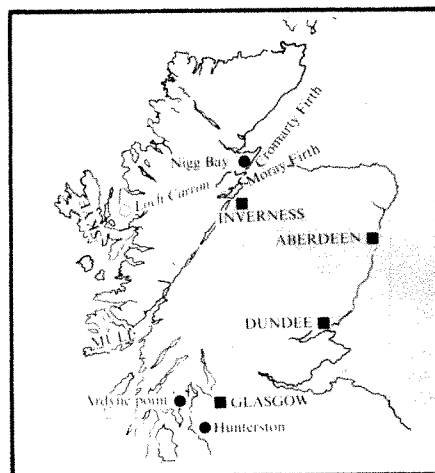
None of the Loch Carron area sites satisfies these criteria so the environmental groups opposing the Loch Kishorn application are hopeful.

But Mr Varley has put the Condeep platform design on his list of 'approved designs' for which he is willing to acquire sites. For his purposes, approved means most favoured by the oil companies. And the only place in Scotland that Condeep can be built is at one of the Loch Carron sites, which have exceptionally deep water close to a flat shore area.

For some time Condeep was thought to be the cheapest and best design for the deepwater oilfields but recent events have proved that this may not necessarily be so, and the image of Condeep as the saviour of the national economy is being challenged from several sides.

A spokesman for the Conservation Society said that as far as the Loch Carron area was concerned, Mr Varley's proposals were "irrelevant" because the society feels that the Condeep platforms are unnecessary. They point to the very successful French design which McAlpine is building at Ardyne Point on the Clyde near Glasgow. This Seatank platform can operate in 600 feet of water but unlike the Condeep does not need very deep water close to the building site, and can be towed some distance for final assembly.

Another blow to claims that the Condeep design is uniquely suitable for deepwater operations came when Shell ordered an Andoc concrete gravity platform for its deepest water site in the Dunlin field and Signal Oil chose a steel design for its Thistle field because seabed conditions proved unsuitable for gravity platforms. Diffi-



culties have also been experienced during the construction of Condeep platforms, at the time when the base is submerged. It was found that when submerged, one of the hollow base cells cracked under the water pressure and the design had to be modified so that the cells could be filled with concrete to overcome this.

Mr Varley's proposals could prove useful, however, in preserving the continuity of work at east coast sites. McDermott at Ardesier near Inverness has no more orders and the workforce faces redundancy as soon as present work is finished in a relatively short time. Mr Varley may find himself faced with the need to use his intended powers of restoration of the site and redeployment of the workforce sooner than he thinks. □

Infrared telescope for space shuttle

THE NASA Ames Research Center has awarded a \$400,000 contract to Hughes Aircraft Co. for a design study to be carried out for an infrared telescope which could be flown in the space shuttle. The contract specifies a pointing accuracy of 1', and is expected to have an aperture in the range 1 to 1.5 m, rather larger than the 36-inch instrument which is the main tool of NASA's existing flying observatory and is mounted in a C-141.

According to a report in *Aviation Week & Space Technology* (August 19), Michel Bader, deputy director of astronautics at Ames, describes the shuttle facility as "an extension of the C-141 programme"; but the new instrument will have cooled optics and detectors, as well as appropriate changes for zero-g operation and other problems specific to the shuttle.

Grumman Aerospace Corporation will be working with Hughes on some aspects of these problems, and the study will take at least a year. Work on the hardware may not begin until well into 1977. □

Sweden's reactor problems

from Wendy Barnaby, Stockholm

THE Swedes have not yet been notably successful in the efficiency of their nuclear technology. Current frustrations in the eight-reactor building programme now under way include altering the cooling systems of two reactors, replacing a fatigued metal part in another and putting up with delays in construction in a fourth. And there was, of course, the famous Marviken reactor, whose design was so faulty that it could never be made operational and was converted to work on oil. (Critics of this waste of money labelled the project "the first oil-fuelled nuclear reactor in the world"; a designation quickly adjusted, during the oil crisis, to "the world's first wood-fuelled-oil-fuelled nuclear reactor"). But now it seems that Sweden's nuclear power programme is running into difficulties of quite another sort.

Until the last couple of years, the large-scale development of nuclear energy in Sweden was accepted by the country without controversy. The consumption of electric power was predicted to increase three-fold by 1990, but expansion of hydro-power, which provides 70% of all Sweden's electricity, was regarded as a non-starter for environmental and economic reasons. Coupled with Sweden's poverty in coal, oil and natural gas, this problem put a high priority on the development of alternative energy sources. Nuclear power was the obvious choice. The government drew up plans to build 24 reactors by 1990. The country was to approach the next century as the world's leader in reactor capacity per capita.

All this had reckoned without the activities of an increasingly vocal anti-nuclear power group, whose agitation caused parliament in May 1973 to suspend approval of the official plan until it had been presented with more comprehensive information about reactor safety and radioactive waste. The parliamentary decision did not affect the reactor in operation since 1971 or those already under construction, but postponed consideration of the future of the other sixteen until next year. The advanced state of the programme has, in effect, solved the question of whether to have nuclear power. At issue now is how much Sweden should have in the light of current safety measures.

The fact that Sweden has not yet signed a safeguards agreement with the International Atomic Energy Agency under the Nuclear Non-proliferation Treaty, and the significance that could have in a large-scale peaceful nuclear

programme (especially after the explosion of India's bomb), has not been at issue. To begin with, the Swedes are expected to initial a safeguards agreement next month; but, more importantly, all the parties to the dispute agree that Sweden will not manufacture nuclear explosives.

The dispute has caused an odd political line-up. The ruling social democrats and the conservatives both favour the large-scale development of nuclear energy, while the agrarian and communist parties are against it. The anti lobby claims widespread public support, quoting as evidence a survey commissioned by the State Power Board and private producers of energy last January, at the height of the fuel crisis, to find out whether people favoured nuclear power or not. The results were not made available to the press. Not until a 'Friends of the Earth' group forced their publication in June could it be ascertained that 59% of the sample had in fact opposed nuclear power. In spite of this support, however, the anti group is worried about the plentiful funds being made avail-

able for pro-nuclear pamphlets and educational material which will be circulated to union study-groups this autumn.

Autumn will also bring publicity for the anti group, however. A number of citizens living near a new reactor site will appeal against the decision of a so-called 'Water Court' to allow the construction of the reactors planned for the site provided that certain measures are taken to safeguard the environment. Under a law outdated since these events began, such a court was obliged to review the potential dangers threatened by any proposed building to the water environment before permission could be given for the building to be constructed. The action will take place in the court of appeals dealing with questions involving water, and the argument will of necessity be limited to those aspects of nuclear power dependent on water. The public can therefore expect to hear a lot about dangers involved in a failure of the emergency cooling system and the pollution of waterways with radioactive material, but little about other nuclear hazards.

Inflation and Imperial College

by Roger Woodham

AT a time when many British universities, notably Leeds, are reporting that they are in the red by hundreds of thousands of pounds, the Imperial College of Science and Technology in London is still firmly in the black, thanks to some wise financial management a year or so ago.

In the year ended July 1973, for example, the college spent some £11.8 million but managed to salt away £635,000 to swell its reserves to £767,000. This was achieved by obliging all departments to reduce their budgets by 6%. The result was that earlier this year, with plenty of money in the bank, the college was able to embark on a modest expansion programme when many other universities were watching their margins closely.

The position now is that the reserves stand at about £250,000 and the college is faced, like other universities, with a government decision to be less than generous about supplementing the University Grant Committee's recurrent grant to allow for inflation. The government, assessed the situation several months ago on the basis of the rise in costs during the calendar year 1973, with a view to paying the extra

money during the academic year 1974-75. The first estimate of the increase in costs came out at 7%—some £13.5 million—and the government declined to give the universities any more money at all. But the increase in the index was subsequently revised upwards to 10%+, representing more than £21 million. At this stage the government announced that it would provide £4 million which, together with about £3 million which the UGC had at its disposal for emergencies, just kept the total deficit to the level the government originally envisaged.

Although the Committee of Vice Chancellors and Principals welcomed the government decision to make the extra money available, its view is that the recurrent grant should be restored to its proper level and its value then maintained in real terms.

What Imperial College is now waiting to find out is how much of the £4 million will come its way. On previous experience the amount will be in the region of £150,000 to £200,000, but Mr M. J. Davies, secretary of the college, said last week that the extra money would at best keep the ship afloat and would not allow for any growth. He also described the college's remaining surplus of around £200,000 as a cushion which is rapidly disappearing. If things get much worse, he said, Imperial College will be in the same situation as other universities.

Binary weapons voted out

by Colin Norman, Washington

THE Pentagon's plan to replace its ageing stockpiles of chemical weapons with a new generation of so-called binary nerve agents received a severe blow in the House of Representatives last week, and it now seems likely that Congress will eventually kill the plan entirely. Virtually unnoticed in the welter of events being played out in Washington, the House voted to deny funds for the Army to begin producing special 155-mm artillery shells for the binary weapons.

The vote, which came on an amendment to the Defense Appropriation Bill, represents sweet victory for a group of congressmen, led by Wayne Owens of Utah, who have fought the binary programme for months on the basis that if it were allowed to go ahead it would torpedo international chemical disarmament negotiations now taking place in Geneva.

The Pentagon—or, more accurately, the Army Chemical Corps—had requested \$5.8 million this year to begin producing binary shells in the Pine Bluff Arsenal in Arkansas, with a view to replacing part of its nerve gas stockpiles with binaries in 1977. The Army began touting the advantages of the weapon late last year, by arguing that since they consist of two "relatively non toxic" components, they will be safe to store, transport and use. The idea is that the two components would be kept apart until needed on the battlefield, then they would be loaded into the binary shell and mixed together to form a lethal nerve agent.

But the House was persuaded last week that if the United States now launches a massive new chemical weapons programme, the credibility of United States negotiators in Geneva would be destroyed and the chances of reaching agreement on international chemical disarmament would be negligible. Last month Mr Nixon and Mr Brezhnev signed an agreement in Moscow to launch new initiatives to help the negotiations along, but the binary weapons programme is scarcely seen as a helpful initiative.

Another factor in last week's vote was that the Pentagon had asked for funds from Congress before the binary programme had even been approved by the Administration, a situation which several congressmen regarded as putting the cart before the horse. In fact, since Congress started considering the funding request, the Administration has been carrying out a review of the necessity for binary weapons, and the programme has already picked up strong opposition from the Arms Con-

trol and Disarmament Agency. Thus, the Pentagon's strategy seemed to be to try to get approval for the programme from Congress and then use that to bolster its case with the Administration.

Last week's House vote was, however, far from the last word on the matter, for the Senate has yet to consider the Pentagon's budget request. But it is generally believed that the anti-binary forces in the Senate will be strong enough to uphold the House's action. For one thing, an amendment will be offered in the Senate Appropriations Committee to cut off funding of binary weapons, and if that fails a number of influential Senators led by Edward M. Kennedy are prepared to take their case to a vote on the Senate floor. □

Windfall for natural resources

THE Wolfson Foundation recently announced its £1 million programme of support for research into the more efficient use of natural resources. The object of the programme is to come up with at least some answer to the problems of Britain's dependence on imported food, raw materials and energy.

This is a departure from the foundation's previous practice of giving grants for university research linked specifically to industry. The foundation received more than 150 applications for money, and in choosing the 18 projects which make up the present programme it was particularly anxious that ideas should be quickly and easily realisable commercially if the research was successful.

The projects chosen cover a wide field. Predictably, studies on various sorts of recycling and uses of wastes are much in evidence, and on the energy front there are two concerned with the use of solar energy. But money has also been found for work on the impact of motorways on agricultural land, and on cultivating the scallop, a potentially valuable export.

The Department of Agriculture at the University of Reading gets the largest slice of the cake. It has been given £250,000 for two pieces of work: one to improve the production and direct use of green leaf protein, and one for improving the production of oil and protein from seed crops. Together with a complementary study at the University College of Wales, Aberystwyth, on breeding grasses and legumes for upland areas, these lines of research could eventually lead to far more home-grown feedstuffs for Britain's animal and even human population. □

Ecological railway line

from Vera Rich

THE new branch of the Trans-Siberian Railway, which is to run via Ust-Kut on the Lena, skirting the north of Lake Baikal, and across some 1,500 km of the Siberian taiga to join the existing tracks at Komsomolsk on the Amur, is clearly being treated as a prestige project by the Soviet media, which faithfully report every step in its progress.

This publicity is, perhaps, not surprising, since the projected line will run through some of the most promising of the undeveloped areas of Siberia. The line will, however, pass through a region which is a constant focus of attention for ecologists.

Lake Baikal has, of course, been subject to railway 'pollution' since the trans-Siberian route was envisaged in 1891. Indeed, in the early days, the track actually ran across the lake—rails were laid on the ice in winter and ferry boats were used during the summer (with a twice-yearly break in service during the thaw and freeze-up).

Conservation has now become an emotive concept, and the planners of the new line have not only taken it into account in their proposals for the line, but have also taken considerable care to be seen to be taking it into account.

Mikhail Reks, the chief engineer of the Baikal project, has assured world opinion (through the Novosti agency) of the environmental protection measures to be taken. No tree felling is to be permitted on slopes with gradients exceeding 15°, or in valley bottoms, to obviate erosion caused by avalanches and mud streams. Special dams and trenches in permafrost areas will protect the soil—as well as ensuring the safety of the railway itself. The banks of the reservoir on the River Zeya are to be reinforced, and it is stated that the massive bridge-building programme involved (over 140 bridges in all) will tend to stabilise the channels of the Siberian river beds, and thus reduce erosion.

The wild life of Siberia is to be left as undisturbed as possible—although Reks admitted that the line will to a certain extent "press back" many species. One wonders however, how the construction workers themselves will react to the ruling on insect life. For, in order to disturb the ecological balance as little as possible, no insecticides may be used, not even against the traditional curse of Siberian life—mosquitoes. The only means of self defence permitted, according to the official ruling, will be mosquito nets and special repellent creams. □

correspondence

Chile: for . . .

SIR,—I have recently read your editorial of May 3, on academic freedom in Chile, and would like to make some comments on it.

This is the third occasion I have come to Chile to lecture, the previous visits being in 1970 (before Allende's election) and 1971 (under the UP government). I also lectured here in 1965 (shortly after Frei's election) but not with a contract. I cannot claim to know the state of all the branches (*sedes*) and faculties of the University of Chile or other universities, but I am tolerably well acquainted with what goes on here. I can assure you that as far as this department is concerned, your article is almost totally untrue.

I have seen no military in or near the Faculty in my two months here, although I do understand that they searched it for arms a couple of days after the coup, since when they have not returned. Not one member of staff of this department has been dismissed for political or any other reasons.

Actions 'such as not taking part in the anti-Allende demonstrations' are not 'now retrospectively considered illegal'. Hardly anyone in the department ever took part in such actions. On the contrary a considerable number of the staff and perhaps 50% of the students regularly took part in pro-Allende meetings or demonstrations. To all those UP sympathisers or militants (not to mention the apolitical) who stay unchanged in their academic posts, your suggestion that they 'remain at the universities in puppet roles' is insulting. You conclude the phrase by adding 'organising research according to the junta's decree, teaching what the junta thinks fit to be taught'. The courses here are submitted to no one, military or otherwise, for approval. My own course has not been reviewed even by the director of the department.

Your report must certainly be true for some academic institutions (because I cannot believe the entire international press is misinformed) but it is nowhere near the whole truth. Either you too easily believe the statements made in Europe by refugees, genuine or otherwise, without attempting to verify them, or you are not concerned with straight reporting and are motivated by political considerations. If the latter is true and *Nature* has ceased to be a scientific journal to become a political one, may I, as an Irishman,

suggest you focus your concern about military misbehaviour on your own army for which you are indirectly, and many British scientists directly responsible, rather than on the army of a country on the other side of the globe where you apparently have never set foot.

I am no apologist for the military junta; on the contrary I am a strong supporter of the ideals of social justice which Allende claimed to support. However honesty forces me to admit that the most charitable interpretation of the cause of his regime's collapse was its catastrophic incompetence. The chaos that reigned in 1973 would have provoked a coup in any other Latin American country, and most European ones too. That said, and recognising the impertinence of making comments on the internal affairs of a foreign country, I unreservedly condemn the brutalities, vengeful actions and plain stupidities attributed to the authorities at various levels since September 11. Perhaps, following the above, I can best demonstrate my conviction that academic freedom and 'intellectual life' in Chile is *not* 'dead' by signing myself openly

Yours faithfully,

W. F. L. PURSER

*Universidad de Chile,
Santiago de Chile*

. . . and against

SIR,—The Chilean replies to the letters published in *Nature*, criticising the regime, although insulting the intelligence of your readers, underline the spirit of a totalitarian regime, a trivial phenomenon these days. However, there is no necessity to refute arguments which only underline how ideological fanaticism can deform judgement.

It seems more important to stress the need for the scientific community, which is rightly reluctant to become involved in political controversy, to assert that it cannot remain indifferent when individual freedom is at stake under any politico-economical order, religious or social prejudice. It is a matter of dignity for scientists to know that the torturers' representatives be at least excluded from their community, if more drastic action cannot be taken.

As for those, whether nationals or 'multinationals', who support regimes of the Chilean or Czechoslovakian type, they would be well advised to have the decency to spare us the nausea of their

public apologetic justifications. They might also be advised to consider that Portugal and Greece are perhaps not just mere accidents.

Yours faithfully,

DIMITRI VIZA

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Faculté de Médecine Pitié-Salpêtrière,
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Publish or perish

SIR,—How can we escape from the tyranny of the Science Citation Index (how many citations did your papers get last year?) and the general publish-or-perish rat-race?

I suggest a procedure by which a stable and well-established department might contract out. Let all papers from the department be published under the same fictitious name, as is done by the pioneer French school of mathematicians who are Nicholas Bourbaki — a general who, when defeated, tried to shoot himself but missed. What effects might follow?

We might build one substantial scientist out of several mediocre ones, whose success might encourage the others. As Blackett has pointed out: "a first-class laboratory is one in which mediocre people can do outstanding work".

The Matthew Principle (Matthew, 25: 29) of R. K. Merton, "to every one that hath shall be given . . ." will be turned to general advantage since $(A + B + C + \dots)^x > A^x + B^x + C^x \dots$ (if $x > 1$).

The general standard of papers might be increased and their numbers reduced by taking off some of the pressure on the individual to rush into print.

Multiple subscriptions to journals and societies could be reduced.

The promotion scheme based on published papers would be confounded, perhaps forcing the consideration of persons as persons.

There has been much talk of the commonwealth of science, but who will be the second to set up a scientific commune? I am sure that common scientific property will be as strongly opposed by the establishment as the commonality of the property of Christians was opposed by the Church of England (Article 38).

Yours faithfully,

ALAN MACKAY

*Department of Crystallography,
Birkbeck College,
London*

news and views

Transfer RNA revisited

It is now some six years since the first successful crystallisation of transfer RNA was reported by a group of workers at the MRC Laboratory of Molecular Biology, Cambridge (*Nature*, **219**, 1222; 1968). Subsequently a large number of both mixed and purified tRNA crystals have also been obtained. There has, however, been a central problem in obtaining crystals of sufficient quality. Only this would enable an X-ray structural analysis to provide a detailed picture of the molecular architecture of tRNA. In 1971, Kim, Rich and their associates, at the Massachusetts Institute of Technology, reported that they had obtained highly diffracting crystals of the tRNA from yeast which codes for phenylalanine (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 841; 1971). These workers then proceeded to analyse these crystals, and reported obtaining electron density maps at successively 5.5 Å and 4.0 Å resolution, culminating in a 3.0 Å map using the standard isomorphous replacement methods of protein crystallography (*Nature*, **248**, 20; 1974). Each improvement in resolution enabled the form of the molecule to be visualised more clearly, and their final electron density map showed the molecule to have an L shape, with two stems of double helical oligonucleotides in each arm. The polynucleotide chain was clearly seen, except in the nonhelical loop regions of the molecule.

The tRNA species that the MIT group has been investigating crystallises in many (up to twelve) distinct crystal forms. This intense polymorphism raises the question whether it is a reflection of merely alternative packing modes within the crystal lattice, or a consequence of conformational changes within the tRNA molecule. Cramer *et al.*, in a thorough examination of crystallising conditions for eight of these polymorphs, have remained undecided on this question (*Biochim. biophys. Acta*, **349**, 351; 1974). The MIT group has attempted an answer by extending its original analysis, on an orthorhombic form, to the monoclinic form. Both of these polymorphs diffract X rays well, to a resolution of better than 3 Å. So it is reasonable to suppose that the molecules in both forms are packed reasonably well. Furthermore, the two forms are related in terms of unit cell dimensions, suggesting closely related structures. Kim *et al.* used simple molecular replacement procedures in order to see how their orthorhombic molecule fitted the data from the monoclinic crystals. They obtained reasonably good fits and concluded that the structure was essentially unchanged on going from one form to another. The only proviso was that there were possibly some small undefined changes in the region of the anticodon loop.

The Cambridge (England) group has now reported their independent 3 Å analysis of the monoclinic form (Robertus *et al.*, *Nature*, **250**, 546; 1974). Again, the method of isomorphous replacement has been used to solve the structure. The outline shape of the molecule was obtained by tracing the ribose phosphate backbone, particularly in the four stem regions where it was most discernible. Clearly, compared with a polypeptide backbone in a protein, polynucleotide chains are considerably more difficult to follow. This is mainly because of the close similarity of ribose, phosphate, purine and pyrimidine groupings when seen at a resolution of 3 Å. It did not prove possible to trace unambiguously and

completely the chain in the non double-helical D and T ψ C loops; the MIT group has similar difficulties in these regions of the molecule. The monoclinic form is very similar in overall shape to the Cambridge (Massachusetts) orthorhombic findings. The fact that the former shape has been called a T, and the latter an L, is to some extent a matter of semantics. There are, however, a large number of significant differences between the two structures. Perhaps the most important concerns the orientation of one of the double-helical stems, the so-called D stem, which differs in the two by 90° in one direction. It is noteworthy that the main helix directions now all agree well with Levitt's helix search locations (*J. molec. Biol.*, **80**, 225; 1973). The relative placing of the other three stems is not disputed. There is a disagreement about the fitting of the nucleotide sequence to the chain, by one (and sometimes two) residues, virtually throughout the entire molecule. Robertus *et al.* consider that these shifts in the chain assignments have necessitated revision of many of the earlier structure conclusions. For example, whereas the helical stems were previously considered to be irregular, they are now found to conform to the standard classes: the amino acid and the T ψ C stems together form a twelve base-pair-long double helix of the 'A' form. The functionally important anticodon loop seems to have more order than previously described with stacking on the 3' side of its stem. This, together with the quasihelical geometry of the anticodon bases, is one of the many satisfying features of the new model. The Cambridge (England) group detail the features of the molecule which help to maintain its structure. These are additional base pairings, and also base triplets—many of a novel nature—as well as numerous stacking and intercalative interactions.

These tertiary interactions cumulatively have the effect of folding and maintaining the familiar tRNA cloverleaf into a molecule with essentially three major interlinked substructures. These are, firstly, the long, almost perfect double helix formed by stacking the acceptor and T ψ C stems. The D stem is augmented by several unusual base pairs and postulated triplets, some involving their own loops, and some the variable loop, and is attached to the long helical segment almost at the middle. This forms the T junction. The anticodon stem then extends outwards from the augmented D helix, with the helices separate and non-collinear. Indeed, the anticodon helix (with its loop at the far end of the molecule) seems almost to be hinged on to the D stem, with a consequent implication of mobility, possibly during protein synthesis.

This new model has also been examined for consistency with the extensive chemical modification data which now exist for tRNAs (Robertus *et al.*, *Nucleic Acids Res.*, **1**, 927; 1974). It has now been assumed that bases susceptible to modification are those not involved in tertiary interactions. On this basis, double-helical residues would be unaffected, as indeed they are. Similarly, those bases involved in the tertiary interactions detailed in the X-ray model would not be modified, as indeed they are not. The reactive bases are the ones that are not only uninvolved in tertiary interactions but are on the surface of the molecule. The model is also of interest in that it suggests that many of its structural features are common to all tRNAs. The bases which are either invariants in all tRNAs, or are conserved as purines or pyrimidines, are mostly involved in the tertiary interactions. Thus, the pattern of

molecular folding, so dependent on these, may well be at least similar for the majority of tRNAs.

The Kim and Rich group have very recently made an additional contribution to the subject of tRNA structure (*Science*, **185**, 435; 1974). In what may be seen as a counter to the criticisms of their structural model, they have presented a comprehensive analysis of its tertiary structure. This necessitated revision of a number of the residue assignments, although the overall chain tracing remains generally unaltered. The tertiary base-pairing and stacking interactions now found are mostly identical to those in the Cambridge (England) model, although a number of differences in interpretation still remain. In general the two models are now very similar. Consequently, it is now possible to state, with some considerable measure of confidence, that the basic structure of a transfer RNA has been established.

STEPHEN NEIDLE

Molecular neuroanatomy

NEUROANATOMY has relied for many years on a limited number of classical staining methods, the chemical bases of which have remained largely obscure. During the past few years, however, the field has seen a quiet revolution as several powerful new techniques have developed as a result of a greatly increased understanding of the special biochemical properties of nerve cells. Many of these new methods make use of the fact that neurones of different types are biochemically differentiated in the sense that each type manufactures and stores only one of a series of potential transmitter substances. Great progress has stemmed, for example, from the development of a histochemical fluorescence technique for visualising catecholamine and indolamine-containing neurones and their fibres (Falck, *Acta physiol. scand. (second suppl.)*, **197**; 1962).

Such specific staining methods have so far not been available for neurones using other transmitters. One approach of general applicability is the development of immunofluorescent histochemical techniques, using antibodies directed against some protein that is characteristically localised in a given transmitter-specific type of neurone. Thus, antibodies to the enzyme dopamine- β -hydroxylase have been used to identify noradrenaline-containing neurones in tissue sections (Fuxe *et al.*, *Progr. Brain Res.*, **34**, 127-128; 1971). Progress in the application of such methods to other transmitter types has been reported recently. Saito *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 269-273; 1974) describe the use of antibodies to the enzyme glutamic decarboxylase for the immunofluorescent identification of neurones that use gamma-aminobutyrate (GABA) as their transmitter and Eng *et al.* (*Nature*, **250**, 245-247; 1974), McGeer *et al.* (*J. Pharm.*, **5**, Suppl. 1, 54; 1974) and Rosser *et al.* (*FEBS Lett.*, **36**, 43-48; 1973) have prepared antibodies to purified choline acetyltransferase and described their use for the identification and mapping of cholinergic neurones in the central nervous system (CNS). These neurones have hitherto been identifiable only by histochemical methods for the enzyme acetylcholinesterase; the localisation of the biosynthetic enzyme choline acetyltransferase, however, promises to be a more specific and reliable biochemical marker for such structures, since this enzyme occurs exclusively in cholinergic neurones in the CNS.

These new methods for identifying transmitter-specific neuronal pathways have been and will continue to be of immense value for mapping the distribution of such pathways in the CNS. At the same time two other approaches,

which represent more general means of determining the axonal projection of groups of neurones in the CNS, have been developed. Both approaches make use of the specialised transport systems that exist for the movement of substances along nerve axons. Protein synthesis in neurones is confined largely to the cell body region of the cell, and there is continuous export of newly synthesised material from the cell body along the axon processes to the nerve terminals. This orthograde flow of material can be used to trace the axonal projections of neurones by pulse-labelling the cell bodies with a radioactively-labelled amino acid and then following the transport of labelled protein into such projections. This usually involves a local microinjection of isotope in the region of a particular brain nucleus, followed by autoradiographic examination of the distribution of label in various potential areas remote from the site of injection (Cowan *et al.*, *Brain Res.*, **37**, 21-51; 1972). Another more recently recognised transport mechanism operates by as yet unknown means to transport certain proteins from the nerve terminals in the retrograde direction towards the cell body regions of neurones. By using an easily identified marker protein not normally present in the CNS, such as horseradish peroxidase, it is thus possible to determine the cells of origin of fibre tracts in CNS. This substance is applied by local injection into a projection area, and then peroxidase-containing cell bodies are identified in remote brain areas. (La Vail *et al.*, *Brain Res.*, **58**, 470-477; 1973).

Apart from methods that allow the mapping of populations of neurones organised in specific pathways, important developments in techniques that reveal the detailed anatomical features of individual neurones have also been made. Stretton and Kravitz (*Science*, **162**, 132-134; 1968) found that microinjection of the fluorescent dye procion yellow into single neurones of the lobster nervous system allowed the subsequent visualisation of even the finest branches of the axonal and dendritic tree emanating from such neurones. Procion yellow diffuses readily into such processes, and the neurone remains electrically excitable during the process. This technique has been widely applied to invertebrate and vertebrate neurones, with results of often breathtaking clarity (*Intercellular Staining Techniques in Neurobiology*, edit. by Kater and Nicholson, Springer-Verlag, Berlin, 1973).

Autoradiography has also been used to trace the dendritic ramifications of spinal cord motoneurones following intracellular injection of radioactively labelled amino acids (Globus *et al.*, *Brain Res.*, **11**, 440-445; 1968). In last week's edition of *Nature* (**250**, 655-658; 1974) Pentreath and Cottrell described a further modification of this approach which depends on the injection of radioactively-labelled transmitter or transmitter precursor into single cells. This has the advantage that the transport of transmitter molecules along axons occurs more rapidly than that of labelled proteins and a higher proportion of the injected material may be transported over quite long distances in this manner. Pentreath and Cottrell worked with a large neurone in the cerebral ganglion of the snail *Helix pomatia*. There is one such giant neurone on each side of the ganglion, and the cells are known from previous neurophysiological and biochemical studies by the authors to use 5-hydroxytryptamine as their transmitter. After the microinjection of tritiated 5-HT or the precursor 5-hydroxytryptophan, using electrophoretic expulsion from the tip of a fine glass electrode inserted into the cell body, the labelled amine was rapidly transported away from the cell body into the axonal system. Within 10-15 h after injection label had penetrated into the fine terminals of the neurones, some of which were several centimetres from the site of injection. Using autoradiographic analysis of serial tissue sections the entire axonal projection could be mapped. The projection from each giant neurone was found to be complex, consisting of several large axonal processes and numerous smaller

branches. Some axons projected to the buccal ganglion, in which 'follower' cells have previously been identified in neurophysiological studies, others crossed the midline, and several terminated in various muscles concerned with feeding behaviour in the mollusc.

In addition to light microscope autoradiography, Pentreath and Cottrell were also able to use electron microscopic autoradiography to investigate the fine structure of some of the labelled terminals. All of the injected label appeared to be confined to the injected neurones, and this method thus offers a relatively rapid and highly specific means of

determining the various presynaptic terminals emanating from an identified single neurone.

It is clear that neuroanatomy has felt some of the impact of the molecular biology era, and that the battery of new techniques that have recently become available will stimulate an upsurge of activity in this area. Such a revival will come none too soon if one is to begin to understand the functional significance of the various chemically specific pathways of neurones that are now known to exist in the CNS.

L. L. IVERSEN

Clock and calendar in SI units

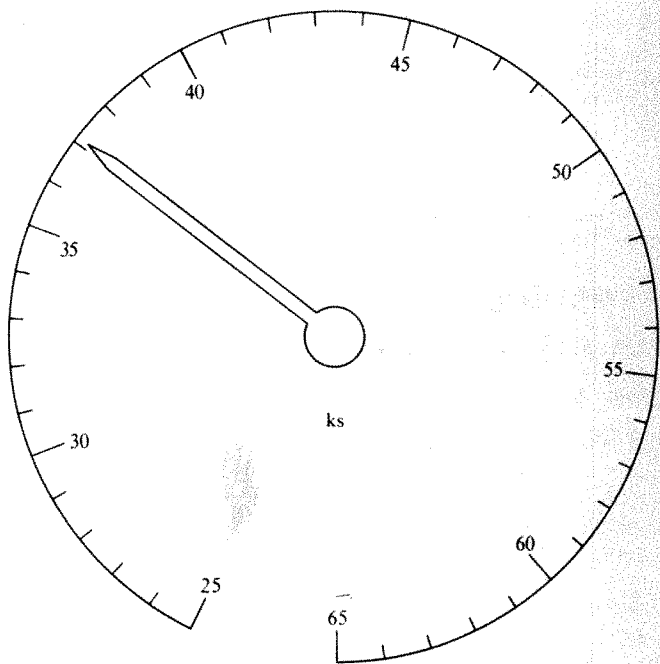
IN the SI system, one gives the time of day and date in kiloseconds, megaseconds and gigaseconds. The appropriate daytime clock has no minute hand and has a new face graduated 25–65 ks (see figure); if the range 0–86.4 ks was completed, the clock would also be suitable for night use. One's schedule might be: 25 ks, breakfast; 30 ks, start work; 45 ks, lunch; 60 ks, stop work; 65 ks, supper; 80 ks, retire.

On the calendar (see Table), one dates by the megasecond, stating a number under 100 with one decimal place. This increases daily except Sunday by 0.1 Ms. Thus, the eight days July 3–10, 1974, are 77.6, 77.7, 77.8, 77.9, 77.9+, 78.0, 78.1, 78.2 Ms. A + distinguishes Sunday. 0.1 Ms, however, must also be added on six Sundays out of 125 (since 1 week = 0.6 Ms = 4.8 ks). The megasecond date then never differs from the actual time each day begins by more than 90.4 ks. After making a correction for this difference, one adds the time of day (see figure) to fix any instant absolutely.

This continuous calendar does not begin anew each year. Subtracting the megasecond at the new year from the current megasecond gives the time of year, useful in astronomy, weather, biology and farming. To place a date in history, one states the gigasecond to the nearest 0.1; for 1972–74 this is 62.2 Gs.

The many advantages include elimination of timers graduated in diverse units, and of unsystematic derivatives such as revolution per minute and kilowatt hour. Moreover, 62 278.2756 Ms has fewer symbols and easier arithmetic than 11:40 a.m. July 10, 1974. Neither change in work schedules nor internationally agreed reform are needed; scientists and engineers can begin to use a SI clock and calendar today.

CHARLES E. CHAFFEY



Partial SI calendar								Date 1974
	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	
Ms	77.3+	77.4	77.5	77.6	77.7	77.8	77.9	July 6
ks	69.6	56.0	42.4	28.8	15.2	1.6	-12.0	
Ms	77.9+	78.0	78.1	78.2	78.3	78.4	78.5	July 13
ks	74.4	60.8	47.2	33.6	20.0	6.4	-7.2	
Ms	78.5+	78.6	78.7	78.8	78.9	79.0	79.1	July 20
ks	79.2	65.6	52.0	38.4	24.8	11.2	-2.4	
Ms	79.1+	79.2	79.3	79.4	79.5	79.6	79.7	July 27
ks	84.0	70.4	56.8	43.2	29.6	16.0	2.4	
Ms	79.7+	79.8	79.9	80.0	80.1	80.2	80.3	August 3
ks	88.8	75.2	61.6	48.0	34.4	20.8	7.2	
Ms	80.4+	80.5	80.6	80.7	80.8	80.9	81.0	August 10
ks	-6.4	-20.0	-33.6	-47.2	-60.8	-74.4	-88.0	

Add 62.2 Gs. New Year was 61.8 Ms. The beginning of each day is the sum of the large figure (in Ms) and the small figure (in ks).

Primitive but not simple

THE study of human embryonic haemoglobin, initiated principally by Huehns and his collaborators, starts from the demonstration that biosynthesis of a 'primitive' or ε polypeptide chain precedes that of the γ chains of human foetal haemoglobin (Hb-F, $\alpha_2\gamma_2$). At birth Hb-F normally comprises some 80% of the total haemoglobin, the remainder being adult haemoglobin (Hb-A, $\alpha_2\beta_2$), as the biosynthesis of β chains is switched on, and that of γ chains starts to decline at 6 months' gestation. The ε chain occurs as Hb-Gower 1 (ε_1) and Hb-Gower 2 (ε_2) and its formation has completely stopped at a gestational age of less than 3 months. There is also evidence that synthesis of the α chain is preceded very early in gestation by another type of chain, originally denoted as x but now designated ξ . This chain is found in the haemoglobin species Hb-Portland 1 with composition $\xi_2\gamma_2$, believed to be present in early human embryos, and also found in stillborn infants

with erythroblastosis foetalis caused by homozygous α -thalassaemia 1, where α -chain synthesis is completely inhibited.

The practical problems associated with the study of human embryonic haemoglobins can be circumvented in animal studies, and Melderis, Steinheider and Ostertag, of the Max-Planck Institute for Experimental Medicine, Göttingen, now report (see page 774 of this issue) evidence for a unique x-type globin chain in early mammalian embryos (BALB/c mice and New Zealand white rabbits). The x chains of these two species have closely related sequences, similar to that of the human ξ chain of Hb-Portland 1. Detailed analysis of the complex systems—three embryonic species in the mouse and six in the rabbit—leads to the conclusion that there are at least two different ε chains, and that x- and α -chain synthesis in both species is inversely correlated, hence the first chain takes the place of the second during early embryo-

genesis. The x chain is now to be regarded as a α -type chain, and there is more sequence similarity between x chains of different species, than between the x and α chains of the same species. This indicates an early evolutionary divergence of x- and α -chain genes and constitutes a further example of globin gene duplication during evolution; compare the findings of Schroeder, Huisman and colleagues on the duplicated $^6\gamma$ and $^A\gamma$ genes for the γ -chain of human Hb-F.

The Göttingen work confirms the indication from the earlier work on human embryonic haemoglobins that there is an α -type x (or ξ) chain synthesised in the nucleated erythroid cells derived from the yolk sac during early stages of mammalian embryogenesis, and preceding the synthesis of α -chains proper. The presence of an ε_1 species in human but not in mice or rabbit embryos remains to be explained.

From a Correspondent

Topology of crystal grains

from Robert W. Cahn

It is one of the paradoxes of the history of science that the rigorous quantitative treatment of the behaviour of large populations of molecules preceded by many years the similarly rigorous description of the individual molecules themselves; yet the kinetic theory of gases implies the extraction of orderly and predictable behaviour from myriads of random motions and collisions, whereas molecules are all identical. The resolution of the paradox lies in the fact that in the kinetic theory, molecules are treated virtually as independent, featureless particles, and therein lies the tractability of the whole approach. A million particles spell simplicity; one molecule spells complexity.

With crystals, the sequence is reversed. A large number of crystal structures had been accurately determined before the collective behaviour of populations of crystals began to be understood. The metallurgist recognises two distinct forms of such collective behaviour: first, there is 'Ostwald ripening', a poetically metaphorical term for the Matthew principle applied to a population of spherical crystallites of varying radii dispersed in a matrix; the larger ones grow at the expense of the smaller, essentially because of a radius-

dependence of the solubility of the particles in the matrix. (Water droplets in a cloud behave in an essentially similar way.) The rigorous treatment of this very important metallurgical process is now well understood.

The second form of collective behaviour is grain growth. A piece of a pure metal consists of a population of crystal grains all sharing the same crystal structure but of different sizes, shapes and orientations. The grains grow from independent nuclei and as they impinge, grain boundaries are formed. The interfaces may be plane or curved, and bear no relation to the regular lattice arrangement of atoms: "There's no art to read the grain's construction in the face". Here, also, the Matthew principle operates: if a piece of metal is heated, the larger grains grow at the expense of the smaller through the migration of grain boundaries, and the average grain size progressively increases.

Grain growth has been studied micrographically for many years, both for its intrinsic interest and because of its practical influence on metal working, on the behaviour of nuclear fuel elements, in powder metallurgy and elsewhere. It has long been recognised that the mean grain diameter varies with time according to $\Delta D = kr^n$, where n falls in the range $1/3$ – $1/2$. The problem is to understand why. It is a conceptually more difficult problem than the kinetic theory of gases, because

crystal grains are not independent and separate particles, but connected polyhedra. When one grain changes shape and size, the neighbours of necessity change too. An assembly of grains is like a nuclear reactor: a change in neutron absorption in one small corner soon leads to a change in neutron flux throughout the reactor. Similarly, an instability at one corner of one grain quickly spreads through the population.

The key to an understanding of the problem is its topology. A population of polyhedral grains can be characterised by the number of grains, faces and vertices, and by the relations between these quantities. In particular, interest attaches to the mean number of faces per grain and the distribution of this number among the grain population. Topological principles were first applied, to assess the stability of a grain population, by C. S. Smith in 1952. He recognised that grain growth stems from disequilibrium at edges where three grains meet and at four-grain corners. Unless three boundaries meeting at an edge are mutually inclined at 120° the edge must move and the boundaries become curved. The curvature itself introduces instability; indeed, curvature and non-equilibrium edge and corner configurations are the fuels of grain growth. The nearest approach to equilibrium obtains in a population of fourteen-sided polyhedra of a particular shape.

In a brilliant paper Rhines, Craig and

Dehoff (*Metall. Trans.*, **5**, 413; 1974) apply topological analysis to grain growth in a highly illuminating way. Their article is based upon an experimental study of successive stages of grain growth in aluminium, using the technique of serial sectioning; the technique depends on a thorough mastery of the theory of quantitative metallography which allows two-dimensional measurements in the microscope to be converted into reliable three-dimensional geometrical statistics. In their laboratory at the University of Florida, the authors have honed this technique to a fine cutting edge. They are able to determine the total grain boundary area, mean boundary curvature, distribution of the number of faces per grain, as well as the true mean volume per grain, as a function of time.

By combining experiment with topological analysis, Rhines *et al.* are able to introduce the concept of a "structural gradient" which is essentially a measure of the mean deviation from equilibrium of the entire grain structure. It is equal to the product of the total mean curvature and the surface area per grain. This structural gradient determines the rate of steady-state grain growth, and remains unchanged because the form of the distribution of different grain shapes remains unaltered as grain growth proceeds. All this is made topologically clear by the authors. They also show in a simple and elegant fashion that the number of grains eliminated when a grain boundary sweeps through unit volume of material is a constant, independent of the mean grain size. This very important theoretical result will have a number of important applications in materials science (for instance, in connection with sintering of powders, in which grain growth plays a crucial part).

By putting the various parts of the analysis together, the authors find both theoretically and experimentally that grain growths follow the law $\Delta D = kt^{1/3}$, when the 'diameter' D is expressed as the cube root of the mean grain volume. The apparent grain diameter derived from intercept analysis on a single two-dimensional section gives an index rather higher than $1/3$, which indicates that good quantitative metallography requires more expertise than most investigators possess. The authors point out that "it has not been required, in developing the foregoing rate law, to introduce any arbitrarily adjustable parameter, as has been done in the usual expression of grain growth kinetics". Theirs is an impressive achievement.

A recent attempt to apply purely statistical principles to grain growth (Louat, *Acta Metall.*, **22**, 721; 1974) is an instance of the type of analysis at which the Florida authors, by implica-

tion, cock a sceptical eyebrow. This is not to say that the statistical approach has no value: for instance, Weaire (*Metallography*, **7**, 157; 1974) shows how statistical arguments can be combined with topological ones to assess whether a distribution of grain shapes deviates locally from randomness.

Another recent article of striking originality on a related theme is by Morrall and Ashby (*Acta Metall.*, **22**, 567; 1974) who consider an assembly of fourteen-sided polyhedra in near equilibrium, as per Smith's specification, and then introduce a number of thirteen or fifteen-sided grains, or other more serious 'grain defects'. The grain structure is then denoted by joining the centres of all neighbouring grains, through their common boundaries, by a network of lines. These lines form a lattice ('lattice graph') with dislocations wherever there is a grain defect. These dislocations can move conservatively (using the term in the special sense of dislocation theory); this corresponds to grain displacement of the type found in superplasticity, without change in the number of grains. Dislocations can also move by climb, which implies the disappearance of some grains in 'real space' and therefore corresponds to grain growth. The authors build on an earlier analysis by Hillert of grain growth in two dimensions, where a set of perfect hexagons is disturbed by some rogue pentagons, and the like. For three dimensions, they relate, as did Hillert for two dimensions, the rate of grain growth to defect density, that is, to the density of dislocations in the lattice graph. They predict that if the defect density is constant, $\Delta D = kt^{1/2}$, while the index $< \frac{1}{2}$ if the defect density decreases during grain growth.

Morrall and Ashby's analysis represents an interesting link between the purely topological and purely statistical approaches. Since the Florida authors showed experimentally that the grain shape distribution is invariant with time (that is, the grain defect population is invariant too) Morrall and Ashby's analysis predicts $\Delta D = kt^{1/2}$, which is inconsistent with the experimental findings of the Florida group. The problem of grain growth has plenty of mileage left for those investigators who can master the requisite degree of subtlety.

Spartina's success in salt marshes . . .

from Peter D. Moore

THE grass genus *Spartina* is a remarkable one, both in terms of its worldwide success as an intertidal, salt-marsh species, and because of its very high production rates in such situations.

In the British Isles the spread of *Spartina anglica* (= *S. townsendii*), particularly during the early part of this century, is well known. The success of this species may be attributed to its remarkable capacity for strong growth over a wide range of levels in the salt marsh as well as to its salinity tolerance. Once established it forms dense clumps, which Ranwell has estimated may expand at a rate of 2% per annum on an area basis (*J. Ecol.*, **52**, 95; 1964). It is thus a powerful competitor, often forming dense, single species stands over wide areas.

On the east coast of North America there are several species of *Spartina* which show a tendency towards localisation within specific zones of the salt marsh. For one of these species, *S. alterniflora*, there are now a number of estimates for annual shoot production. Production rates, as indicated by above ground standing crop at the end of the growing season, are greatest in the southern States: for example, Teal (*Ecology*, **43**, 614; 1962) found standing crops of 1,290 g m⁻² dry weight in Georgia, whereas in Rhode Island Nixon and Oviatt (*Ecol. Monogr.*, **43**, 463; 1973) found 840 g m⁻². These figures are fairly representative of the falling production of the species as one moves north (see the literature review by Keefe, *Mar. Sci.* **16**, 163; 1972).

More detailed studies of the seasonal changes in productivity and the chemical composition of *S. alterniflora* in a New Jersey salt marsh have been carried out by Squiers and Good (*Chesapeake Sci.*, **15**, 63; 1974). Production in early spring was 8 g m⁻² d⁻¹ rising to 26 g m⁻² d⁻¹ in early summer. The peak standing crop was 1,592 g m⁻², but in late summer this trend was reversed and there was a net loss of dry matter at a rate of 13 g m⁻² d⁻¹. This loss could be caused by enhanced respiration rates relative to photosynthesis or, more likely, the death and loss of lower leaves. Since *Spartina* is not heavily predated by herbivores, the fall of litter represents the most important channel whereby the energy fixed in photosynthesis is passed on to heterotrophic organisms.

There is also a build up of protein, reaching maximum levels in the early spring. Presumably the plant has a strong demand for soil nitrogen at this period since this nutrient could become limiting to growth later in the season. Indeed, Sullivan and Daiber (*ibid.*, **15**, 121; 1974) have found that production rates in the dwarf form of *S. alterniflora* are limited by nitrogen supply in the growing season.

These data confirm the assertions of Odum that the salt marshes of the eastern United States are among the most productive habitats in the world. They also point to *Spartina* as a key

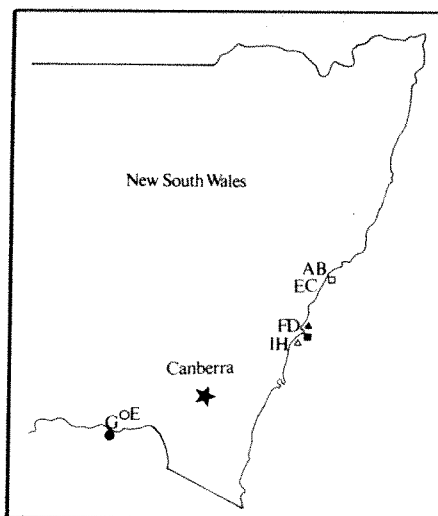
species in this high productivity. Recent studies on the physiology of *S. anglica* in Britain have suggested that this is one of the few temperate grasses which has adopted the C_4 system of carbon assimilation (see *Nature new Biol.*, **246**, 98; 1973), a mechanism frequently associated with high productivity. It is likely that this propensity for CO_2 fixation by the C_4 pathway is shared by other members of the grass *Spartina* which could account, in part, for its success in temperate salt marshes.

... and alligator weed spreads in Australia

from John Hockley

ALLIGATOR weed (*Alternanthera philoxeroides*), a normally harmless aquatic weed, is spreading rapidly in the rivers and canals of eastern Australia; it is blocking irrigation channels and is threatening the fishing, prawning and oyster industries along the Georges River near Sydney in New South Wales. The natural home of this species of weed, which belongs to the family Amaranthaceae (Duke, *Ann. Mo. bot. Gdn.*, **48**, 43; 1961), is South America and it was first noticed in Australia in the mid-1940s when it was seen growing on heaps of ballast dumped ashore at Carrington near Newcastle (see map). It seems to be spreading rapidly because although its seeds are infertile in Australia, it can reproduce vegetatively, its natural insect enemies are absent and it is able to flourish in a variety of habitats—it does particularly well in rivers enriched with sewage effluent.

The weed has been reported from places as far apart as Grafton, on the north coast of New South Wales; Williamstown, north of Newcastle, where it is blocking drainage canals; and Albury on the Victorian border, where it is not only a menace along irrigation canals, but also a possible threat to the Murray and Murrumbidgee Irrigation Areas. The largest



Recorded occurrences of alligator weed in New South Wales. 1946(A), 1962(B), 1965(C), 1969(D), 1970(E), 1971(F), 1972(G), 1973(H), 1974(I). □, Newcastle District (Williamstown, Carrington and Maitland); ▲, Carnarvon Golf Club (Lidcombe); ■, Duck Creek (Clyde); △, Georges River (Casula, Glenfield and Liverpool); ○, Holbrook; ●, Lake at Woomargama (Albury).

infestation is along the Georges River on Sydney's south-western boundary, where the optimum environment, one containing a high level of nitrogen, is provided by the effluent from the many sewage treatment works that empty into the river. In addition to the threat to the fishing industries, recreational activities such as swimming, water skiing and rowing along the river are being affected. Along the Georges River, alligator weed will grow on land as well as in freshwater and is tolerant to salt water (up to 10% salt by volume).

Various control measures have been tried with mixed success. Mechanical control is costly and usually has only a short term effect. Chemical control is only partially successful because only that part of the plant above the water can be killed. The stem that floats just below the surface of the water remains to shoot again. Trees, such as eucalypts

and she-oaks, and other plant life on the banks are very susceptible to the herbicide used to control alligator weed. Another undesirable aspect of this 'knock-down' type chemical control is the unpleasant smell produced by the rotting leaves that sink to the bottom of the river. The decaying leaves take oxygen out of the water, rendering it unsuitable for fish life.

Introduction from South America of two small insects that are the natural enemies of alligator weed may assist in controlling the infestation. The adults and larvae of the flea beetle (*Agasicles* spp.) are specific to alligator weed and feed on its leaves. The phycitid beetle (*Vogtia malloi*) bores into and eats the hollow stem of the weed allowing water to enter. After a period of time the remains of the stem become waterlogged and the plant drowns, as the leaves sink below the surface of the water. It seems likely that alligator weed will be controlled by biological means when its natural insect enemies are introduced to Australia in the near future.

Hedging one's evolutionary bets

from Montgomery Slatkin

ALTHOUGH population geneticists are not yet sure how natural selection acts on most of the genome—or even if it affects that much of the genome—there have been many predictions of the consequences of different kinds of selection. The hope has been that such models would help one to understand the dynamics of the gene pool of a population and would identify the kinds of natural selection which have been most important in evolution. Such models, however, despite fifty years of development are still far from that goal because of the inherent complexity of genetic systems and the large number of possible starting assumptions. Nonetheless, progress is being made and current models, of which Gillespie's is an example (*Genetics*, **76**, 601; 1974), are more realistic and potentially more useful than their predecessors.

One of the main problems in models of selection is the assumption about what is actually selected. Traditionally, and in keeping with Darwinian principles, the various genotypes have been assigned fitnesses which represent their relative contributions to the next generation. The fitness differences can be the result of either differential fecundity or mortality or both. Such models, however, do not take into account many of the effects produced by different patterns of reproduction. For example, in the simplest models, there would be no difference in fitness between two individuals one of which



Alligator weed on the Georges River near the Liverpool Golf Club at Casula in November 1973. The river is almost covered by mats of the weed. Earlier spraying has proved ineffective. This matted mass of 1 million tonnes of alligator weed was swept down the river by the mid-summer floods of 1974.

had ten offspring at once and the other of which had ten offspring one at a time: the average contribution of each individual to the next generation would be the same. It is obvious, however, that there would be a great difference between them; one is investigating its reproductive effort in one clutch and the other in ten clutches. If it were certain that a clutch would survive, regardless of its size, then clearly the variability in the number of offspring of the second individual would be much smaller than that of the first.

Until the publication of Gillespie's article, selection resulting from differential variance in offspring number had been ignored by theoreticians despite its obvious importance to the evolution of complex life histories. Gillespie shows that an allele which produces the same mean number of offspring but a smaller variance will increase in frequency and he argues that an important mechanism for reducing offspring variance is repeated reproduction and smaller clutch size. Gillespie deals only with a haploid population and, even then, the analysis is somewhat difficult. His simplification of the original model certainly needs more careful examination.

Although further mathematical work on the problem is needed, other results should support Gillespie's conclusion. Although many geneticists have assumed otherwise, the evolution of complex life histories involves more than simply the maximisation of reproductive potential.

OSSO

from our Chemical Physics Correspondent
No, the title of this piece is not an acronym for the Office for Senior Scientists' Obsolescence, that secret body which arranges for its 'clients' to be shunted into administration at 35 to make way for the next generation of research workers. It is rather the genuine chemical structure of a molecule recently characterised by Lovas, Tiemann and Johnson (*J. chem. Phys.*, **60**, 5005; 1974). It is formed, under conditions controlled as much by art as science, in the fairly high pressure, 10^4 Pa (100 mm Hg), microwave discharge in SO_2 . SO is a major product and the yield of it and its dimer seems to be a function of wall conditions as well as other components in the gas mixture. Careful lifetime studies have not yet been reported, but the half life is of the order of a few seconds under the flow conditions used. Polymeric deposits formed on the walls and some OSSO appeared in the gas phase when the surface was exposed to oxygen under discharge conditions.

The identification of OSSO was by means of a microwave spectroscopic

study which established (1) that the molecule was planar; (2) that it had a large dipole moment, 10.7×10^{-30} C m (3.17 debye), parallel to the intermediate axis of inertia; (3) that alternate rotational levels were absent, being forbidden by the spin statistics of a symmetrical molecule containing the bosons ^{16}O and ^{32}S ; (4) that there was a vibrational state, probably torsional, at about 140 cm^{-1} ; (5) that an analogue containing ^{34}S could be detected and that in it the full set of rotational levels was allowed. These facts, together with the values of the rotational constants, clearly show the species to be *cis*-OSSO, planar with C_{2v} symmetry.

The geometrical structure is then established with $r_{\text{SO}} = 145.8$ pm (1.458

Å), $r_{\text{SS}} = 202.45$ pm (2.0245 Å), and the SSO angle = 112.7° . This is a short S-O bond and a fairly long S-S bond so that the formula, $\text{O}^--\text{S}^+=\text{S}^+-\text{O}^-$, which matches classical valence rules, is in fact not very realistic, and the distribution of the six π electrons over the four centres is more delocalised than the above structure might imply. The characterisation of this molecule with its comparative stability is likely to catalyse further work on its electronic structure, on its vibrational and electronic spectra, and on a search for a possible *trans* form which, having no dipole moment, would not appear in the microwave spectrum. There could also be interest in isoelectronic species such as OSPF and FPPF.

Is history of science good for one?

from Robert Olby

In a recent article in *Science* (**183**, 1164-1172; 1974) the historian of physics, Brush, has described the "new look" which the history of science has acquired in recent years. Writing under the provocative title "Should the History of Science be rated X?", Brush pictures the old history of science as motivated by the search for approaches to modern scientific knowledge in the works of the past, thus to expose to view the progressive character of the development of science and its objectivity. Now the picture has changed and what Brush sees as emerging is the subjective manner in which scientific work and ideas are accepted or rejected. The roles of simplicity, analogy, unity and purpose seem to have been more important than mere empirical verification. To the believer in Galileo as the founder of the experimental method it comes as a shock to learn that the words "by experiment" were inserted in the English translation of the famous sentence "I have discovered by experiment some properties of [motion]"; or to see how the caloric theory of heat was overthrown not by the experiments of Count Rumford and Davy but by the acceptance of the wave theory of light and the analogies between light and heat.

The subjective and social features of scientific activity have been embodied in Kuhn's division of science into "normal" and "revolutionary". In a state of "normal science" the onus for proof lies with those who wish to overturn the established body of theory and practice (the

"paradigm") and not with those who uphold the paradigm. In this way the choice of problems as well as the significance accorded to experimental results tends to be determined by factors which are not purely rational and objective.

This type of approach to the history of science may be seen as subversive of the cult of scientific objectivity, so long regarded as essential to a scientist's education. Brush suggests not only that it furnishes a "more realistic picture of the behaviour of scientists", but also that it serves to soften the hard image of the "robot-like scientist lacking emotions and moral values".

Brush has, of course, expressed reservations to the "new look", he describes. He recognises that so eminent an American historian of science as Gillispie has argued for the objectivity of science. In Britain it is doubtful that an extreme relativist view of scientific knowledge has been established outside the gamut of the sociology of science. The British tend to believe that sufficient elements of such knowledge survive Kuhnian revolutions to justify the use of the words "development" and "progress". They would, at least, side with Bernard who judged progress in science by the criterion of the degree of control over the phenomena. They also recognise the distinction between what is irrational (counter to reason) and what is rational but devoid of any empirical supporting evidence. It is very doubtful that any but the most narrow-minded educators and the most gullible of students are discouraged by the historian's exposure of the rich and varied foundations upon which science has been erected.

Embryonic 'cold nose'

from a Correspondent

ANYBODY who opens a boiled egg at the blunt end will know there is an air space between the shell in this region and the rest of the egg contents. The usual explanation given for the function of this space is that it serves as a region for respiratory exchange between the embryo and the outside world; furthermore, the chick punctures the air space with its beak before hatching—a procedure which initiates respiration through the lungs and thus prepares the chick for its life outside. An additional function, which experiments on eggs of the domestic fowl and Japanese quail support, has recently been proposed by Simkiss (*J. Zool., Lond.*, **173**, 225; 1974). He argues that since loss of water by evaporation is one of the main problems associated with the development of an embryo in a closed or 'cleidoic' egg, any measure which limits such a loss would be of value.

When the parent bird is sitting on the nest the temperature of the egg and the humidity of the surrounding

air are both kept high so there is little loss of water vapour. But when the parent leaves the nest—and many birds are off the nest for periods of 5 to 15 minutes, 20–40% of the day—the temperature and humidity of the air surrounding the egg fall rapidly and, because the watery contents of the egg cool only slowly, there is a concentration gradient for water vapour to pass out of the egg; this loss of water from a warm wet body to cool dry air only stops, of course, when the parent returns or if the egg cools completely. The basis of Simkiss's argument is that the air held in the air space, because it has a low specific heat, cools rapidly when the parent leaves the nest thus reducing the gradient for the loss of water vapour through the shell. He estimates that in the climatic conditions of Britain the loss of water vapour per unit area of shell is 50% greater in those regions which overlie the liquid egg contents than in the region of the air space; with the air space region occupying about 40% of the area of the shell, he calculated a saving of about 15% during the period when a parent is not sitting.

It is, as Simkiss admits, difficult to assess whether this water-conserving

effect of the air space was more or less important in evolution than the respiratory advantages. Nevertheless, the comparative physiologist may well be able to throw light on the relative importance of the proposed functions, for one can think of climatic conditions in which some species breed but in which an air space would be of no advantage in water conservation, and species in which the egg is always kept warm by the parent.

Lasers probe the hydrogen atom

from B. P. Kibble

THE Doppler effect, which causes the apparent alteration in wavelength of the light emitted by moving atoms, has always limited the accuracy with which spectral line wavelengths, particularly those of atomic hydrogen, could be measured. But now this limitation has been overcome by saturated absorption techniques using lasers whose light can be tuned over a range of wavelengths. The first accurate measurements of a line in the hydrogen spectrum are reported by Hänsch and his colleagues at Stanford University in a recent issue

Lipmann's birthday party

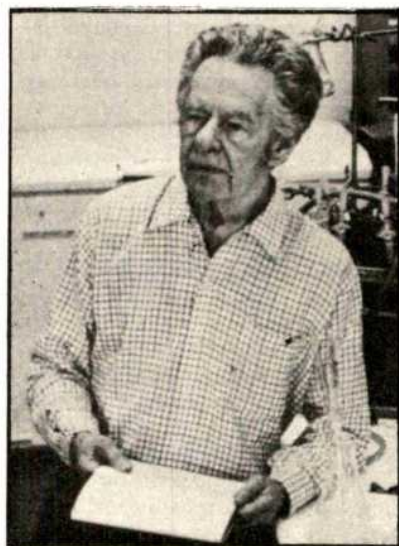
from a Correspondent

THE name of Fritz Lipmann is such a commonplace in textbooks of biochemistry that many students of basic science may well be surprised to learn that, unlike Dalton and Archimedes, he is still alive and has in fact just celebrated his 75th birthday. To mark this event, eighty of his former students and colleagues attended a special symposium organised in his honour at the Max-Planck-Institut für Molekulare Genetik in Berlin-Dahlem, on July 7–9, to take part in a relaxing mixture of history, sentiment and science.

The Institut für Molekulare Genetik was an appropriate venue, for it was literally only a couple of hundred yards away that Lipmann's career really began, as an unpaid graduate student in the old Kaiser-Wilhelm-Institut in Dahlem. The institut, founded in 1910, had somehow managed to survive both the First World War and the German hyperinflation of the early 1920s, when Lipmann joined Meyerhof's laboratory there in 1927. At that time, the institut, which was of course the forerunner

of today's Max-Planck-Institut, was quite a small organisation and Lipmann and his contemporary Sir Hans Krebs gave an entertaining account of life there in the late 1920s, under the "benevolent dictatorship" of Meyerhof and Warburg, two rather forbidding characters. For instance, Sir Hans was advised on leaving the institut to take up another career, as his "chances of success in biochemistry were slight".

In 1930, Lipmann, with his wife and three years of postgraduate experience, set out for America to take up a one-year fellowship at the Rockefeller University. After that he worked for a few years in Copenhagen before returning to the United States to settle. First in Boston and later at the Rockefeller University, he proceeded to pour out his classical work on protein phosphorylation, "energy-rich bonds", coenzyme A, and a host of other topics. This diversity of interest was reflected in the scientific sessions of the symposium, in which the Lipmann alumni discussed on subjects ranging from bioenergetics to immunity in insects, and from ribosome structure to molecular biology in the reign of the Chinese Emperor Fu Hsi. The only common feature was the warmth of the tributes which



Fritz Lipmann

all the speakers paid to their former mentor. Finally a special lecture was given by Feodor Lynen of the Max-Planck-Institut für Biochemie on the isolation and characterisation of the biotin enzymes. This was the first 'Fritz Lipmann Lecture', a new event in the academic calendar, which will now be given annually in Germany as part of the programme of the Gesellschaft für Biologische Chemie, under the sponsorship of the Boehringer-Mannheim Corporation.

of *Physical Review Letters* (32, 1336; 1974), following their demonstration of the phenomenon in *Nature Physical Science* (235, 63; 1972).

In their experiment they studied the light absorbed rather than emitted by atoms. The intense light from a tuneable wavelength laser is shone on the atoms and is absorbed by a set of atoms whose component of velocity in the direction of the light enables the Doppler-shifted wavelength to be matched to their absorption. A weaker probe beam from the same laser, and therefore having the same wavelength, is made to travel in the opposite direction so it will be ignored by this set of atoms but absorbed by another set whose members have an equal and opposite velocity component. There is a special case when the wavelength is tuned to be such that the atoms which absorb the light are moving at right angles to the light paths and hence have a very small Doppler shift. Both sets then comprise the same atoms and these being partially saturated by the intense beam, absorb less of the probe beam. It is this decrease in absorption which is detected as a narrow signal without any appreciable Doppler width as the laser wavelength is tuned through the spectral line.

There is a double advantage in all this. First, the line-broadening Doppler effect, which previously blurred together the closely spaced fine structure components of the spectral line, is eliminated. Second, as this technique entails absorption of nearly monochromatic laser light the interferometer used to measure the wavelength can have a very small spectral range and a high resolving power; previously all the fine structure components were excited simultaneously in a lamp and the spectral range of the interferometer had to be large enough to encompass them all, with a consequent loss of resolution.

Hänsch *et al.* measured the absolute wavelengths of some fine structure components in the Balmer- α lines of hydrogen and deuterium to determine a value for the Rydberg constant, which characterises the wavelength of the spectral lines, and a value for the isotope shift between hydrogen and deuterium, which essentially measures the ratio of the mass of the electron to that of the proton. The accuracy achieved was an order of magnitude greater than that obtained by the emission technique.

Physicists in the past have used new techniques to measure accurately the spectrum of the hydrogen atom and in each successive instance the results have not agreed with the current theoretical description. Each consequent improvement of the theory has revolutionised physics—witness Bohr's

concept of stationary states which led to quantum theory. And again, cooling the hydrogen lamp in liquefied gases narrowed the spectral lines and revealed the fine structure which was ultimately explained by Dirac's relativistic formulation of the quantum theory. Here the requirement of negative energy states suggested the existence of the positron and brought about the science of elementary particle physics. Further minute discrepancies (Lamb shifts), confirmed by another new technique of radio-frequency spectroscopy, resulted in the quantum theory of radiation fields and particles, quantum electrodynamics (see Series, *The Spectrum of Atomic Hydrogen*; Oxford University Press 1957). Now measurements of increasing refinement can be anticipated using this latest technique of saturated absorption, particularly as what I have described is only one of a number of allied methods in which the natural resonances of atoms which give rise to their spectral lines can be explored by tuneable wavelength lasers. (One of these methods which is especially promising is two-photon absorption which has recently been demonstrated experimentally, see, for example, *Physics Today*, 27, 17; 1974.) Modern physics owes much to the exploration of the hydrogen atom at successively higher levels of precision and there is no reason to suppose that this process cannot continue.

Missing link in folding of trypsin inhibitor

from Barry Robson

A PARALLEL could be drawn between the folding of a globular protein and biological evolution in that both represent a transition from a state of disorder to one of order and function. Moreover, both processes have their missing links without which it is impossible to verify hypotheses concerning mechanism. The difficulty of finding the missing links in the folding of a globular protein is because the process is, for most proteins, approximately two state, involving unstable and short-lived intermediates which cannot normally be isolated. Three articles by Creighton (*J. molec. Biol.*, 87, 563, 579, 603–624; 1974) describe a procedure for trapping and characterising such intermediates.

As an experimental system, Creighton chooses bovine pancreatic trypsin inhibitor, a small protein of only 58 amino acid residues which inhibits the catalytic function of certain other proteins, including trypsin and chymotrypsin. The native structure of the inhibitor as obtained from living tis-

sue has been well characterised by X-ray crystallographic analysis (Huber *et al.*, *Naturwissenschaften*, 57, 389–392; 1970). As in the case of other globular proteins, the native structure is a compact, relatively rigid conformation maintained by non-covalent interactions (that is by van der Waal's, electrostatic, hydrogen bond and hydrophobic interactions), as well as by covalent disulphide bridges between cysteine residues. Trypsin inhibitor has three such disulphide bridges.

Since this compact, biologically active structure is to be the end point of the folding process, Creighton first unfolds the inhibitor in a concentrated solution of guanidinium chloride, which breaks the non-covalent interactions, and dithiothreitol, which breaks the disulphide bridges. In such conditions it is known that typical protein molecules assume highly flexible, open conformations which are, strictly speaking, not single states at all but a whole collection of conformational states of roughly equivalent energies separated by low conformational energy barriers. The importance of starting with this collection of states is that all the information for directing the folding process towards the native conformation must initially reside only in the sequence of amino acid residues characteristic of trypsin inhibitor and ultimately coded for in the chemical structure of the gene. It is therefore regrettable that the conformational disorder of the inhibitor was not actually proven before refolding, but only assumed by analogy to the behaviour of other proteins in these conditions. Although Creighton's indirect evidence for initial conformational randomness does not, however, exclude the possibility of a fairly high degree of residual conformational structure, nobody would deny that the absence of such structure is a reasonable bet which one hopes will be verified by future hydrodynamic tests.

Creighton initiates refolding of this assumed random structure by removing the guanidinium chloride by dilution and adding a disulphide reagent such as oxidised dithiothreitol. The refolding reaction is then quenched at different times by the addition of iodoacetate or iodoacetamide which stop the further making and breaking of disulphide bridges. The species so trapped at various stages of refolding are subsequently analysed by acrylamide gel electrophoresis.

When any remaining unbridged residues of the trapped intermediates are carboxymethylated, the relative mobilities of the intermediates in the acrylamide gel reveal that some contain one and some two disulphide bridges. Consideration of the time course of the appearance and disap-

pearance of these intermediates shows that the folding can be considered to occur in three steps, each associated with the appearance of a further disulphide bridge. The first step is the formation of one-disulphide intermediates which are in rapid equilibrium with each other. The rate-limiting step in their disappearance is the formation of two-disulphide intermediates which are rapidly converted by the formation of the third bridge into the biologically active inhibitor.

Those intermediates with one disulphide bridge are more closely investigated, largely because results concerning the two-disulphide intermediates are less reproducible. The most abundant species of single disulphide intermediates has a disulphide bridge between cys residues at positions 30 and 51 in the amino acid sequence, and constitutes about half the population of single-disulphide molecules. The next most abundant species has a disulphide bridge between cys-5 and cys-30, and constitutes about one quarter of the population. Two minor species, involving bridges between cys-30 and cys-55 and between cys-5 and cys-51, are also detectable.

Returning to the analogy with biological evolution, the importance of the predominating species with a bridge at cys-30 to cys-51 is that this bridge constitutes a 'living fossil' in the sense that it survives to appear in the native, folded structure. Contemporary theories of protein folding lay great emphasis on the importance of such 'living fossils'. It is widely held that local arrangements of the backbone of the protein, such as α helix and β -pleated sheet, form early during the folding but survive to appear in the native structure because of their intrinsic stability. Since none of the other detectable disulphide bridging arrangements appears in the native structure, it is interesting to consider whether the 30-51 bridge fits in with this notion. Creighton points out that in the native structure cys-30 occurs in the middle of a β -pleated sheet region and cys-51 in the middle of an α -helical region. Since the initial formation of helix and pleated sheet regions is generally supposed to be followed by mutual association, the early appearance of a bridge between cys-30 and cys-51 is consistent with the early appearance of the helix and pleated sheet features in which they occur.

A further bonus is that the early formation of the β -pleated sheet region would account for the correct threading of the backbone. Correct threading is a particular problem for trypsin inhibitor because to arrive at the native conformation the backbone must be passed through the loop formed by the 30-51 bridge. Without the initial

formation of the β -pleated sheet, it would be hard to see the factors which select for correctly threaded molecules.

Because of the emphasis on disulphide bridging it might seem paradoxical that the usefulness of Creighton's results depends on cys residues not having a special role in the folding of proteins. In fact it is crucial that disulphide bridges form only when the conformation of the molecule, determined by other factors, will permit. Hence the trapping of the disulphide intermediates is intended merely to be a device for freezing the folding process at any moment in time and it is hoped that the disulphide bridges which form are an effect, never a cause, of the conformational preferences of the intermediates. In order to satisfy this ideal it is necessary that an interaction between cys residues is no stronger than non-covalent interactions between other sidechains at the time of folding. Although the covalent nature of the disulphide bridge would seem to make this an unrealistic ideal, the disulphide bond is in fact a labile association whose stability is determined by the reducing potential of the environment. It is therefore very pertinent that, as pointed out by Creighton, trypsin inhibitor is synthesised biologically in conditions where the reducing potential actually disfavors disulphide bridge formation. Evidence summarised by Creighton would seem to suggest that cys residues are cunningly placed by nature so as to form stabilising disulphide bridges in an extracellular oxidising environment, but that they have no relevance to the normal folding process.

The most general conclusion of this work is that a non-random mixture of conformations can be formed early during the folding process, and that some of the features of the most abundant intermediates may survive to appear in the native conformation. The most important result specific to trypsin inhibitor is that the missing link from the early stages of folding is represented by the disulphide bridge cys-30 to cys-51.

Meteorites and rabbits

from David W. Hughes

THE collection of meteorites in the Australian Museum has increased enormously in the past few years, and a large portion of the new material has resulted from the activities and interests of a group of rabbit trappers who criss-cross the Nullabor Plain on motor cycles. In fact, out of 36 meteorites recovered from the plain 24 were found by the members of one family, the Carlises. The added involvement of the Kalgoorlie School of

Mines and the Western Australian Museum has increased the rate of detection of meteorites from 16 per decade, which remained constant between 1897 and 1966, to around 80 per decade since 1966.

Until the latest count (*Rec. Austr. Mus.*, 29, 169; 1974), reported by Mason (Smithsonian Institution, Washington DC), irons formed the most numerous group of Australian meteorites. This is in marked contrast to general world statistics, in which stones predominate. A possible explanation for this is that meteoritic iron may have provided raw material for swords and ploughshares. Iron meteorites would therefore have been consumed rapidly once a native people had acquired the facility for working metal. Significantly, that facility is not possessed by the Australian aborigine. Stone meteorites, which had no practical use, were invariably worshipped. Moslem pilgrims to Mecca pay homage to the sacred black stone of Kaaba, and in Japan the Ogi meteorites were worshipped for 150 years in the belief that they were weights which had fallen from the loom of the Goddess Shokujo who lived on the shores of the Heavenly River (the Milky Way).

The problems of estimating the influx of meteorites and the ratio between stones and irons have been discussed by Nininger (*Out of the Sky, an Introduction to Meteoritics*, Dover New York; 1952) who searched for witnessed and unwitnessed meteorite falls for more than 30 years. Campaigns among rural populations in the United States were designed to acquaint farmers and ranch dwellers with the appearance and importance of meteorites. Directions were given for simple field tests to distinguish meteorites from terrestrial rocks and a price was offered for specimens as an inducement to all to keep on the lookout and report them. The programme was very successful: the number of finds increased dramatically and eventually the content of the Nininger Collection nearly mirrored the prevalence of meteorites in space (where irons make up 6% by number, stony-iron 2%, and stones 92%).

In Australia the intensive prospecting for gold and other minerals led people to expect a reasonable yield of meteorites. But, unfortunately, the prospectors avoided the flat, sandy, desert regions of the interior plains where meteorites would be more obvious. Stockmen and ploughmen may have found meteorites but, unlike the prospector, they generally had no interest in unusual rocks. On the Nullabor Plains, however, the personnel at the Kalgoorlie School of Mines have encouraged an active interest among the rabbit trappers.

articles

Nitrosoguanidine mutagenesis during nuclear and mitochondrial gene replication

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The timing of individual nuclear gene replication during S phase in an eukaryote can now be studied by nitrosoguanidine mutagenesis. Using this technique, results also suggested that mitochondrial DNA replication is synchronous and a late event in the cell cycle.

In prokaryotes the mutagen N-methyl-N'-nitro-N-nitrosoguanidine (NG) is thought to induce mutations selectively at the DNA replication fork^{1,2} and NG mutagenesis at different stages of the cell cycle has provided information on the order and timing of individual gene replication³.

In eukaryotes, techniques do not exist for determining the ordering of DNA replication at the gene level. We have therefore tested whether NG induces mutations during DNA replication in yeast and whether the temporal ordering of replication of genes on individual chromosomes and the timing of replication of chromosomes relative to each other is feasible using the NG mutagenesis technique.

The budding yeast, *Saccharomyces cerevisiae*, is an appropriate test organism. Nuclear DNA synthesis occurs during a restricted period of the cell-cycle which can be divided into G1, S, G2 and M phases⁴. Yeast populations can be synchronised or fractionated according to cell age^{5,6} in the division cycle. An adequate genetic map has been developed with most of the 17 known chromosomes well marked⁷ and NG has been shown to be an effective mutagen⁸.

In addition to the nuclear genetic complement, yeast mitochondria contain mitochondrial DNA (mit DNA). Using the best available estimate⁹, each haploid cell contains about 50 mit DNA molecules. A few markers on the mitochondrial genome have been identified and progress has been made in constructing a genetic map¹⁰. The pattern of mit DNA synthesis during the yeast cell cycle has been the subject of conflicting reports. Cottrell and Avers, using *S. cerevisiae*, and Smith *et al.* with *Saccharomyces lactis*, concluded that the replication of mit DNA occurs over a restricted period at a different stage in the cell cycle to nuclear DNA replication^{11,12}. Williamson and Moustacchi, however, observed that mit DNA synthesis was continuous throughout the cell cycle in *S. cerevisiae*¹³.

If NG treatment of yeast results in enhanced mutation at the replication fork for both nuclear and mit DNA it should be possible to delineate both periods of synthesis within the cell cycle. If mit DNA synthesis is synchronous and occurs during a restricted part of the cell cycle different from that for nuclear DNA synthesis, then mutations showing mitochondrial inheritance should be induced by NG only during that part of the cell cycle. ••

To study the effect of NG on both nuclear and mit DNA, readily detectable forward mutations are required. Those for acquisition of resistance to antibiotics affecting mitochondrial functions are suitable because resistance to erythromycin¹⁴ or oligomycin¹⁵ can arise by nuclear- or mitochondrially-inherited mutation.

As yeast cells increase in size throughout the cell cycle and can be separated according to size by zonal rotor centrifugation, the cell cycle is represented across the rotor after centrifugation of an exponential culture.

Cell age during the cell cycle is approximately linearly related to fraction number obtained from the zonal rotor in our conditions. This was shown by measuring mean cell volumes during growth of a synchronous culture and in a different experiment in fractions obtained after zonal rotor

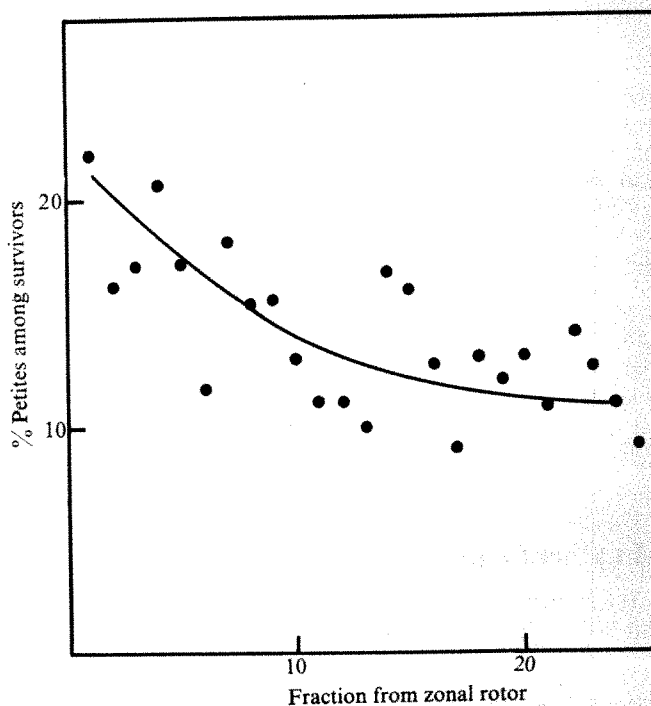


Fig. 1 Average total DNA content per cell as a function of cell age in the cell cycle. Exponential cultures of strain ID-1 grown to log phase in YEPG at 30°C were separated into fractions of various cell cycle age by zonal rotor centrifugation. For each fraction total DNA content was estimated by the Giles and Myers method²⁰ and cell concentration was determined by electronic particle counting. Fraction number from the zonal rotor is a linear function of cell age in the cell cycle.

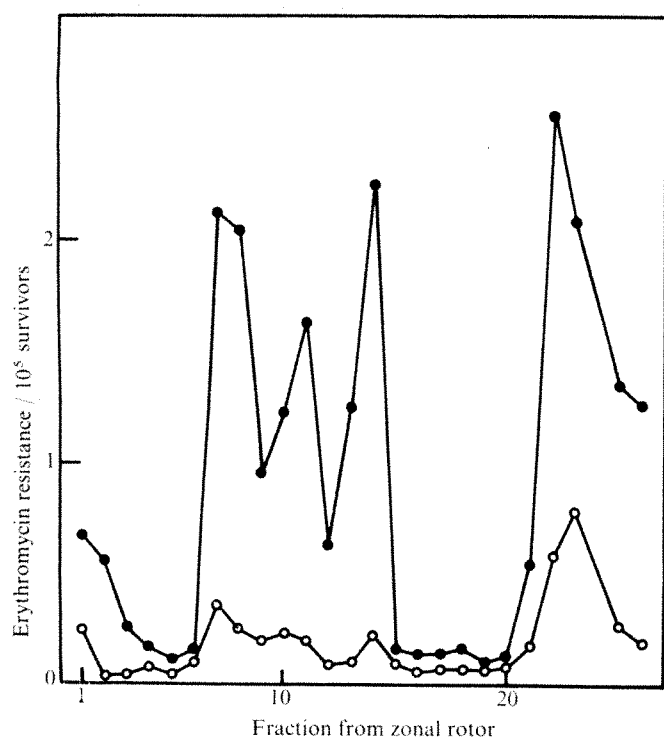


Fig. 2 NG-induced mutagenesis to ER^+ as a function of cell age. An exponential population of strain ID-1 grown to 10^7 cells ml^{-1} on YEPG at $30^\circ C$ was washed by centrifugation in sterile $0.2 M$ potassium acetate (KAc; pH 5.5) and resuspended in KAc containing NG ($0.5 mg\ ml^{-1}$). After 15 min at $30^\circ C$ the cells were centrifuged, resuspended in $40 ml$ sterile water and separated into fractions in the zonal rotor. Viable cell concentration in each fraction was determined by counting on YEPD plates. ER^+ mutants in each fraction were scored after 4 d (○) and 7 d (●) by plating between 10^6 and 10^7 survivors on to each of six YEPG plates containing $0.4 mg$ erythromycin ml^{-1} .

centrifugation of an exponential population. Cell age from the synchronous culture plotted against fraction number in which the mean cell volume was identical gave a near linear curve.

Figure 1 shows the average DNA content per cell for fractions obtained by zonal rotor centrifugation of strain ID-1 growing exponentially in YEPG medium (1% yeast extract, 2% bactopectone, 3% glycerol; mean generation time: 3.5 h). As the zonal rotor separates yeast populations into fractions according to stage in the cell cycle, and the majority of DNA in the yeast cell is nuclear DNA (ref. 4), it is clear that nuclear DNA replication in ID-1 takes place mainly during the second quarter of the cell cycle, as in other strains of *S. cerevisiae*.

Mutation frequency as function of cell age

An exponential culture of ID-1 growing on YEPG was exposed to NG ($0.5 mg\ ml^{-1}$) for a brief part (15 min) of the cell cycle (3.5 h) and separated into fractions according to cell age. The distribution of mutants resistant to $0.4 mg\ ml^{-1}$ erythromycin (ER^+) as a function of cell position on the zonal rotor is shown in Fig. 2. These data were plotted for mutants appearing on plates after 4 d and 7 d incubation. As similar results were obtained on plotting mutants per total cells in each fraction NG inactivation was not markedly cell cycle dependent.

NG-induced mutation to ER^+ occurred at an increased frequency in two regions of the cell cycle (represented by fractions 7-14 and 22-25 in Fig. 4). Three peaks of suscept-

ibility to mutagenesis to ER^+ (fractions 7, 11 and 14) were found in the initial period of mutant induction coincident with nuclear DNA synthesis (Fig. 1). In contrast, ER^+ mutants induced later in the cell cycle were found in a single large peak (fractions 22 and 23) in a region of the rotor containing cells close to cell division. Mutants induced in later fractions of the zonal rotor appeared earlier on selective plates than most of those in fractions 7 to 14, indicating possible differences in these two groups of mutants.

If NG acts mainly at the replication point in *S. cerevisiae*, ER^+ mutants induced in fractions 7-14 during S phase should show normal Mendelian segregation of the resistance marker from the cross to a sensitive strain. Results of the relevant genetic tests are summarised in Table 1. Most (seven of nine tested) of the mutants in fractions 7, 11 and 14 were the result of a nuclear mutation, the other two were cytoplasmic. All mutations in fractions 22 and 23 were cytoplasmically-inherited on the basis of the following criteria. First, the segregation of stable mutant and wild-type diploid cells during vegetative division of the diploid formed from a cross of the mutant to a sensitive strain; second, the tetrad ratios of 4:0 and 0:4 (sensitive:resistant) following meiosis of diploids formed in a cross with a sensitive strain; third, most ρ^- -petites derived from the resistant mutants by continuing exposure to ethidium bromide led to sensitive diploids on crossing with a sensitive strain.

By testing 16 mutants from each fraction, it was found that nuclear ER^+ mutations in fraction 14 differed from

Table 1 Characteristics of ER^+ mutants from the zonal rotor fractions

Fraction/mutant		Cross to sensitive strain Diploid phenotype*	Ascus segregation of resistance	Effect of ethidium bromide†
7	1	Mixed	4:0 and 0:4	Loss
	2	Sensitive‡	2:2	Retention
	3	Sensitive‡	2:2	Retention
11	1	Sensitive‡	2:2	Retention
	2	Sensitive‡	2:2	Retention
	3	Mixed	4:0 and 0:4	Loss
14	1	Resistant	2:2	Retention
	2	Resistant	2:2	Retention
	3	Resistant	2:2	Retention
22	1	Mixed	4:0 and 0:4	Loss
	2	Mixed	4:0 and 0:4	Loss
	3	Mixed	4:0 and 0:4	Loss
23	1	Mixed	4:0 and 0:4	Loss
	2	Mixed	4:0 and 0:4	Loss
	3	Mixed	4:0 and 0:4	Loss

* Mutants were crossed to an ER^+ strain and diploid clones tested for resistance or sensitivity to the antibiotic.

† Mutants were treated with ethidium bromide to produce petites and these were crossed to a grande sensitive strain. Diploids so formed were tested for presence or absence of resistance allele directly or after tetrad dissection.

‡ Diploids showed slight growth on selective plates.

those in the other two nuclear peaks in being completely dominant to the ER^+ allele. If the peaks in susceptibility to mutation during nuclear DNA replication represent mutation at different loci, recombination between them should be possible. Random spore analysis of the cross between haploids carrying mutations 11.2 and 14.1 (derived from the $ER^+ \times ER^+$ dissections) indicated that linkage was not detectable by this method as approximately 25% ER^+ recombinants were recovered (8 from a total of 35 spores). Analysis of the crosses of mutants derived from fraction 7 with those from 11 or 14 was not satisfactory

because of poor viability of spores and inability to disrupt tetrads in these crosses, although several ER^+ spores (two out of five) were recovered from the cross 7.2 with 14.1.

Mutation to oligomycin resistance

As mitochondrial mutation to ER^+ represents mutation in a restricted region of the mitochondrial genome it is important to study other mitochondrial loci distinct from those for resistance to erythromycin. Those for resistance to oligomycin, a mitochondrial ATPase inhibitor, are suitable as they are not linked to the segment of the mitochondrial genome specifying functions of mitochondrial ribosomes¹⁰.

Figure 3 shows the frequency of induction of OL^+ and ER^+ mutants as a function of cell age. An exponential culture of ID-1 was treated with NG, separated into fractions according to cell age and assayed for mutants resistant to either antibiotic. OL^+ is induced at the same stages of the cycle as ER^+ , particularly for the later part of the cell cycle in which OL^+ and ER^+ mutants reached peak concentration in the same fraction. During the S phase there were two main peaks of susceptibility to mutation to OL^+ . These occurred within the same cell age range as those for resistance to erythromycin although in one case at least the OL^+ mutation was induced in cells of different age from those for ER^+ . This result is expected as the sites of action of the two antibiotics differ, erythromycin inhibiting mitochondrial protein synthesis whereas oligomycin inhibits the mitochondrial inner membrane ATPase complex. Nuclear mutations conferring resistance to one antibiotic need not necessarily confer resistance to the other.

Preliminary characterisation of the various fractions had led to the identification of cytoplasmically-inherited OL^+ mutations from the late peak of mutants in Fig. 3. These

were mixed with a fairly high background of nuclear mutants, in contrast with the ER^+ mutants in which the nuclear region was contaminated with a low background of those of cytoplasmic origin. The majority of ER^+ mutants tested were OL^+ .

At concentrations similar to those used for yeast mutagenesis, NG induces up to 40% petites in *S. cerevisiae*. If this is due to a direct mutagenic effect of NG at a few specific loci, as seen in the induction of drug resistance, the fraction of petites in survivors from NG mutagenesis should

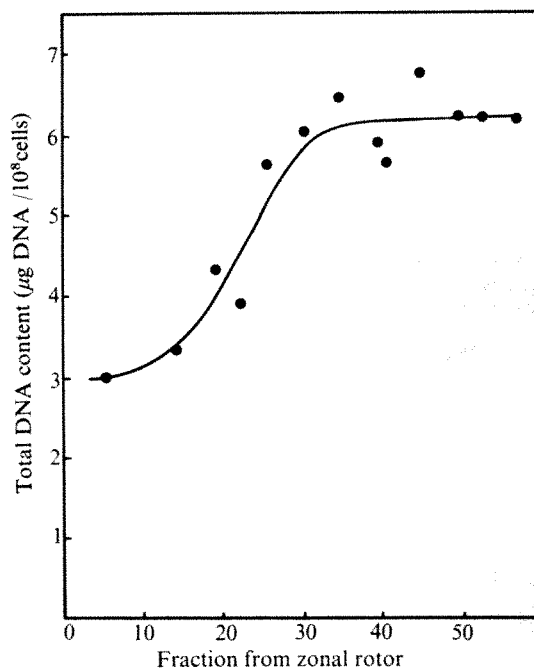


Fig. 4 Petite induction by NG during the cell cycle. Plates used for estimating viable cell concentrations in the experiment shown in Fig. 2 were used to determine the percentage of petites among survivors by the triphenyltetrazolium chloride agar overlay technique of Ogur *et al.*²¹.

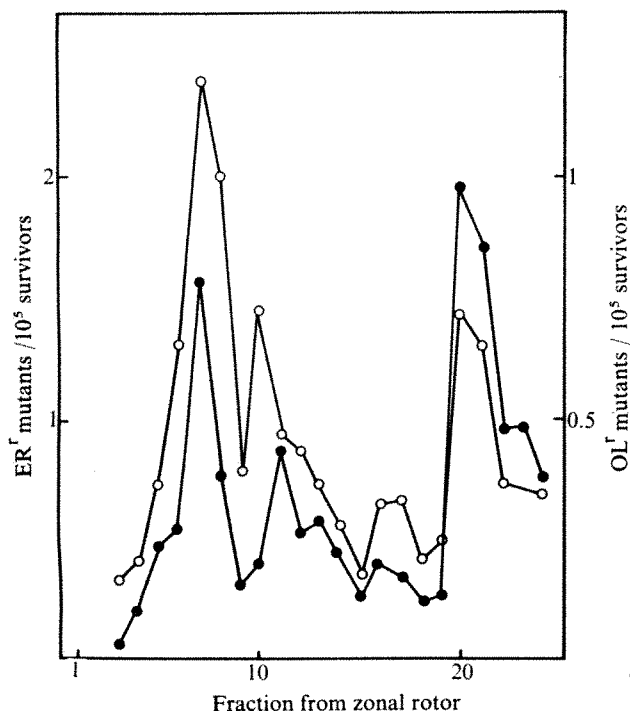


Fig. 3 Comparison between induction of OL^+ and ER^+ mutants by NG during the cell cycle. Experimental details were as those in the legend to Fig. 2. OL^+ mutants (\circ) were scored after 4 d incubation at 30°C on YEPG plates containing 6 μ g oligomycin ml^{-1} . ER^+ mutants (\bullet) were scored after 5 d.

show similar variations with cell age. Petites were not induced by NG in the same way as drug resistance but were found in all fractions from the zonal rotor at a frequency of 20% in young cells continuously decreasing to 10% in doublets (Fig. 4).

DNA replication and NG mutagenesis

Evidence that NG-induced mutation occurs primarily at the DNA replication point derives mainly from prokaryotic studies and is based on the observation in synchronous cultures of a correlation between the time during the cell cycle of induced reversion at a locus with its map order on the bacterial chromosome¹. Furthermore, in *Escherichia coli* and *Salmonella typhimurium*, NG induces double mutations at high frequency and these usually show close linkage².

In eukaryotes, NG-induced mutation at enhanced rates during DNA replication has been shown in *Chlamydomonas reinhardtii*¹⁷. In this system a slight increase in mutation at a cytoplasmic locus was found during chloroplast DNA replication. The results with yeast extend these observations to show that mutation at a specific nuclear locus (for example that conferring 'dominant ER^+ ') occurs only during a brief interval of the S phase, and that different loci are sensitive at different points in the S phase. We presume that

this is a result of mutagenesis in loci replicating at different times during S phase. This differential sensitivity, and its correlation with the period of nuclear DNA replication, makes it unlikely that selective NG mutagenesis is the result of changes in membrane permeability or detoxification of the mutagen during the cell cycle.

Regardless of the molecular mechanism by which NG mutagenesis is correlated with the process of DNA replication, it may now be feasible to map the temporal ordering of chromosomal replication in an eukaryote in much more detail than hitherto has been possible, as mutations in different loci replicating at different times during S phase can be resolved by zonal centrifugation.

Autoradiographic studies of a number of eukaryotes have shown that chromosomal replication follows a sequential and heritable pattern¹⁸. For example, certain chromosomes and regions of chromosomes consistently replicate late in successive cell cycles and in *Physarum* DNA replicated at a particular point in one S period is replicated at the same point in the next S phase¹⁹. Our experiments extend this observation to the level of individual gene replication. In spite of the existence of 17 chromosomes in *S. cerevisiae* and the probable existence of multiple replication origins on each chromosome, duplication of a particular locus always occurs at a fixed time in the S phase of the cell cycle since it is possible to distinguish separate peaks of NG-induced mutation at different nuclear loci after zonal centrifugation.

Mitochondrial DNA replication

Haploid yeast cells apparently contain approximately 50 circular mit-DNA molecules, each 25 μ m long⁴. If NG acts selectively during mit DNA replication, the simplest interpretation of our data on coincident induction of cytoplasmic ER⁺ and OL⁺ mutations is that all copies of these two genetically distinct regions of the mitochondrial genome are replicated during the same brief interval late in the cell cycle. It is possible that all copies of the complete mitochondrial genome are replicated synchronously late in the cell cycle.

The hypothesis that mit DNA replication is periodic and a late event in the cell cycle is consistent with some biochemical data for diploid *S. cerevisiae*¹¹ and *S. lactis*¹² but differs from that of Williamson and Moustacchi who reported continuous synthesis of mit DNA in diploid *S. cerevisiae* populations synchronised by alternate feeding and starving¹³. The direct test of these alternatives will come from biochemical assays of mit DNA replication.

These discrepancies may be due to strain differences, or may result from varying approaches in studying the cell cycle. Our experiments examine a normal cell cycle as exponential cultures are treated with the mutagen and then separated according to stage in the cell cycle. Feeding and starving is a form of induction synchronisation and may not result in a normal cell cycle because artefacts resulting from metabolic disturbance and failure to synchronise all cell activities¹⁸ can occur during induction methods. Mitchison has shown the cell cycle is comprised of two cycles, the growth and the DNA-division cycles. The former is not synchronised by induction methods, and some events which normally occur periodically during the cell cycle appear continuously¹⁹.

Our results show a striking difference between petite induction and mutagenesis to antibiotic resistance. Petites were induced at all stages of the cell cycle, at a rate 10,000 times greater per survivor than mutation to ER⁺. This could be indicative of a fundamental difference between the mode of petite induction and the mechanism of mutagenesis by NG. NG is known to alter the activity of proteins²² and if as

suggested by Williamson⁹, a repression mechanism operates to regulate mitochondrial integrity, NG could act in petite induction by causing a phenotypic alternation in the activity of a repressor. This would be distinct from a mutagenic process as is the induction of petites by 5-fluorouracil⁹.

It is frequently difficult to demonstrate activity of mutagenic compounds in induction of mitochondrial mutation. The recovery of a peak of mitochondrial mutants at late stages in the cell cycle following NG treatment indicates that this compound is an effective mutagen for the mitochondrial genome.

NG mutagenesis, in conjunction with techniques for separation of populations according to the stage reached in the cell cycle provides a powerful selective technique for enrichment of particular mutants, and the ability to 'direct' mutagenesis discussed by Cerdà-Olmedo *et al.*¹ may now be possible in eukaryotes.

NG induces mutations conferring resistance to antibiotics affecting mitochondrial function at two stages of the cell cycle. One stage is coincident with nuclear DNA replication while the other is late in the cell cycle and may be that at which mitochondrial genes are replicated. A particular nuclear gene is susceptible to NG mutagenesis only at a specific time during S phase and different unlinked nuclear genes are sensitive at different times during S phase. NG is presumed to act during gene replication, and it should therefore be possible to determine in yeast the temporal pattern of chromosomal replication at the gene level.

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- ¹ Cerdà-Olmedo, E., Hanawalt, P. C., and Guerola, N., *J. molec. Biol.*, **33**, 705-719 (1968).
- ² Guerola, N., Ingraham, J. L., and Cerdà-Olmedo, E., *Nature*, **230**, 122-125 (1971).
- ³ Hohlfeld, R., and Vielmetter, W., *Nature new Biol.*, **242**, 130-132 (1973).
- ⁴ Williamson, D. H., in *Cell Synchrony* (edit. by Cameron, I. L., and Padilla, G. M.), 81-101 (Academic Press, New York and London, 1966).
- ⁵ Carter, B. L. A., Sebastian, J., and Halvorson, H. O., *Adv. Enzyme Regulation*, **9**, 253-263 (1971).
- ⁶ Sebastian, J., Carter, B. L. A., and Halvorson, H. O., *J. Bact.*, **108**, 1045-1050 (1971).
- ⁷ Mortimer, R. K., and Hawthorne, D. C., *Genetics*, **74**, 33-54 (1973).
- ⁸ Nordström, K., *J. gen. Microbiol.*, **48**, 277-281 (1967).
- ⁹ Williamson, D. H., *Symp. Soc. exp. Biol.*, **24**, 247-276 (1970).
- ¹⁰ Faye, G., *et al.*, *Biochimie*, **55** (in the press).
- ¹¹ Cottrell, S. F., and Avers, C. J., *Biochem. biophys. Res. Commun.*, **38**, 973-980 (1970).
- ¹² Smith, D., Tauro, P., Schweizer, E., and Halvorson, H. O., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 936-942 (1968).
- ¹³ Williamson, D. H., and Moustacchi, E., *Biochem. biophys. Res. Commun.*, **42**, 195-201 (1971).
- ¹⁴ Thomas, D. Y., and Wilkie, D., *Genet. Res.*, **11**, 33-41 (1968).
- ¹⁵ Stuart, K. D., *Biochem. biophys. Res. Commun.*, **39**, 1045-1051 (1970).
- ¹⁶ Avner, P. R., Coen, D., Dujon, B., and Slonimski, P. P., *Molec. gen. Genet.*, **125**, 9-52 (1973).
- ¹⁷ Lee, R. W., and Jones, R. F., *Molec. gen. Genet.*, **121**, 99-108 (1973).
- ¹⁸ Mitchison, J. M., *The Biology of the Cell Cycle* (Cambridge University Press, 1971).
- ¹⁹ Mitchison, J. M., in *The Cell Cycle in Development and Differentiation* (edit. by Balls, M., and Billet, F. S.) 1-11 (Cambridge University Press, 1973).
- ²⁰ Giles, K. W., and Myers, A., *Nature*, **206**, 93 (1965).
- ²¹ Ogur, M., St. John, R., and Nagai, S., *Science*, **125**, 928-929 (1957).
- ²² Cerdà-Olmedo, E., and Hanawalt, P. C., *Molec. gen. Genet.*, **101**, 191-202 (1968).

Educability and group differences

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Professor Jensen replies to criticism of his book Educability and Group Differences made by Professor J. M. Thoday in a review published in Nature.

I WISH to reply to the two main points of criticism made in the review of my book, *Educability and Group Differences*, (Methuen, London, 1973) by Professor J. M. Thoday in *Nature* (245, 418–420; 1973).

I had reported that when white and negro children were matched for a particular IQ score, say 120, the siblings of the two racial groups differ in average IQ, and that the difference is consistent with the phenomenon known as 'regression to the mean'. The white siblings regress toward the white population mean IQ of 100, the negro siblings toward the negro population mean of 85. The amount of regression is predictable in both racial groups from a genetic model in which the genetic correlation between sibs is 0.05 and the heritability, h^2 , of IQ is 0.80. The same model, using different empirically estimated values for h^2 , is applicable to any other continuous traits, such as height, weight, and fingerprint ridges. (In the present example, approximately the same amount of sibling regression was found for height as for IQ, and the same equation predicts as well for negroes as for whites.) All these findings are consistent with an already existing polygenic model which has proven theoretically valuable in understanding variation in metrical characteristics, physical and behavioural.

Factor X

But Thoday claims that a finding such as I reported "... adds nothing whatsoever to the strength of the genetic hypothesis" on the ground that the evidence is also as compatible with an explanation in terms of a hypothetical environmental "factor X" as in terms of the genetic hypothesis. But "factor X" is, of course, a purely *ad hoc* hypothesis. No previous environmentalist theory or model has been put forth which would have predicted the quantitative aspects of these findings, nor the linearity of regression throughout the middle 98% of the IQ range, nor the similarity of regression for IQ, height, and weight, nor the fact that the same regression equation works equally well for both racial groups. All these points, which are consistent with a much larger body of genetical theory and findings applicable to all organisms, would have to be regarded as coincidences in terms of the purely *ad hoc* hypothesis that some as yet unidentified environmental "factor X" is responsible. The findings, of course, cannot prove or disprove any *ad hoc* hypothesis which is invented expressly to explain them. But the fact that they are consistent with a genetic model which is not *ad hoc* is a point in favour of the genetic explanation. Philosophers of science, I believe, would support my contention. In fact, a forthcoming article, "Progress and Degeneration in the IQ Debate" by Dr Peter Urbach (*Br. J. Phil. Sci.*) argues that the chief weakness of the environmentalist position is its extreme recourse

to *ad hoc* explanations, often mutually inconsistent, of findings which were predicted by, or which easily fit into the framework of, already existing genetical theories supported by a growing internally-consistent network of evidence.

Thoday's second criticism is intended as an example of uncritical acceptance by me of some evidence which seems to favour a genetic hypothesis.

It involves my reference to a published study by DeLemos, which shows that a sample of full Australian aboriginals performed significantly less well on several of Piaget's tests of conservation than did part aboriginals (with the average genetic equivalent of one Caucasian greatgrandparent), despite the fact that the two groups shared much the same general environment without any distinguishable systematic environmental differences between the full and part aboriginals. Since the Piagetian tests, which are intended to reflect changes in mental maturity, are sensitive to age differences, and DeLemos's subjects ranged in age from 8–15 yr, Thoday conjectures that the findings reported by DeLemos could be an artefact of her not having controlled for age. The much more detailed presentation of the data and other analyses in DeLemos's PhD thesis (460 pages), of which I obtained a microfilm copy in 1967 and on which her later published article was based, however, shows that Thoday's statement that "the data cannot be regarded as demonstrating that the ancestry difference has significant effects" is not borne out by the evidence. Nor did I notice any other likely artefacts in my reading of DeLemos's thesis. My personal discussion of this research with Dr DeLemos, in 1969, added to my confidence in her conduct and analysis of the study.

Partial correlations

The invalidity of Thoday's conjecture can be demonstrated perhaps most simply by a reanalysis of the original data provided by DeLemos, using partial correlations. I have performed this analysis, based on all 80 subjects from the Hermannsburg group (42 full and 38 part aboriginals) ranging in age from 8–15 yr. Intercorrelations were obtained between age in months, the exact percentage of Caucasian ancestry, and total score on the six tests of conservation (each test scores as 0, nonconservation, 1, transitional; 2, conservation). The zero order correlations among these variables are: age \times %Caucasian ancestry: $r=0.192$ ($P<0.05$); age \times total conservation score: $r=0.350$ ($P<0.01$); %Caucasian ancestry \times total conservation score: $r=0.478$ ($P<0.01$). If the correlation between ancestry and conservation score depends upon the correlation of each of these variables with the third variable, age, for example, then the partial correlation between ancestry and conservation, with the effect of age statistically held constant, should be reduced to a value not significantly greater than zero. If, on the other hand, the partial correlation is significant, it means that ancestry makes some contribution to the conservation score independently of age. The partial correlation between Caucasian ancestry and conservation score turns out (independent of age) to be 0.448, which is significantly greater than zero at the 1% level of confidence. A more

complex type of analysis (ANOVA of total conservation scores, with ancestry nested within 1 yr age groups), too involved to present here but which does not make any assumptions about linearity of regressions as is implicit in partial correlations, fully supports this conclusion that DeLemos's finding is not attributable to age differences between the full and part aboriginals. Also, it can be shown that the sex of the subjects has no significant relationship to any of the other variables in DeLemos's study.

The fact that another study of conservation in full and part aborigines, by Dasen (published in 1972, after my citation of the DeLemos study was in press), failed to find a significant relationship between ancestry and conservation

performance does not automatically invalidate the DeLemos study, which appears methodologically at least as sound as the Dasen study. The latter involved certain procedural and sampling differences, so that it cannot be regarded as a true attempt at replication of the DeLemos study. Dasen's discrepant findings do mean, however, that the findings by both DeLemos and Dasen are not clearly understood in terms of the procedural variables affecting performance and that neither's results are generalisable to the general population of aboriginals or to other tests of conservation. The only answer for this state of affairs, which is of course a common occurrence in empirical research, is to systematically pursue further investigations in the same vein.

letters to nature

Absence of soft X rays from Eta Carinae

THE X-ray telescopes on OAO-Copernicus have been used to search for X-ray emission from η Carinae. The instrumentation has been described elsewhere¹⁻²; briefly, it comprises two paraboloidal X-ray telescopes operating in the energy ranges 0.5–1.5 and 1.5–4.6 keV, with a separate collimated proportional counter operating from 2.5–7.5 keV.

The energy source of η Car, first seen in the optical spectrum³ and now in the infrared⁴, is not yet entirely clear⁵⁻⁶. Thackeray⁵ suggested that it belongs to a new, slow class of supernovae associated with the birth of an expanding stellar association, and Ostriker and Gunn⁷ have developed a supernova model of large mass ($\approx 50M_{\odot}$) energised by a central neutron star and emitting synchrotron radiation from the surrounding nebula in the optical and infrared⁸. Alternatively⁶, the object may be a very massive star ($600\text{--}100M_{\odot}$) which is vibrationally unstable and has ejected a fraction of a solar mass to form the observed nebulae and condensations⁹; or it could be a very young massive star approaching the main sequence^{3,6}. On either of these last two hypotheses the radiation is entirely thermal: the optical continuum results from a hot central star or from two-photon emission from metastable hydrogen, and is distorted by reddening in a circumstellar dust cloud¹⁰ which re-emits the absorbed radiation in the infrared^{11,12}. The thermal model, more probably involving a hot central star, is strongly supported by the relative intensities of emission lines of hydrogen¹³ and permitted¹⁴ and forbidden¹⁰ FeII, by the detailed analysis of Davidson¹⁵ and by the presence of silicate bands near $10\text{ }\mu\text{m}$ (ref. 16); but measured intensities of [S II] lines do not show the expected intrinsic reddening¹⁷.

On the synchrotron model, inverse Compton scattering may lead to an observable X-ray flux¹⁸, which would probably be accompanied by intense synchrotron X rays if the 'pulsar' mechanism were operative. On either model the observations of an expanding shell moving out into the surrounding medium must imply the presence of a shock wave with compression and heating of the ambient gas to a temperature at which the emission of X rays becomes important. An observation of η Car in X rays would therefore be of great value in helping to decide between the two models and in setting constraints of the physical parameters involved.

A soft X-ray source found in a scanning rocket experiment¹⁹ was located somewhere near the galactic equator and within 0.3° of the galactic longitude of η Car, with which it was tentatively identified. This unconfirmed identification led Davidson and Ostriker²⁰ to comment on the parameters of the thermalised shock front which precedes the expanding shell and which was assumed to account for the X rays observed below

2.7 keV. They concluded that the shell must be moving into a surrounding medium of density $\sim 2,000\text{ cm}^{-3}$, which was rather difficult to account for, because it implied that η Car had not cleared a cavity around itself by mass outflow before the large outburst of 1843. Another possible consequence of this model is that the green coronal line [FeXIV] λ 5,303 may be present in the visible spectrum²¹. A spectral tracing from the Radcliffe Observatory indicates that this line, if present at all, is considerably weaker than predicted, but this negative result cannot be treated as a very conclusive test of the shock model. The pulsar model seems, on the other hand, to have been ruled out by the steep slope and modest intensity of the observed X-ray flux²⁰, even if the identification¹⁹ with η Car were correct.

Eta Car was observed by the X-ray telescopes on board OAO-Copernicus on May 25, 1973. Both telescopes used the largest field of view (equivalent beam width 12 arc min) and were pointed 'on' and then 'off' the source for six sets of observations of about 14 min each. The slew 'off' the source was of about 5° in range, and provided a reliable background estimate. No statistically significant difference in count rate was observed in either telescope, which leads to the upper limits (at the 2σ level) shown in Table 1. Similarly, no significant count rate was

Table 1 Upper limits to the X-ray flux from η Carinae

Energy band (keV)	0.5–1.5	1.5–4.6	2.5–7.5
Total count rate (s^{-1})	<0.01	<0.13	<0.025
Maximum energy flux in band erg $\text{cm}^{-2}\text{s}^{-1}$	1.6×10^{-11} *	4×10^{-11} *	2×10^{-11} †

* Assuming thermal spectrum¹⁷ $kT = 0.26\text{ keV}$, and hydrogen column density $N_H = 3 \times 10^{21}\text{ cm}^{-2}$

† Assuming a synchrotron spectrum, $\alpha = 0.8$ (ref. 16).

recorded in the collimated proportional detector, which has a 3° field of view; the corresponding upper limit (Table 1) is slightly below the threshold of the third Uhuru catalogue of X-ray sources²², which is $3.4 \times 10^{-11}\text{ erg cm}^{-2}\text{ s}^{-1}$ (2 Uhuru units) over the energy range 2–6 keV. An upper limit to the X-ray luminosity L_x of the source, between 0.5 keV and 7.5 keV can be obtained by removing the assumed effect of a column density $N_H = 3 \times 10^{21}\text{ cm}^{-2}$ on the maximum flux observed in the 0.5–1.5 keV band and extrapolating the continuum to higher energies. Assuming a distance of 2 kpc, this gives $L_x < 2 \times 10^{34}\text{ erg s}^{-1}$; the result is approximately the same whether the thermal ($kT = 0.26\text{ keV}$) or synchrotron ($\alpha = 0.8$) spectrum is assumed, and does not vary significantly for N_H values between 2.5 and $4.8 \times 10^{21}\text{ cm}^{-2}$. The interstellar cross sections used here are those of Brown and Gould²³.

The low energy flux (0.5–1.5 keV) is at least an order of magnitude below that expected from the Livermore source.¹⁹ Possible explanations for this disparity are:

- (1) that the source found by Hill *et al.*¹⁹ was not η Car but was instead a soft source some distance away in galactic latitude;
- (2) that the observed emission¹⁹ came from an extended region around η Car at least 1° in diameter
- (3) that the X-ray flux from η Car is variable.

In the second case the low energy flux within the field of view of our telescopes would have been undetectable, and at high energies the source was not detected in either of the two experiments. The existence of such an extended source seems very unlikely, however, in view of the optical morphology of the region: η Carinae is embedded in Herschel's 'Keyhole Nebula' NGC3372, and there is no evidence for any extended supernova remnant. The third possibility also seems very unlikely if the X rays come either from a shock region outside the $10''$ visible shell or from the volume inside the shell. We can see no observational evidence for any variable source with a time scale of the order of 1 min over 40 min of observation; a source as strong as that found by the Livermore group¹⁹ would have been observed in a few minutes.

We shall therefore assume that the first hypothesis is correct. The present upper limit on soft X-ray emission then removes the problem encountered by Davidson and Ostriker²⁰ of a high ambient density around the object, and it also removes any basis for expecting coronal lines in the visible spectrum. The pulsar model, already unlikely on the basis of the Livermore observation²⁰, now seems even more unlikely, because the total X-ray luminosity which, on the basis of Ostriker and Gunn's model⁷, should exceed that of the Crab Nebula ($\sim 10^{38}$ erg s⁻¹ above 1 keV), is at least three orders of magnitude lower. On the Compton model¹⁸, the predicted flux density in the neighbourhood of a few keV is of the order of 10^{-28} erg cm⁻² s⁻¹ Hz⁻¹ for the 'most likely' model with relativistic electrons with a low energy cut off at $\gamma = 10^3$ and a magnetic field of about 10^{-3} gauss, but it can be very much less if the cutoff energy is greater. The corresponding upper limit from our observations (averaging 1.5–7.5 keV) is 4×10^{-29} erg cm⁻² s⁻¹ Hz⁻¹, which could be taken as indicating a higher low energy cutoff on this model.

The balance of the evidence, however, reinforced by the failure to detect any X rays, favours strongly the view that the spectrum of η Carinae is of thermal origin, with the bulk of the energy coming out from dust grains.

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- ¹ Bowles, J. H., Patrick, T. J., Sheather, P. H., and Eiland, A. M., *J. Phys., E*, **7**, 183 (1974).
- ² Sanford, P. W., *Proc. R. Soc.*, (in the press).
- ³ Gratton, L., in *Star Evolution*, (edit by Gratton, L.), 297 (Academic Press, London, 1963).
- ⁴ Neugebauer, G., and Westphal, J. A., *Astrophys. J. Lett.*, **152**, L87 (1968); Westphal, J. A., and Neugebauer, G., *ibid.*, **156**, L45 (1969).
- ⁵ Thackeray, A. D., *Observatory*, **76**, 103, (1956).
- ⁶ Burbidge, G. R., *Astrophys. J.*, **136**, 304 (1962).
- ⁷ Ostriker, J. P., and Gunn, J. E., *Astrophys. J. Lett.*, **164**, L95 (1971).
- ⁸ Rodgers, A. W., and Searle, L., *Mon. Not. R. astr. Soc.*, **135**, 99 (1967).
- ⁹ Gaviola, E., *Astrophys. J.*, **111**, 408 (1950).
- ¹⁰ Pagel, B. E. J., *Nature*, **221**, 325 (1969).

- ¹¹ Pagel, B. E. J., *Astrophys. Lett.*, **4**, 221, (1969).
- ¹² Burbidge, G. R., and Stein, W. A., *Astrophys. J.*, **160**, 573 (1970).
- ¹³ Pagel, B. E. J., in *Atoms and Molecules in Astrophysics* (edit. by Carson, T. R. and Roberts, M. J.), 341 (Academic Press, London, 1972).
- ¹⁴ Ade, P., and Pagel, B. E. J., *Observatory*, **90**, 6 (1970).
- ¹⁵ Davidson, K., *Mon. Not. R. astr. Soc.*, **154**, 415 (1971).
- ¹⁶ Robinson, G., Hyland, A. R., and Thomas, J. A., *Mon. Not. R. astr. Soc.*, **161**, 281 (1973); Sutton, E., Becklin, E. E., and Neugebauer, G., *Astrophys. J. Lett.*, **190**, L69 (1974).
- ¹⁷ Rodgers, A. W., *Astrophys. J.*, **165**, 665 (1971).
- ¹⁸ McCray, R., *Astrophys. J.*, **147**, 544 (1967).
- ¹⁹ Hill, R. W., Burginyon, G., Grader, R. J., Palmieri, T. M., Seward, F. D., and Stoering, J. P., *Astrophys. J.*, **171**, 519 (1972).
- ²⁰ Davidson, K., and Ostriker, J. P., *Nature Phys. Sci.*, **236**, 46 (1972).
- ²¹ Pagel, B. E. J., *Observatory*, **92**, 214 (1972).
- ²² Giacconi, R., *et al.*, *Astrophys. J. Suppl. Ser.*, **237** (1974).
- ²³ Brown, R. L., and Gould, R. J., *Phys. Rev. D*, **1**, 2252 (1970).

Radio emission from Hen1044

CONTINUUM radio emission has been detected from several early-type emission-line objects which show a strong infrared excess^{1–9}. Here we report the detection of radio emission from a Southern Hemisphere compact emission-line object Hen1044 (He2-113).

Observations were made at 3.0 and 6.0 cm using the CSIRO 64-m telescope at Parkes, which has half-power beamwidths of $2'.5$ and $4'.0$ at these two wavelengths. At both wavelengths beam-switching was used to reduce atmospheric effects, the beam separations being 6.2 and 2.8 half-power beamwidths respectively. At 3.0 cm (9.86 GHz) a mixer receiver¹⁰ was used with a predetection bandwidth of 500 MHz and a system noise temperature of 650 K. At 6.0 cm (5.0 GHz) a cryogenically cooled parametric amplifier was used with a predetection bandwidth of 300 MHz and a system noise temperature of 80 K. An on-on source technique of observing similar to that described previously⁴ was used, except that here the two beams were separated in azimuth.

Radio emission was detected from Hen1044 at both 3.0 and 6 cm wavelengths. The flux density determined from 10 independent measurements made during the period 1973 August 24 to 27 was 0.24 ± 0.03 Jy at 3 cm. Observations at a wavelength of 6.0 cm were made during the period 1973 October 17 to 22. Two independent measurements yielded a flux density of 0.11 ± 0.03 Jy. Scanning observations were also made at this wavelength. The means of five right ascension scans and 11 declination scans are shown in Fig. 1. The radio source is unresolved and the region is reasonably free of confusion. The differences between the

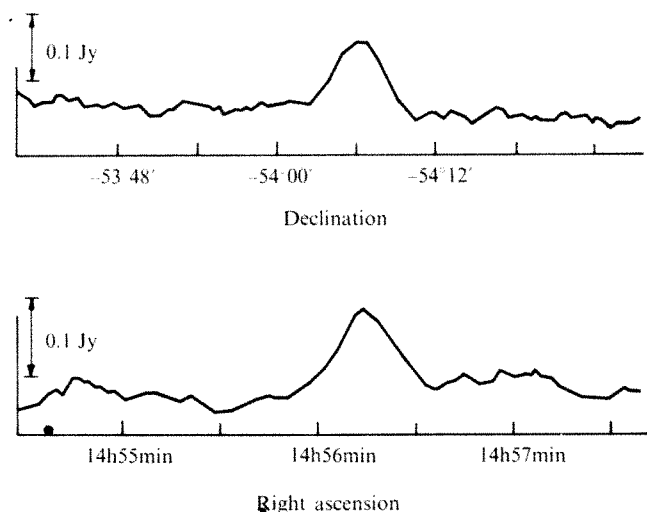


Fig. 1 Declination and right ascension scans of Hen1044 at 6 cm; the epoch for both coordinates is 1950.

optical position (RA 14 h 56.3 min dec. $-54^{\circ}06'$, epoch 1950) and the radio position are 4 s in right ascension ($36''$ on the sky) and $20''$ in declination. These differences are within the pointing errors of the telescope.

The object Hen1044 has a stellar appearance¹¹, with "no obvious surrounding nebulosity"¹². Its optical spectrum, which has shown no appreciable change between 1962 and 1973 (refs 12,13), is that of a low-excitation planetary nebula associated with a cool WC star. The object is one of only four which have been classified¹² as WC 10. Continuing mass outflow is indicated by the presence of P Cygni profiles. These characteristics are suggestive of an embryonic planetary nebula. Hen1044 is very similar to the WC 9 object HD167362, from which radio emission has recently been detected^{5,14}.

The continuum radio spectrum of Hen1044 is not well defined by the two flux density measurements reported here. But if it is assumed that the radio emission did not vary significantly between the two dates, and that the emission is produced by the bremsstrahlung mechanism, then the emitting region has an optical depth of 1 at ~ 8 GHz, indicating an emission measure of $\sim 10^8 \text{ cm}^{-6} \text{ pc}$. If the radio emission is associated with circumstellar gas at a temperature $\sim 10^4 \text{ K}$, then the angular size of the radio source is $\sim 1''$, consistent with the upper limit to the optical size. Very similar results have been found¹⁴ for the bright central component of HD167362.

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- ¹ Braes, L. L. E., Habing, H. J., and Schoenmaker, A. A., *Nature*, **240**, 230 (1972).
- ² Altenhoff, W. J., and Wendker, H. J., *Nature*, **241**, 37 (1973).
- ³ Hughes, V. A., and Woodsworth, A., *Nature phys. Sci.*, **242**, 116 (1973).
- ⁴ Purton, C. R., Feldman, P. A., and Marsh, K. A., *Nature phys. Sci.*, **245**, 5 (1973).
- ⁵ Purton, C. R., Feldman, P. A., and Marsh, K. A., *Nature phys. Sci.*, **245**, 6 (1973).
- ⁶ Feldman, P. A., Purton, C. R., and Marsh, K. A., *Nature phys. Sci.*, **245**, 7 (1973).
- ⁷ Feldman, P. A., Purton, C. R., and Marsh, K. A., *Nature phys. Sci.*, **245**, 39 (1973).
- ⁸ Baldwin, J. E., *Observatory*, **93**, 182 (1973).
- ⁹ Feldman, P. A., Purton, C. R., and Marsh, K. A., *Bull. Am. astr. Soc.* (in the press).
- ¹⁰ Fourkis, N., Howarth, C. D., and Dunn, D. C., in *Convention Digest of IREE Aust. 14th Natn. Radio Electron. Engng Conv.*, 14 (Melbourne 1973).
- ¹¹ Westerlund, B. E., and Henize, K. G., *Astrophys. J. Suppl. Ser.*, **14**, 154 (1967).
- ¹² Webster, B. L., and Glass, I. S., *Mon. Not. R. astr. Soc.*, **166**, 491 (1974).
- ¹³ Carlson, E. D., thesis, Northwestern Univ. (1968).
- ¹⁴ Seaquist, E. R., and Gregory, P. C., *IAU Circ.*, No. 2563 (1973).

marily from extragalactic sources. In an intermediate group there are those³ who think that perhaps only the most energetic particles ($E > 10^{18} \text{ eV}$) are derived from extragalactic sources.

The observation^{4,5} of cosmic γ rays has given hope for a distinction between the Galactic and extragalactic models, at least for the energies of cosmic ray protons ($1 \text{ GeV} < E < 10 \text{ GeV}$ mainly) whose collisions with interstellar gas atoms are considered to give rise to the π^0 mesons from which the γ rays are derived. The experimental data, which relate to the intensity of γ rays above 100 MeV as a function of Galactic longitude, indicate rather clearly that there is an increase in the emissivity of γ rays (defined as the rate of production per unit volume) towards the Galactic centre (GC) with a peak in the region of radial distance from the GC, R , of about 5 kpc. There is some argument as to the actual variation of emissivity with R : Puger and Stecker⁶ derive a very sharp peak at $R=5$ kpc whereas one of the present authors, Strong⁷ (to be published) derives a rather more gradual variation of emissivity. Figure 1 shows

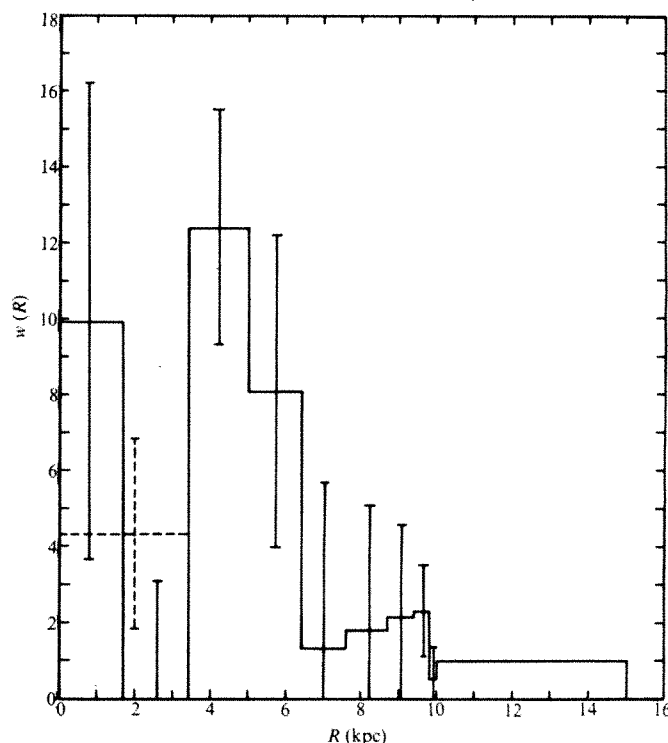


Fig. 1 The variation of γ -ray emissivity relative to that at the Sun $w(R)$, as a function of distance from the Galactic centre (R), deduced from observations by the OSO-III and SAS-II satellites. Cylindrical symmetry about the centre has been assumed in order to unfold the experimental data. The quoted errors are statistical only. — — Average emissivity for the first two bins of radial distance.

the average relative emissivity derived by the present authors using Strong's method for the longitude range 270° – 90° .

So far the interpretations have suggested that there is an increase in the flux of cosmic rays towards the GC corresponding to cosmic rays of Galactic origin. A number of models have been proposed: an increased containment time arising from an enhanced magnetic field⁷, structured acceleration in the hydrodynamic shock driven by the expanding gas in the "3 kpc arm"⁸ and, very recently, an increased yield in the spiral arms resulting from an assumed correlation between the cosmic ray flux and the neutral hydrogen density in the arms (Bignami and Fichtel⁹).

In all these models it is assumed that the predominant interaction responsible for the parent π^0 mesons is between

Relevance of cosmic gamma-rays to origin of the cosmic radiation

THE controversy over the question of the origin of energetic cosmic rays is well known. There are those who believe that the bulk of the radiation comes from Galactic sources, such as supernovae¹ and others² who find rather good reasons for supposing that the radiation comes pri-

cosmic-ray protons (with a small admixture of α particles) and the protons from neutral hydrogen atoms. The variation of atomic hydrogen density with R has been derived from 21 cm data which shows (see Mezger⁹ for example) that the average surface density of neutral hydrogen in the range $0 < R < 5$ kpc is about one half that at $R \sim 10$ kpc (the location of the Solar System). In all the calculations some allowance has been made for the presence of unseen gas, presumed to be mainly molecular hydrogen, by multiplying the predicted γ -ray yields by a factor of ~ 1.5 , this having been thought to be a 'reasonable' factor.

It has been noted before that the distribution of γ -ray emissivity appears to correlate quite well with that of giant H II regions (ref. 8 and A. W. Strong, unpublished). Now it is well known that dense ($\sim 10^3$ cm⁻³), massive ($\sim 10^4$ – $10^5 M_\odot$) molecular clouds are found in association with such H II regions¹⁰, and such clouds will be copious sources of γ rays. (The possibility that nearby clouds may be observable as point sources has in fact been suggested by J. H. Black and G. G. Fazio, unpublished). What appears not to have been realised until recently is the fact that the contribution of the molecules to the gas density at $R \sim 5$ kpc may be very large: according to Solomon¹¹ and N. Z. Scoville and P. M. Solomon (private communication), the smoothed molecular hydrogen density in the region 4–6 kpc from the Galactic centre may be as high as 10 cm⁻³, compared to a total gas density near the Sun of only about 1 atom cm⁻³. (It should be remarked that the importance of the correlation between the molecular hydrogen distribution and that of γ rays has also been stressed recently by Solomon and Stecker¹².)

As is clear from Figure 1, the increase in molecular hydrogen density is quite adequate to account for the observed γ -ray intensities with a uniform cosmic ray flux if, as would seem likely, the molecular clouds have a distribution perpendicular to the Galactic plane not too different from that of the H II regions (with a full width of about 100 pc¹³). Explanation in terms of molecular hydrogen was in fact suggested some time ago by Stecher and Stecker¹⁴, although at that time there was no direct evidence for an increase in the molecular hydrogen density towards the Galactic centre and later explanations by Stecker and coworkers have concentrated on cosmic-ray gradients such as would result from Galactic sources of the cosmic-ray protons or at least Galactic acceleration of the particles.

The importance of the new result is that the necessary constancy of the cosmic-ray flux would be more consistent with a largely extra-galactic origin of the cosmic rays rather than a Galactic one.

In conclusion, it is clear that, unless the molecular hydrogen measurements are seriously in error the γ -ray data do not now necessarily demand explanation in terms of a Galactic origin for the initiating cosmic rays.

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¹ Ginzburg, V. L. and Syrovatskii, S. I., *Origin of Cosmic Rays*, (Pergamon, London, 1964).

² Brecher, K., and Burbidge, G. R., *Astrophys. J.*, **174**, 253–291 (1972).

³ Wolfendale, A. W., *Proc. R. Soc.* (in the press, 1974).

⁴ Kraushaar, W. L., Clark, G. W., and Garmire, G. P., *Astrophys. J.*, **177**, 341–363, (1972).

⁵ Kniffen, D. A., Hartman, R. C., Thompson, D. J., and Fitchel, R. C., *Astrophys. J. Lett.*, **186**, L105–L109 (1973).

⁶ Puget, J. L. and Stecker, F. W., *Astrophys. J.*, **191** (in the press, 1974).

⁷ Strong, A. W., Wdowczyk, J., and Wolfendale, A. W., in *Gamma-Ray Astrophysics* (edit. by Stecker, F. W., and Trombka, J. L. 259–262, Government Printing Office, Washington, 1973).

⁸ Stecker, F. W., Puget, J. L., Strong, A. W., and Bredekamp, J. H., *Astrophys. J. Lett.*, **188**, L59–L61 (1974).

⁹ Mezger, P. G., in *The Interstellar Medium* (edit. by Pinkau, K.) (Reidel, Dordrecht, 1973).

¹⁰ Scoville, N. Z., and Solomon, P. M., *Astrophys. J.*, **180**, 31–53 (1973).

¹¹ Solomon, P. M. Paper presented to American Physical Society, Washington (April 1974).

¹² Solomon, P. M., and Stecker, F. W., *Proc. Eighth ESLAB Symposium on Gamma Ray Astronomy, Frascati, June 1974*, (in the press).

¹³ Murdin, P. and Sharpless, S., in *Interstellar Ionised Hydrogen* (edit. by Therzian, Y.), 249–267 (Benjamin, New York, 1968).

¹⁴ Stecher, T. P., and Stecker, F. W., *Nature*, **226**, 1234–1235 (1970).

¹⁵ Bignami, G. F., and Fichtel, C. E., *Astrophys. J. Lett.*, **189**, L65–67 (1974).

Formation of holes in the solar corona

SOFT X-ray spectroheliograms obtained from Skylab¹ and rocket flights² show many bright filamentary features which map out the magnetic structure of the solar corona. Additional interesting features are the extensive regions from which the emission is extremely low. These long, but relatively narrow, 'coronal holes' can stretch much of the way across the solar disk in a roughly N–S direction. The photosphere and chromosphere beneath are relatively featureless and have low magnetic flux density. The magnetic structure of these lower levels is not untypical of other quiet regions on the Sun where coronal holes have not developed. This suggests that the magnetic configuration associated with the holes might not have emerged from the solar surface with any particularly unusual geometry, but this might have developed later due to some rearrangement within the atmosphere. Changes in field structure within the corona have been regarded for more than a decade as part of the normal process of development during the solar cycle³.

The spectroheliograms indicate the existence of long magnetic-arch structures in the solar atmosphere². It is thought that these arches are produced by merging of smaller structures by cross-connection of field lines, since this results in a reduction of the magnetic energy stored³. How this proceeds depends upon the initial conditions, but the trend is illustrated by the simple case in Fig. 1a. Where the flux tubes touch their neighbours there will be merging to produce the resultant fields shown as solid curves with double arrow heads. Even when the initial conditions are more complex, as when the original arches emerge from the solar surface at different times, the trend should be the same.

Magnetic arches have a preferred alignment on the Sun⁴. In the northern hemisphere the vertical flux tubes tend to lie within NE–SW planes, while south of the equator they stretch roughly SE–NW. (Hereafter, both these directions are denoted as E–W. The description can then remain reasonably general because it is unnecessary to specify which hemisphere is being considered.) At any time the field tends to point from E to W in one hemisphere but from W to E in the other. In both cases the sense reverses between one 11 yr cycle and the next.

The situation discussed so far concerns interaction between the relatively young flux tubes developing within

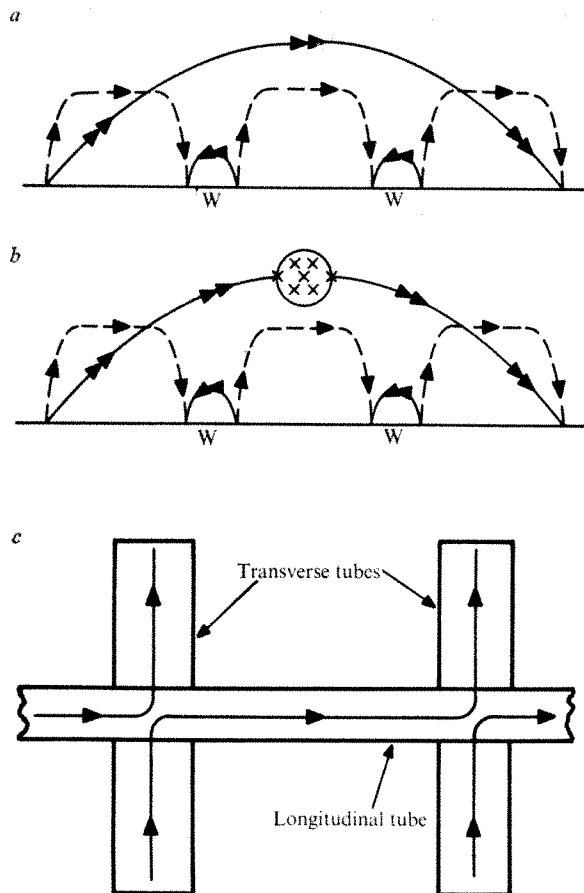


Fig. 1 Each dashed curve represents a magnetic flux tube which passes from the photosphere into the solar atmosphere (presumably related to development at supergranulation). Because these arches have similar polarities they can merge to reduce the magnetic energy. *a*, The solid curves represent the resultant flux tubes; *b*, the situation when an additional flux tube crosses the region perpendicular to the plane of the paper. The resultant loops produced at the regions marked *w* will possibly be carried away³ by the flow of material at the solar surface. In *c* the horizontal flux tube shown in *b* is seen to cross two parallel arches. Curves with arrow heads illustrate how cross connection between field lines from each of the three flux tubes allows a line to pass from one transverse arch to the other.

quiet regions on the Sun. It is necessary to take into account the interaction with other arches produced earlier in the solar cycle, or even during the previous cycle. Some particularly long structures develop in roughly N-S directions. These stretch between pairs of active regions or from active regions to polar caps⁵. It is also possible that flux passes from one pole to the other³. If one of these N-S flux tubes comes into contact with the E-W arch system forming in Fig. 1*a*, merging between the perpendicular fields can proceed⁶. A section through the new system is shown in Fig. 1*b*; the plan view of this structure (Fig. 1*c*) shows how a field line from one of the transverse arches might cross to another by passing along a length of longitudinal flux tube. Cross coupling of field lines which leads to this configuration can be expected because it reduces the magnetic energy stored in the whole structure.

The situation becomes particularly interesting if some of the transverse arches have polarities opposing the others. A simple system containing a pair of arches illustrates this in Fig. 2*a*. A field line crossing between the pair now has both feet to one side of the longitudinal flux tube, so that the relaxation accompanying merging can separate it from the original structure. If cross connection between the pair of arches became complete, a cavity would be

produced in the magnetic field, as shown in Fig. 2*b*. The flux along the N-S tube gets 'eaten away' in this process.

Figure 2*b* shows the simplest situation where a pair of neighbouring antiparallel arches merge together. In practice the horizontal flux tube will cross many arches so that all the field lines from one will be unlikely to pass into a single antiparallel structure. A cavity can be produced nevertheless if the transverse arches towards one end of the N-S flux tube have one polarity, while the other end makes contact with fields of reverse polarity. A formalised representation of this appears in Fig. 3*a*. (Field lines like that in Fig. 1*c*, which cross from one arch to a parallel one on the same side of the boundary, have not been included because these do not represent a state having minimum energy. Such lines continue to interact with flux lying N-S until antiparallel arches are linked). This is just the situation which can produce very long coronal holes if the N-S flux tube is suitably located on the solar disk. Two cases are of interest:

(a) The more obvious case is the one where the longitudinal flux tube crosses the solar equator in the manner of the 'trans equatorial arches' observed by Hansen *et al.*⁵. It has been pointed out already that E-W arches crossing this tube would change polarity across the equator. The general alignment of coronal holes in the N-S direction between hemispheres can therefore be explained in this way.

(b) A stage is likely to be reached at the transition between solar cycles when antiparallel magnetic arches form in a single hemisphere. As solar minimum approaches, polarities associated with the old 11-yr cycle are found near the equator, while reversed fields due to the developing cycle form at intermediate latitudes³. Suitable conditions seem to be developing at present since Gillespie *et al.*⁷ reported the appearance of new cycle polarity configurations during August 1973.

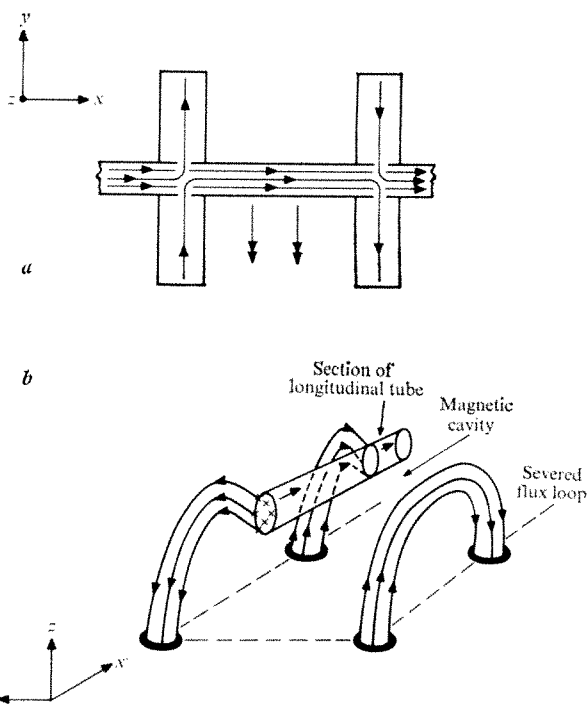


Fig. 2 *a*, is similar to Fig. 1*c*, except that the fields along the transverse arches are now antiparallel. A field line crossing from one of these to the other should relax, in the direction of the double-headed arrows, from the position shown. *b*, The extreme case when all the field lines from one arch pass into the other.

At the stage when the old and new 11-yr cycles are both active, the polarities of arches will change between zones as indicated in Fig. 3b.

In addition to the coronal holes, soft X-ray spectroheliograms show regions of low emission which form along the path of dark filaments (prominences) observed in H α spectroheliograms. One of these filament cavities is labelled in Fig 1 of ref. 1, while several good examples (one of which is Y-shaped) are seen as long thin dark features in Fig. 1 of ref. 2. Such structures could be closely related to coronal holes since there is evidence that dark filaments might have the magnetic configuration drawn in Fig. 1c. It has been suggested³ that condensed material is embedded in a longitudinal field produced by local

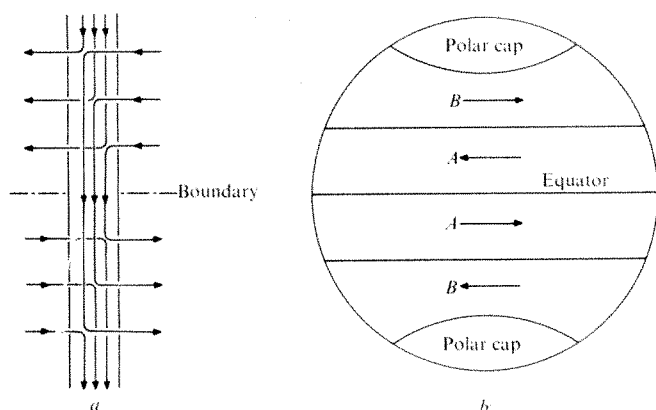


Fig. 3 The situation shown in Fig. 2 is unlikely because it assumes that all field lines pass from one arch to a second neighbouring structure. It is far more probable that there would be interaction between numerous arches crossed by the horizontal flux tube. This could still lead to the formation of a cavity, however, if the transverse arches had different polarities on each side of a boundary. *a*, A formalised representation of how field lines from one or more transverse arches on one side of the boundary can pass into one or more structures of reversed polarity on the other side. *b* shows a number of boundaries on the solar disk across which changes in polarities of arches are likely to occur. The arrows show field directions at one stage during a 22-yr cycle. Regions denoted by *A* have fields associated with the first half of this cycle while regions *B* have polarities of the second half-cycle.

shearing of transverse support arches. Even if such structures are not formed by the exact sequence of events described here, it is still possible that a reversal in the polarity of the support arches along the length would lead to the formation of a cavity.

In the model outlined the flux tubes need only merge on a time scale of the order of one day, over which period changes in the structure of coronal holes occur. Such a rate of merging appears acceptable on theoretical grounds although, as Sweet⁹ points out, it is difficult to explain the far more rapid changes needed to account for solar flares.

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¹ Vaiana, G. S., Davis, J. M., Giacconi, R., Krieger, A. S., Silk, J. K., Timothy, A. F., and Zambbeck, M., *Astrophys. J. Lett.*, **185**, L47 (1973).

² Vaiana, G. S., Kreiger, A. S., and Timothy, A. F., *Solar Phys.*, **32**, 81 (1973).

³ Babcock, H. W., *Astrophys. J.*, **133**, 572 (1961).

⁴ Weart, S. R., *Astrophysics. J.*, **162**, 987 (1970).

⁵ Hansen, S. F., Hansen, R. T., and Garcia, C. J., *Solar Phys.*, **26**, 202 (1972).

⁶ Parker, E. N., *J. geophys. Res.*, **62**, 509 (1957).

⁷ Gillespie, B., Harvey, J., and Livingstone, W., *Astrophys. J. Lett.*, **186**, L85 (1973).

⁸ Tandberg-Hanssen, E., and Anzer, U., *Solar Phys.*, **15**, 158 (1970).

⁹ Sweet, P. A., *A. Rev. Astr. Astrophys.*, **7**, 149 (1969).

Ionospheric effects of the Flixborough explosion

ON June 1, 1974, an explosion of unprecedented magnitude occurred at the Nypro plant at Flixborough, Lincolnshire. A considerable blast wave was produced and evidence is presented here to show that this wave penetrated the atmosphere to ionospheric heights. Our purpose is to report these major ionospheric disturbances since they may yield information regarding the time sequence of the explosion and the amplitude of the blast wave that it created.

Naturally occurring waves in the ionosphere have been studied at Leicester using the high frequency (HF) radio Doppler sounding technique¹. In this method an HF radiowave is reflected from the ionosphere at steep incidence and the frequency of the received signal is monitored². Acoustic-gravity waves propagating through the atmosphere displace the reflection point and a Doppler frequency shift is produced in the reflected signal. The magnitude of the Doppler shift Δf is proportional to the rate of change of phase path of the radiowave according to the expression

$$\Delta f = -(f/c)/(dP/dt) \quad (1)$$

where f is the radiowave frequency and c is the velocity of light. If three spaced reflection points are available then the speed and direction of the disturbance may be determined from the time delays in the Doppler frequency changes observed on the three transmissions.

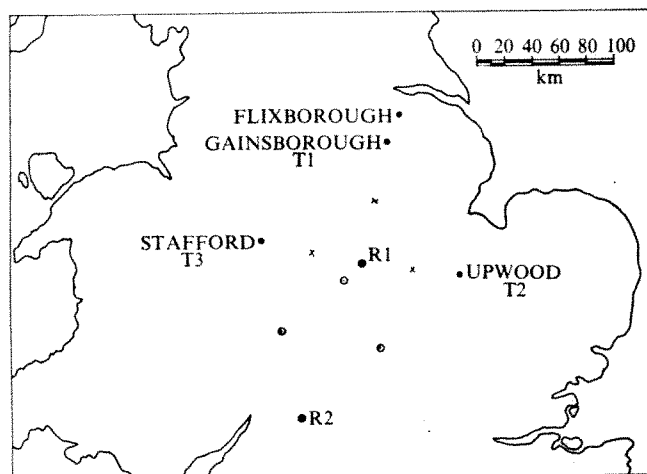


Fig. 1 Location of transmitters and receivers of the Doppler sounding system. T, Transmitter; x and o, propagation path mid points; R, receiver.

On June 1 three transmitters located at Gainsborough, Upwood and Stafford were operational. These were received at two sites, one at Leicester (R1) and one in Wiltshire (R2). Figure 1 shows the locations of the transmitters and receivers together with those of the six reflection points.

At 1600.20 GMT a major disturbance was noted on the Gainsborough-Leicester path and subsequently on the more

southerly transmissions. Recordings of frequency as a function of time were obtained from the two receivers R1 and R2 and the Leicester record is reproduced in Fig. 2. The frequencies of the three transmitters are offset by 3 Hz relative to each other so that they may be distinguished on the frequency-time recording. The principal feature on all six transmissions is the large quasisinusoidal oscillation starting at 1601.11 GMT on the transmission closest to the explosion (Gainsborough-Leicester). The feature approximates to two cycles of a sinusoidal oscillation with a period of about 50 s. The initial positive Doppler shift indicates that the electron-density

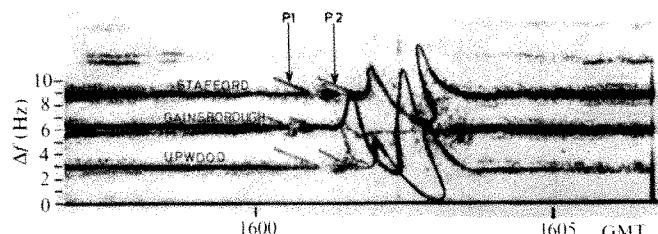


Fig. 2 Time variation of Doppler frequency shifts recorded by the Leicester receiver (R1) showing large-scale disturbances between 1600.20 and 1603.38 GMT.

is first enhanced, thus producing a lowering of the reflection level. This is followed by a negative Doppler shift indicating that the height increases rapidly. During the next half cycle the frequency shift is again positive showing a further period of decreasing height. Finally there is a very small negative Doppler shift as the height returns to its undisturbed position. The maximum Doppler frequency shift is 4.5 Hz which corresponds to a rate of change

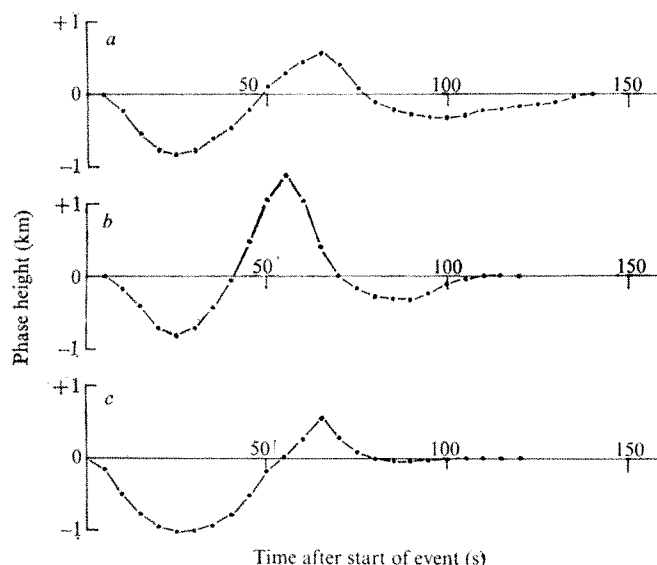


Fig. 3 Phase height changes produced by the disturbances, calculated from the Leicester observations. Time measured from the start of each disturbance. a, Stafford; b, Gainsborough; c, Upwood.

of 142 m s^{-1} in the reflection height. This frequency deviation is a factor of four greater than those produced by naturally occurring travelling disturbances in the ionosphere. An interesting feature of the Leicester recordings are the two precursors, marked P1 and P2 in Fig. 2, which precede the main event on all three transmissions. These features are less well defined

on the recordings obtained from R2.

The velocity c of an acoustic wave in the atmosphere is given by

$$c^2 = \gamma g H \quad (2)$$

where γ is the ratio of the specific heat of the gas at constant pressure to that at constant volume, g is the acceleration due to gravity, and H is the scale height of the atmosphere at the height considered. Using the CIRA reference atmosphere³ and equation 2, the time taken for an acoustic wave to travel from Flixborough to the reflection point of the Gainsborough transmission was found to be 10.6 min. The recording in Fig. 2 shows that the major disturbance started at 1601.11 GMT and so the time of the explosion is 1550.35 GMT. This is in fair agreement with the time of 1554 GMT reported in the national press. The presence of the two precursor events P1 and P2 is not yet understood but they clearly indicate that two distinct disturbances were produced in advance of the main event. This could result from two pressure waves separated by about 40 s at ionospheric heights.

The magnitudes of the disturbances recorded by R1 (Fig. 2) are greater than those obtained at R2. The increased amplitude of the disturbances probably results from the slightly greater reflection heights of the signals received at Leicester. The amplitude of the acoustic wave will increase exponentially with height, consequently the disturbance will be greater the higher the reflection point in the ionosphere.

The phase height change (h) produced by the passage of the disturbance has been determined by integrating the frequency-time recording since from equation 1

$$h = P/2 = - (c/f) \int \Delta f \cdot dt \quad (3)$$

The height changes for the various transmissions are shown in Fig. 3. The maximum displacement is about 1.5 km and the period of the wave is about 75 s. This corresponds to a quasi wavelength of 68 km assuming the velocity of sound to be 900 ms^{-1} at the reflection height.

A detailed analysis will be undertaken to determine the exact time of detonation and to estimate the magnitude of the pressure wave at its origin. The presence of the precursor events will be examined and the significance of the double feature investigated.

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¹ Davies, K., Watts, J. M., and Zacharisen, D. H., *J. geophys. Res.*, **67**, 601-609 (1962).

² Jones, T. B., and Wand, I. C., *J. atmos. Terrest. Phys.*, **27**, 1111-1113 (1965).

³ Kallman-Bijl, H., Boyd, R. L. F., Lagow, H., Poloskov, S. M., and Priester, W., *COSPAR International Reference Atmosphere* (North Holland, 1961).

The turbulent boundary layer on the continental shelf

IN the late summer of 1973 we made measurements of the current structure in the thermal boundary layer on the continental shelf. The measurements were taken under conditions of complete neutrality, well away from the influence of local

coastlines. Figure 1 shows the positions of two moorings, 001 ($48^{\circ} 10.5'N$, $07^{\circ} 54'W$), and 002 ($48^{\circ} 16.5'N$, $07^{\circ} 47'W$) separated by 15 km. This compares with a tidal excursion of about 10 km for the surface water. Both moorings were in 180 m of water and more than 200 km from the nearest mainland. The current meters were supported by subsurface buoyancy. The thermal structure of the water column, and the depths at which current meter observations were made in relation to the thermal boundary layer is shown in Fig. 2. The temperature of the bottom 100 m of the water column increases with depth. This marginal increase of temperature at the adiabatic rate ($1.3 \times 10^{-6}^{\circ}C\ cm^{-1}$) is expected of a turbulent boundary layer, because the production of turbulent energy is much greater than the stabilising effect of the buoyancy flux. The absolute accuracy is only $0.02^{\circ}C$, using a mercury-in-glass reversing thermometer as the calibration standard. The resolution is, however, considered quite genuine to $\pm 0.0005^{\circ}C$ (ref. 1).

All but one of the current meters gathered reliable current data during the same seven day period. The meter which was placed 30 m off the bottom, failed after only two days and is excluded from the analysis. For the short period that it was in operation, however, it was consistent with the measurements obtained at a slightly shallower depth, 33.5 m off the bottom. The data reduction was identical for all of the current meter records.

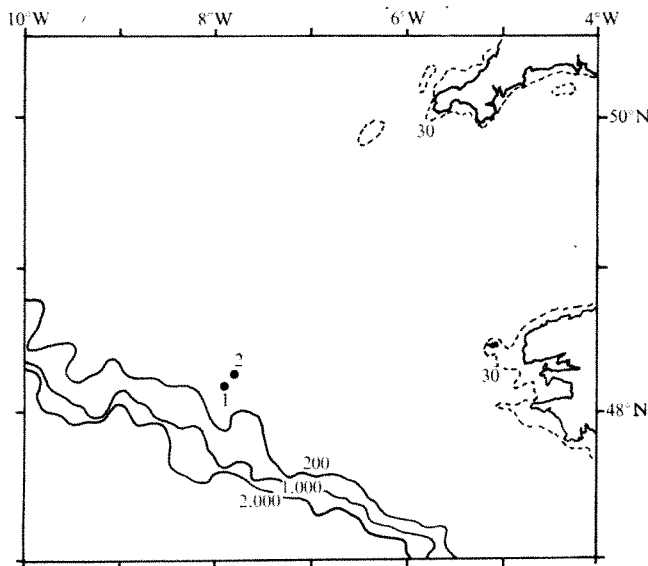


Fig. 1 Positions of moorings (●). Depth contours at 30 m, 200 m, 1,000 m and 2,000 m.

Kinetic energy density spectra for the current records were obtained from 2,048 two inch data points which were sampled every five minutes using a fast Fourier algorithm for complex numbers. Spectra for the current meter records obtained from mooring 001 are shown in Fig. 3. The kinetic energy density is generally less for the current meter records closest to the bottom but is evenly distributed throughout the frequency range except at the highest frequencies. The variance at the high frequencies is greatest on the record furthest from the bottom, which may be because of internal waves travelling along the thermocline. These results were also obtained for mooring 002.

Of the total kinetic energy, 80% is contained between the spectral estimates 11.4^{-1} and 13.2^{-1} cycles per hour, centred on 12.2^{-1} cycles per hour. This represents the bulk of the tidal stream energy. We have chosen this estimate to represent the motion because it represents about 90% of the mean speed.

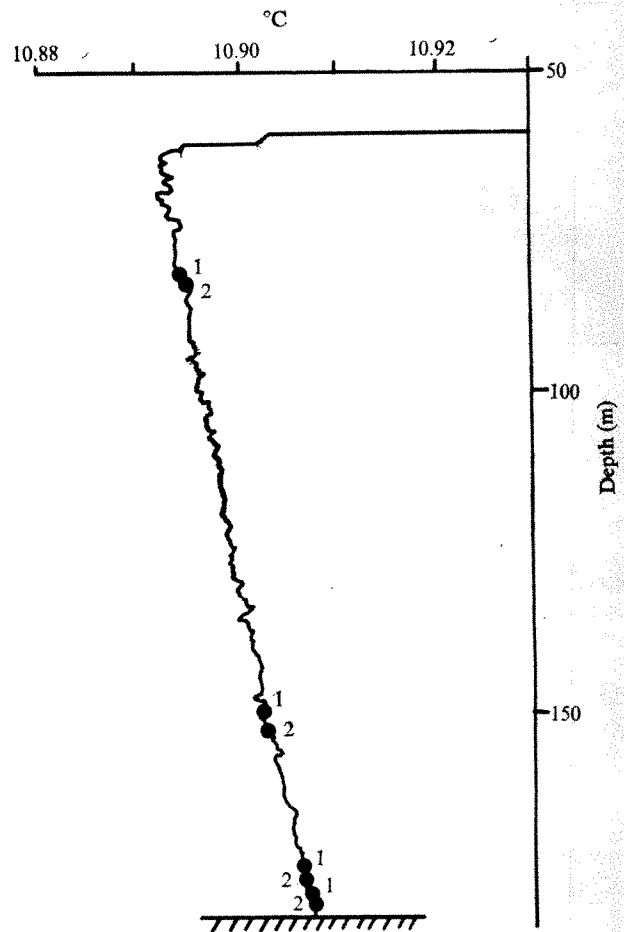


Fig. 2 Thermal structure of the water column in the turbulent boundary layer obtained from a typical temperature profile showing the depths at which current meters were fixed in this layer (●).

To avoid a time varying analysis the kinetic energy density spectra for each record was split into clockwise and anticlockwise spectral estimates. An example of this is shown for the bottom current meter record of mooring 001 (Fig. 3). The vector addition of clockwise and anticlockwise components produces a series of ellipses.

Table 1 summarises the results obtained for the semidiurnal estimate of all the records. At semidiurnal periods 94% of the kinetic energy is contained in the clockwise motion. Table 1 also shows the semimajor (a) and semiminor (b) axes of the tidal ellipse and the ratio $-b/a$. These show that the tidal ellipses are geometrically similar but become reduced in scale as the bottom is approached; a semimajor axis of $\sim 55\ cm\ s^{-1}$ 100 m from the bottom drops to $\sim 32\ cm\ s^{-1}$ 2 m from the bottom. The ellipses are orientated such that their major axes lie roughly parallel with a line drawn through the two mooring positions. A clear difference in orientation exists between the records from different moorings, with mooring 002 rotated about 8° clockwise with respect to mooring 001. The mean phase differences, which are relative to the lower current meter on mooring 001, are less than 3° apart in the bottom 33.5 m of the water column. That is approaching the limits of the absolute accuracy obtainable using different meters. The uppermost current meter on both mooring is, however, lagging about 13° (anticlockwise) behind the lower meters. Mooring 001 as a whole, lags behind mooring 002. That is confirmed by a simple overlay of direction records.

Figure 4 summarises the distribution of clockwise tidal amplitude [$u(-)$] with respect to depth (Z). The logarithmic depth (m) above the bottom is plotted against the clockwise semidiurnal speed ($cm\ s^{-1}$). This plot has been chosen to admit

Table 1 Current meter results

Water depth (m)	Mooring	Height of current meter above bottom (m)	Kinetic energy at semidiurnal frequency (%)	Semidiurnal energy in the clockwise component (%)	Relative phase of current ellipse (degrees)	Semi-major axis, a (cm s ⁻¹)	Semi-minor axis, b (cm s ⁻¹)	$-b/a$	Orientation of major axis (°T)
188	001	98.0	79	96	-013	51.6	-33.2	0.64	031
	001	33.5	80	93	-003	45.1	-25.3	0.56	027
	001	7.5	83	93	-002	38.8	-22.4	0.57	033
	001	3.5	82	94	000	35.2	-21.4	0.61	026
184	002	98.0	79	95	-007	58.0	-35.6	0.61	042
	002	5.0	80	92	+006	36.4	-20.2	0.56	035
	002	2.0	82	93	+006	32.1	-18.6	0.58	034

possible inferences with turbulent boundary layer theory.

In the plot of Fig. 4 the data fits the form

$$u(-)/u_*(-) = (1/k) \log_e Z/Z_0$$

where $k=0.4$, $u_*(-)=1.2$ cm s⁻¹, and $Z_0=3 \times 10^{-4}$ m, for $Z < 33.5$ m.

Such logarithmic plots can, however, be matched with power laws for selected ranges, and the data could have equally been put in the form

$$Z/Z_1 = (u)/(u_1)^n$$

where $n=8$, and at $Z_1=1$ m, $u_1=24$ cm s⁻¹.

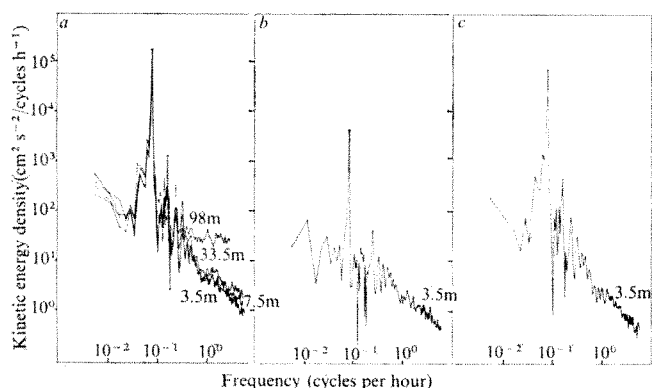


Fig. 3 Kinetic energy density spectra for records obtained on mooring 001. The total kinetic energy density has been split into anticlockwise and clockwise estimates for the current meter nearest the bottom: a, total density; b, anticlockwise; c, clockwise.

Identification of the logarithmic form with a boundary layer, where $u_*(-)$ represents the friction velocity and Z_0 the characteristic length scale relating to the boundary roughness, requires some independent confirmation. Such a form is strictly applicable only to an unaccelerated, one-dimensional flow under negligibly small pressure gradient. In a tidal stream, however, the flow is accelerated and pressure gradient forces, geostrophic forces and centrifugal forces are important. Applicability of the logarithmic form in the unsampled lower two metres may be extended through measurements in the coastal waters of Red Wharf Bay, although we have no values of current direction here². The homogeneous nature of the temperature in the bottom mixed layer suggests that eddy viscosity is related to distance from the boundary.

Two lines have been drawn in Fig. 4 as the values come from different moorings and both suggest that the flow is hydrodynamically rough (the bottom surface is sand and broken and abraded shells). The uppermost records clearly do not fit this logarithmic form and they are undoubtedly influenced by the thermocline and the boundary at the sea surface. Temperature measurements made from the upper two metres showed that the thermocline descended semi-diurnally below the uppermost current meter. This effect was

mainly responsible for the phase lag of the uppermost meter on each mooring. Continuous temperature records obtained from the other current meters showed no vertical temperature differences within instrumental resolution (0.03° C) during the complete seven day period, and this confirms the view that Fig. 2 represents a very persistent state of neutrality within 30 m of the bottom on the continental shelf.

The friction velocity of the anticlockwise component, $u_*(+)$ can be determined from Table 1, which shows that the anticlockwise energy is only ~ 7% of the semidiurnal energy on the records below 33.5 m. This gives $u_*(+)=0.3$ cm s⁻¹. The time varying friction velocity is found by adding:

$$u_*(t) = u_*(-) \exp(-i\omega t) + u_*(+) \exp(i\omega t)$$

where ω is the angular velocity (rad s⁻¹) of the semidiurnal

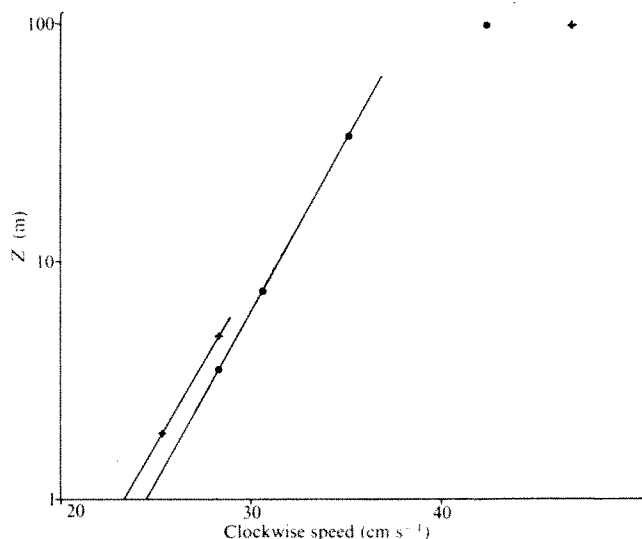


Fig. 4 The logarithmic profile for the clockwise semidiurnal motion.

tide. The friction velocity then varies between 0.9–1.5 cm s⁻¹. Our observations extend from spring to neap tides and so the values of $u_*(t)$ and Z_0 describe an approximate mean situation for the turbulent boundary layer of the continental shelf.

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¹ Pingree, R. D., *Deep Sea Res.*, **16**, 275–295 (1969).
² Charnock, H., *Geophys. J.*, **2**, 215–221 (1959).

Empirical relationship for the critical temperature of some A15 superconductors

A NEW, empirical relationship¹, which links the T_c of A15 superconductors—based on the A_3B stoichiometric ratio—with the atomic mass, M_B , of the B element, indicated that $\log T_c$ is a linear function of $\log M_B$ and that high T_c values can be predicted if M_B is small. A B element is from group IVB (Si, Ge and Sn) and an A element is from group VA (V, Nb and Ta).

The subsequent discovery that stoichiometric Nb_3Ge has the highest known T_c —23.2 K (ref. 2)—provides an opportunity to test further the $\log T_c$: $\log M_B$ relationship. The original prediction for Nb_3Ge was 29–30 K (ref. 1). If the experimental value, 23.2 K, is accepted as correct then the $\log T_c$: $\log M_B$ plot, using the additional data in Table 1, defines for the Nb and Ta series, two accurately parallel lines, with a slope of $-\frac{1}{2}$.

Table 1 Transition temperatures (experimental) of some A15 compounds

	V*	Nb*	Ta*
Si†	17.1(4)‡	Not known	Not known
Ge†	7 (3)	23.2(2)	8 (16)
Sn†	4.3(3)	18.3(17)	6.4(18)

* A elements.

† B elements.

‡ Reference numbers are in brackets.

The deviation of the V alloys from this sequence occurs because some of the T_c data refer not to the stoichiometric A_3B ratio (without which accurate comparisons between systems cannot be made) but to non-stoichiometric compounds with a V content greater than 75 atm. % (ref. 3). Thus, although V_3Si is well characterised at 17.1 K for stoichiometric single phase ordered samples (refs 4 and 5, and B. A. Hatt, unpublished) the compositions of the equilibrium A15 phases in V-Ge and V-Sn are $V_{7.7}Ge_{2.3}$ (ref. 3) and about $V_{8.0}Sn_{2.0}$ (refs 3 and 6), respectively. In both cases additional experimental work would be needed on the metastable, stoichiometric A15 compounds to establish their T_c values, which would

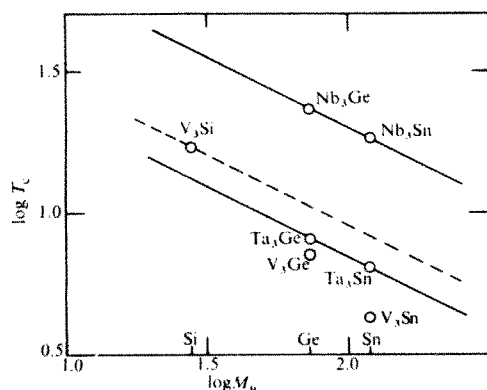


Fig. 1 $\log T_c$ against $\log M_B$ for binary A15 compounds of group VA and group IVB elements, where T_c is the superconducting critical temperature, and M_B is the atomic mass of the IVB element. Lines of slope of $-\frac{1}{2}$ are drawn through the points for the Nb and Ta compounds. The dotted line is drawn with a slope of $-\frac{1}{2}$ through the point for V_3Si .

certainly be higher than those quoted in Table 1. At present it must suffice to use the single point for V_3Si in order to define a dashed line, with a slope of $-\frac{1}{2}$, (Fig. 1), from which the predicted T_c values for V_3Ge and V_3Sn are 10.3 K and 8.3 K, respectively. These are reasonable estimates in the light of what is known of the effect of non-stoichiometry in other systems such

as V_3Si and V_3Ga (refs 4 and 7), and the extrapolation of data³ on V_3Ga – V_3Ge pseudobinary alloys yields a T_c of 10 K for stoichiometric V_3Ge .

Accepting the data used in Fig. 1 it emerges that T_c can be expressed as a function of M_B^{-1} and that when presented in

Table 2 Slope, C , of lines in Fig. 2

C (K $u^{1/2}$)	Group VA element
90	V
198	Nb
69	Ta

this way (Fig. 2) the experimental plots for the Nb and Ta series extrapolate accurately to zero.

From Fig. 2 the transition temperature is expressed in the form $T_c = C/M^{\frac{1}{2}}$. The slope, C , for the three curves is given in Table 2. The relationship does not apply when the B element in A_3B comes from Group IIIB or the transition metals. It remains true, however, that when selected systems are compared, the Nb compound always has the higher T_c (for example, Nb_3Ga —20.3 K, and V_3Ga —15 K; Nb_3Al —18.9 K, and V_3Al —~9 K; Nb_3Au —11 K, and V_3Au —3.5 K

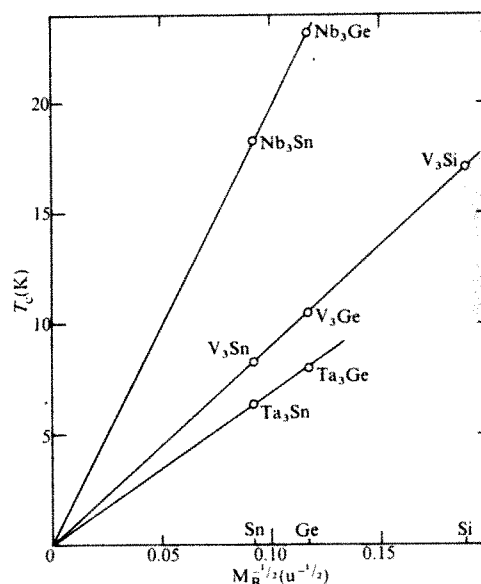


Fig. 2 T_c against $M_B^{-1/2}$ for the compounds shown in Fig. 1. T_c values for V_3Ge and V_3Sn are those estimated for the stoichiometric compounds from the dotted line in Fig. 1.

It is interesting to try to relate the values of C to those of some property of V, Nb and Ta (Table 3). The only property which varies in the same relative order as C , is the critical temperature, T_c (Table 3). C for the compound series has been plotted against T_c for the corresponding element (Fig. 3). A straight line can be drawn through the points. From Figs 2 and 3 it seems that, for an A15 compound A_3B , where A is either V, Nb, or Ta, and B is one of the group IVB elements Si, Ge and Sn, the superconducting critical temperature is given by:

$$T_c = 27.5 (T_A - 2) M_B^{-1}$$

where T_A is the critical temperature of pure A and M_B is the atomic mass of element B.

The existence of this empirical relationship raises three questions: first, what is its physical significance; second, why does it hold only for group IV elements; third, how far can it be used to predict higher critical temperatures?

The theory of Bardeen, Cooper and Schrieffer (BCS) gives

Table 3 Properties of elemented V, Nb, Ta to be compared with C (Table 2)

	$\theta_D(K)$	$T_c(K)$	$\gamma(mJ\ mol^{-1}\ K^{-2})$	$V_{ph}(10^{-2.3}\ eV\ cm^3)$	$T_m(^{\circ}C)$	$K(10^{10}\ N\ m^{-2})$	$Y(10^{10}\ N\ m^{-2})$
V	320–400	5.3	9.04	0.18	1920	15.80	12.76
Nb	240–280	9.2	7.66	0.34	2468	17.03	10.49
Ta	250	4.5	5.84	0.36	3010	19.63	18.57

* θ_D , Debye temperature; T_c , superconducting critical temperature; γ , electronic specific heat coefficient; V_{ph} , electron-phonon interaction parameter (data from ref. 8); T_m , melting point; K , bulk modulus (compressibility); Y , Young's modulus (data from ref. 9).

the critical temperature of a superconductor as

$$T_c = (1.14h/k) \langle \omega \rangle \exp(-1/N(o)V)$$

If it can be assumed that $N(o)V$ does not vary much among the compounds under discussion, the isotope effect must derive from the average phonon frequency, $\langle \omega \rangle$. In a monatomic crystal, the phonon dispersion relationship involves (isotope mass) $^{-1/2}$ and gives rise to the classical isotope effect in some pure metal superconductors. In a polyatomic crystal the dispersion relationship has more than one branch, each of which depends upon some average of isotopic masses, usually of the form $(\sum_i 1/M_i)^{1/2}$. At the zone boundary, however, the relationship for each branch reduces to a form which involves only one of the masses, that is $\omega \propto (1/M)^{1/2}$. Thus, the quoted empirical formula for T_c is rationalised if it can be assumed that the phonon modes involved in the BCS interaction are those close to the zone boundary. This has interesting implications

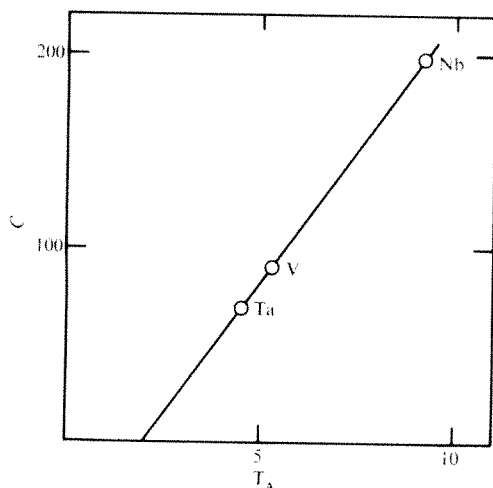


Fig. 3 The slope C , of the lines in Fig. 2, against T_A , the critical temperature of the group VA elements.

for the theory of superconductivity in these compounds. The fact that the important modes are those relating to the B atoms may also explain why, in the ternary compound $Nb_3(AIGe)$, an ordering of the Al and Ge atoms is thought to be necessary to achieve maximum T_c (refs 10 and 11); disorder on the B-atom sites possibly disturbing these modes.

The similarity between elements in group IVB, both chemically and physically, is marked most strongly. They all have the same low temperature equilibrium crystal structure, and electrically are all semiconductors. This is not true of the group IIIB elements, and probably explains why a simple T_c relationship exists for compounds with IVB elements, but not for compounds with IIIB elements in which $N(o)V$ may no longer be a constant. The situation is more complicated when the second element is a transition metal, and the value of T_c depends on the position of the transition metal's d band relative to the Fermi level of the compound³.

Extrapolation of the Nb line in Fig. 2 suggests that if Nb_3Si crystallises in the A15 structure, it should have $T_c \approx 38\ K$, which would make it by far the highest T_c superconductor, with distinct economical advantages. It does not exist in equilibrium because the silicon atom radius is too small; the ratio of the radii of the two component atoms must lie between 0.85 and 1.15 in order for a stable A15 structure to form¹². An ordered (Cu_3Au) structure for this composition, with $T_c = 1.5\ K$, has been reported¹³. Codeposition of the evaporated elements onto a heated substrate has produced a non-stoichiometric A15 phase, with a maximum T_c of 9.3 K at a composition of 21 atom % Si (ref. 14). More recent attempts to form Nb_3Si under high pressures have been unsuccessful¹⁵. It may be possible to get some enhancement of T_c in a ternary compound $Nb_3(GeSi)$. The Ge-Si ratio should be such as to permit ordering on their sub-lattice, but the fraction of silicon atoms should not be so large that a weighted average radius differs by much more than 15% from the radius of the Nb atom. The compound $Nb_3(Ge_{0.75}Si_{0.25})$ fulfils these conditions, and if a weighted average of the Ge and Si atomic masses is appropriate, and if it forms a stable A15 compound, it should have a critical temperature of 25.2 K.

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- Hatt, B. A., *et al.*, *J. Low-Temperature Physics*, **10**, 285 (1973).
- Testardi, L., *et al.*, *Phys. Today*, **26**, 17 (1973).
- Flükiger, R., thesis, Univ. Geneva (1972).
- Bruning, H. A. C. M., *Philips Res. Rep.*, **22**, 349 (1967).
- Brook, G. B., *et al.*, *Fulmer Res. Inst. Rep.*, **R225/5** (1968).
- Marchukova, L. V., *et al.*, *Izv. Akad. Nauk SSSR, Metal.*, **2**, 207 (1973).
- van Vucht, J. H. N., *et al.*, *Philips Res. Rep.*, **19**, 407 (1964).
- Gladstone, G., Jensen, M. A., and Schrieffer, J. R., *Superconductivity* (edit. by Parks, R. D.), 665, (Dekker, New York, 1969).
- Metals Reference Book* (edit. by Smithells, C. J.), (Butterworths, London, 1967).
- Ageev, N. V., Alekseevsky, N. E., and Shamrai, V. F., *Soviet Phys. Dokl.*, **14**, 1023 (1970).
- Kitchingman, W. J., and Birch, A., *Met. Sci. J.*, **7**, 191 (1973).
- Nevitt, M. V., *Trans. Am. Inst. Min. Engrs.*, **212**, 350 (1958).
- Galasso, F., and Pyle, J., *Acta crystallogr.*, **16**, 228 (1963).
- Hazra, S., and Hammond, R. H., *Annual Report from Inorganic Materials Research Division*, 127–8 (Univ. California at Berkeley, 1971).
- Leger, J. M., and Hall, H. T., *J. less common Metals*, **32**, 181 (1973).
- Hanak, J. J., *et al.*, *J. Appl. Phys.*, **41**, 4958 (1970).
- Hanak, J. J., Strater, K., and Cullen, G. W., *RCA Rev.*, **25**, 342 (1964).
- Cody, G. D., *et al.*, *Proc. 7th int. Conf. on Low Temperature Physics* (edit. by Graham, G. M., Hollis Hallett, A. C.), 382 (Univ. Toronto Press, Toronto, 1961).

An interfacial isotope effect

RADIOACTIVE carbon is utilised commonly to tag compounds used in interfacial studies. As is usual in studies with isotopes of carbon, it has been assumed tacitly that the radioactive surfactant behaves exactly like the untagged or inactive compound. During a study¹ of condensed monolayers at the water-air interface, however, a significant kinetic isotope effect was observed in their stability behaviour.

Stearic-1-¹⁴C acid (Dhom Products), which has a specific activity of 58 mCi mmol⁻¹, was mixed with inactive stearic acid to yield a 14.3 mCi mmol⁻¹ mixture with 23.1% ¹⁴C-labelled carboxyl groups. This particular stearic-1-¹⁴C acid sample was selected because, in addition to its purity (99.5% C₁₈, 0.2% C₂₀, and 0.3% C₂₂ saturated acids), its surface pressure-area isotherm (Fig. 1) is almost identical

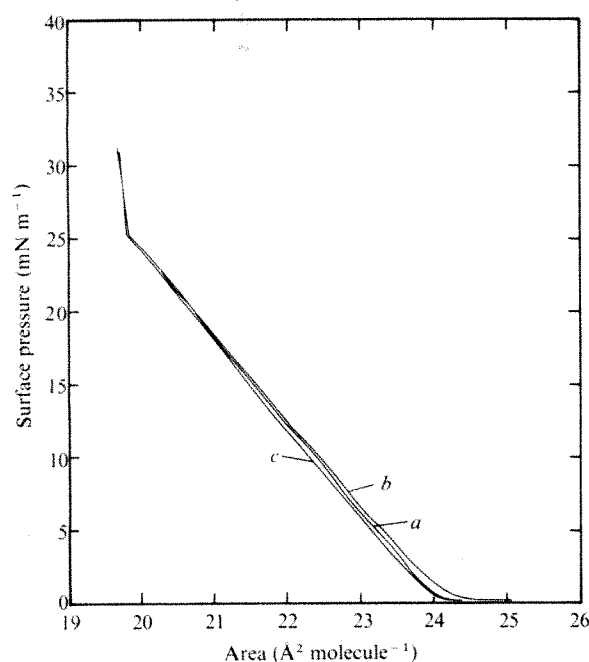


Fig. 1 The surface pressure-area isotherms of stearic acid monolayers on pH 2.00 subsolutions (corrected to have the same area at 30 mN m⁻¹ as inactive stearic acid). a, Inactive stearic acid; b, stearic-1-¹⁴C acid; ¹⁴C-labelled stearic acid. Compression rate=1.5 Å² molecule⁻¹ min⁻¹; temperature, 20.7° C.

to that of the highly purified inactive stearic acid (99.6% C₁₈, 0.2% C₂₀, and 0.2% C₂₂). Stearic acid was deposited from purified hexane spreading solutions on to either calcium free or 10⁻⁴ M CaCl₂ (ultrapure) subsolutions prepared from triply distilled water to which HCl, KHCO₃, or 10⁻³ M KHCO₃ and KOH of AR quality were added to vary the pH of the subsolution. The monolayers were compressed to a surface pressure of 31 mN m⁻¹ with an automatically recording Langmuir-type film balance designed for constant pressure operation. An indication of the monolayer stability was obtained by determining the decrease in the film area as a function of time, at constant surface pressure.

The measurements of monolayer stability in surface films of condensed stearic acid at pH 2.0 and 6.0, are shown in Fig. 2. The stability measurements of Ca-H-St monolayers (stearic acid monolayers on subsolutions containing calcium) were compared at intervals of 15 min and plotted (Fig. 3) as a function of the pH of the subsolution. The large decrease in the film area of inactive unionised

stearic acid films (Figs 2 and 3) result from a slow process of monolayer collapse in which crystals of stearic acid form slowly at the water-air interface. (The stearic acid and Ca-H-St monolayers consist principally of unionised stearic acid molecules below subsolution pH values of 5.5 and 4.2, respectively. The slow collapse of the monolayer is different from the catastrophic collapse observed by a number of other investigators.) Figure 2 also shows that the ¹⁴C-labelled stearic acid monolayer is more stable than the corresponding inactive monolayer. This result is not expected because the isotopic substitution occurs at an atom which is not immediately involved in the bonds that break and form as the fatty acid molecules transfer from the monolayer state to the collapsed bulk phase. This significant difference in the behaviour of the monolayer stability is attributed to a secondary kinetic isotope effect. The rate of change of the film area decrease and the isotope effect (¹²C/¹⁴C) both become greater with increasing time of compression. Figure 3 also shows that there is a difference in the stability behaviour between inactive monolayers and ¹⁴C-labelled Ca-H-St monolayers. At pH < 5.0, where the molecules are dominantly unionised stearic acid, the radioactive Ca-H-St monolayer is more stable; both monolayers possess the same stability in the pH range 5.0-7.8; and then above pH 7.8 a reversal in stability occurs with the inactive Ca-H-St monolayer becoming much more stable.

Several considerations indicate strongly that the observed stability differences do not arise from impurities in the

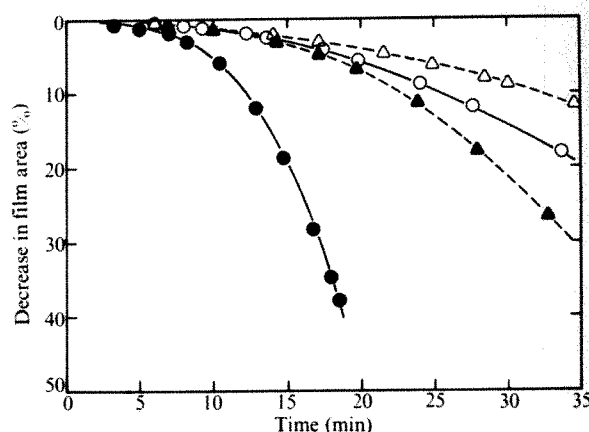


Fig. 2 Stability measurements of stearic acid monolayers at 31 mN m⁻¹. ○●, pH=2.0; △▲, pH=6.0; ●▲, inactive; ○△, ¹⁴C-labelled. Temperature, 20.5° C.

system. The high purity of the stearic acid samples minimises the effect of chemical impurities; and impurities arising from radiolysis would be expected to decrease the stability of the Ca-H-St monolayer throughout the pH range, not just on alkaline subsolutions. Furthermore, the peculiar stability behaviour of the ¹⁴C-labelled Ca-H-St monolayer, which rises to a maximum and then decreases again with increasing alkalinity, and the magnitude of the stability differences seem to rule out the impurity explanation.

The stability increase of ¹⁴C-labelled unionised stearic acid monolayers arises apparently from increased intermolecular forces of attraction between the carboxyl head groups and/or between the carboxyl groups and the water molecules of the subsolution. An increase in the carboxyl-group interactions is not, however, the sole effect of the isotopic substitution, because the stability of ¹⁴C-labelled Ca-H-St monolayers on alkaline subsolutions is not in-

creased similarly. Isotopic substitution of ^{14}C shifts the $\text{C}=\text{O}$ stretching fundamental from 1,715 to 1,637 cm^{-1} because of the difference in isotopic mass². The zero-point energy of the $^{14}\text{C}=\text{O}$ bond is thus lower than that of the $^{12}\text{C}=\text{O}$ bond, and the dipole moments of the two isotopic modifications of the $\text{C}=\text{O}$ bond will be slightly different because of vibrational anharmonicity. Because the dipole moment increases in deuterium substituted molecules³, it is assumed that ^{14}C -isotopic substitution also increases the dipole moment. Consequently, there should be an increase in the hydrogen-bond energy between the carbonyl oxygen atoms of the head groups and the hydrogen atoms of the water molecules and, as observed, an increase in the stability of the monolayer. The stabilising influence of the carboxyl ^{14}C atom seems to have its origin in the hydrogen bonding or dipole-dipole interactions between the carboxyl head groups and the subsolution water molecules.

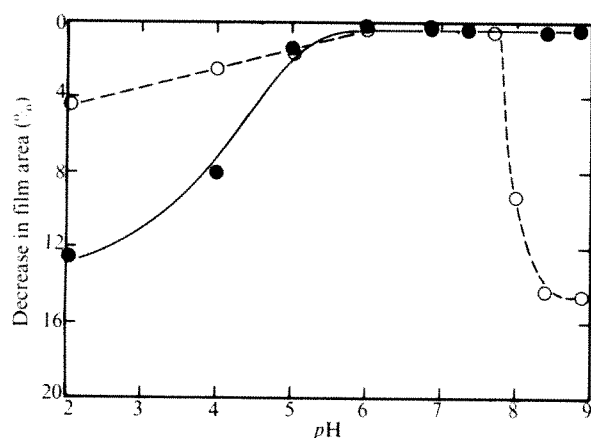


Fig. 3 Comparison of the stabilities of Ca-H-St monolayers at 31 mN m^{-1} , with compression time of 15 min at a temperature of 20.6° C. ●, inactive; ○, active.

The sudden decrease in the stability of ^{14}C -labelled Ca-H-St monolayers above pH 7.8 corresponds to a significant increase in the rate of monolayer collapse¹. Apparently the isotopic substitution affects the charge distribution within the calcium stearate surface micelles so that the molecular interactions between the coordination complexes and the subsolution water molecules are weaker than they are in the presence of untagged carbon atoms (R. D. Neuman, unpublished).

It is conceivable that compounds with a high specific activity, which exist in the highly oriented arrays of the two-dimensional state, may exhibit effects not observed commonly in bulk systems. The observed kinetic isotope effect indicates that the presence of tagged atoms in the head groups of surface active agents may significantly influence interfacial phenomena. Unfortunately, this interfacial isotope effect is not understood completely and needs further clarification.

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¹ Neuman, R. D., thesis, Institute of Paper Chemistry, Appleton, Wisconsin (1973).

² Pinchas, S., and Laulicht, I., *Infrared Spectra of Labelled Compounds* (Academic Press, London and New York, 1971).

³ Halevi, E. A., *Prog. phys. org. Chem.*, **1**, 109 (1963).

Geographic and temporal development of plagues

ALTHOUGH deterministic and stochastic descriptions of localised epidemics abound in the literature of epidemiology and quantitative biology^{1,2}, the geographic spread of epidemics has not been analyzed in such detail. (Although in ref. 2, page 205, Bailey gives equations similar in spirit to my equation (1) they seem to lack physical significance.) Realistic mathematical models of the geotemporal development of plagues could be useful in the study of epizootics (that is, in ecology, wildlife management or veterinary medicine), of social phenomena (such as the spread of drug abuse or fads), and of history. Needless to say, such models should also be applicable to public health questions.

I report here the results of an investigation of the propagation of epidemics using a simple, but plausible, deterministic model, and compare them with Langer's data on the Black Death of 1347-50 (ref. 3). First I describe the model, then explore its mathematical properties and in particular its ability to sustain wave-like (propagating pulse) solutions, compare its predictions with existing data³, and finally discuss its shortcomings and suggest directions for further research.

To describe the progress of plagues as simply as possible, it is convenient to assume only two interacting populations which can be taken to be infectives and susceptibles, although one might equally well consider hosts and parasites (for example, rats and fleas) or humans and vectors. We can express the change with time of the number of infectives within a small area as the rate of transitions from the susceptible population, less the removal rate (defined as the mortality, plus recovery, plus the net physical outflow from the area). The temporal variation of the susceptible population can be expressed similarly, except that the transitions from susceptible to infective appear as a net loss, and that the rate of removal is merely the net outflow. It is reasonable to suppose that, under the conditions obtaining during plagues, the net outflows of the infective and susceptible populations result from random walks rather than mass migrations, and thus can be represented as simple diffusion. The resulting equations are:

$$\frac{\partial I}{\partial t} = KIS - \mu I + D \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) I \quad (1a)$$

$$\frac{\partial S}{\partial t} = -KIS + D \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) S \quad (1b)$$

where S and I are the susceptible and infective population densities, μ is the mortality rate (which for simplicity I have represented as a Poisson-distributed process) and the transition rate from I to S is proportional to the rate of binary encounters between infectives and susceptibles. The transmissibility coefficient K may be expressed in terms of more fundamental quantities:

$$K = 2 \int_0^\infty db b \int_0^\infty dv v \Phi(b, v) f(b, v) = 2 \langle bvf \rangle \quad (2)$$

where $f(b, v)$ is the infection probability as a function of distance of encounter, b , and velocity v , and $\Phi(b, v)$ is the distribution function for encounters. This K represents the average area of infection swept out by an infective per unit time, and KS is therefore the number of new infectives produced per unit time, per infective. The diffusion constant, D is assumed the same for both groups, since there is no good reason to assume it is not the same. The model represented by equations (1) and (2) is usually described as deterministic although it incorporates probabilistic ideas in three places (the rate of binary encounters, the random walks leading to a diffusion term, and the Poisson-distributed mortality) because it admits of numerically precise solutions and gives no idea of what the variance of I and S should be. (Although we should clearly expect the variances of the numbers of individuals to be found in the area dA to be roughly $I dA$ and $S dA$, respectively, by analogy with other counting experiments.)

Since we are interested primarily in the simple case in which the initial susceptible population density is uniform, $S(\vec{x}, 0) = U$, we can reduce equation (1) to dimensionless form by scaling:

$$\begin{aligned} I &\rightarrow UI \\ S &\rightarrow US \\ x &\rightarrow (D/KU)^{1/2} x \\ t &\rightarrow (KU)^{-1} t \end{aligned}$$

and so

$$\frac{\partial I}{\partial t} = IS - \lambda I + \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) I \quad (3a)$$

$$\frac{\partial S}{\partial t} = -IS + \left(\frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} \right) S \quad (3b)$$

where $\lambda = \mu/KU$.

Langer's map, in which contours of the progress of the black Death are drawn for equally spaced time intervals (Fig. 1), strongly suggests wave-like propagation of this disease. For this reason I have investigated whether equation (3) can support propagating one-dimensional (plane) waves. Letting $I(\vec{x}, t) = I(x - vt)$ and $S(\vec{x}, t) = S(x - vt)$, we obtain the coupled, second-order ordinary differential equations

$$-vI' = IS - \lambda I + I'' \quad (4a)$$

$$-vS' = -IS + S'' \quad (4b)$$

These equations constitute a nonlinear eigenvalue problem. The interesting question is, for what values of λ will the equations have solutions satisfying the conditions

$$\begin{aligned} I(-\infty) &= I(\infty) = 0 \\ 0 \leq S(-\infty) &< S(\infty) = 1 \\ I, S, v &> 0? \end{aligned}$$

Adding equations (4a) and (4b), we find

$$\frac{d}{dx} [I(x) + S(x)] = \lambda e^{-vx} \int_{-\infty}^x e^{vx'} I(x') dx' > 0 \quad (5)$$

so that

$$I(x) + S(x) < I(\infty) + S(\infty) = 1 \quad (6)$$

On the other hand, if we integrate (4b) from $-\infty$ to $+\infty$ we obtain (note $S'(-\infty) = S'(\infty) = 0$)

$$\Delta S = S(\infty) - S(-\infty) = \frac{1}{v} \int_{-\infty}^{\infty} dx I(x) S(x) \quad (7)$$

However, integrating (4a) from $-\infty$ to $+\infty$ we find

$$\lambda \int_{-\infty}^{\infty} dx I(x) = \int_{-\infty}^{\infty} dx I(x) S(x) = v \Delta S \quad (8)$$

and since $0 < S(x) < 1$ for all x ,

$$\int_{-\infty}^{\infty} dx I(x) > \int_{-\infty}^{\infty} dx I(x) S(x) \quad (9)$$

or

$$\frac{v}{\lambda} \Delta S > v \Delta S$$

That is, wave-like solutions can only exist if

$$\lambda = \mu/kU < 1 \quad (10)$$

This criterion which the ratio of the rate constants, μ and KU , must satisfy has an obvious physical interpretation: if the infectives die too rapidly, they cannot spread the disease; whereas if the population density multiplied by the transmissibility factor K is too small, the plague cannot be transmitted either. (I should add that it is straightforward to show that outward-propagating circular waves are also solutions of equations (3); see also ref. 2, page 205.)

There are various ways one might attempt to find an approximate solution of equations (4) and thereby estimate v as a function of λ . I thought it more interesting to consider the original partial differential equation in order to see explicitly how wave-like solutions arise. I have therefore solved numerically the (one-dimensional) equations

$$\frac{\partial I}{\partial t} = IS - \lambda I + \frac{\partial^2 I}{\partial x^2} \quad (11a)$$

$$\frac{\partial S}{\partial t} = -IS + \frac{\partial^2 S}{\partial x^2} \quad (11b)$$

on a spatial mesh of 100 points. The development of and final form of the propagating solutions is essentially independent of the initial distributions $I(x, 0)$ and $S(x, 0)$, as long as they bear some resemblance to a plague locus entering a previously uninfected population of susceptibles. (When the ratio μ/KU exceeds the critical value, unity, initial perturbations rapidly die away.) A typical propagating solution is shown in Fig. 2. The (approximate) velocities of propagation, the maximum density of infectives, and surviving populations are given in Table 1 for various values of μ/KU .

At the time of the Black Death (1347 AD) the population density U of Europe was about 50 mile⁻². To try to get a rough idea of what D should be, we note that communication was such that one might expect minor news or gossip to diffuse a distance on the order of 100 miles in a year, that is $D \sim 10^4$ mile² yr⁻¹ (ref. 4). The area swept out in ambulation at a nominal 1 mile h⁻¹ slow walk, assuming $\langle fb \rangle \sim 0.5$ foot (corresponding to a 5-foot flea-hop times a 10% average transmission probability) is 0.4 mile² yr⁻¹ and so $KU \sim 20$ yr⁻¹. Assuming a mortality rate of $\mu \sim 15$ yr⁻¹ (corresponding to a 2-week infectious period) we expect a velocity of propagation of 200–400 mile yr⁻¹. This velocity is in substantial agreement with that which can be estimated from Fig. 1. (All the numbers given above were educated guesses, but the resulting speed is insensitive to their values since $v \sim \sqrt{KUD}$. My object in making these guesses was primarily to indicate the reasonableness of the model and its predictions.)

The results reported here can be summarised as follows. The gross features of the geographical progress of epidemics can be described by means of a deterministic model which is strongly reminiscent of models which have been used to describe chemotaxis⁵, propagation of neural impulses⁶, complex organic chemical reactions^{7,8}, and the advance of advantageous genes along a one or two-dimensional habitat⁹⁻¹¹. The model presented here gives rise to propagating solutions whose velocities are (primarily for dimensional reasons) comparable with the observed velocity of the Black Death of 1347 AD when the physical constants in the model are set to reasonable values. It is particularly important that, given the infectious period of the disease (essentially the time for removal of a diseased individual from the population of infectives) and its transmissibility, K , we can estimate the



Fig. 1 Approximate chronology of the black death, 1347 to 1350. (From *The Black Death* by William L. Langer. Copyright © 1964 by Scientific American, Inc. All rights reserved.)

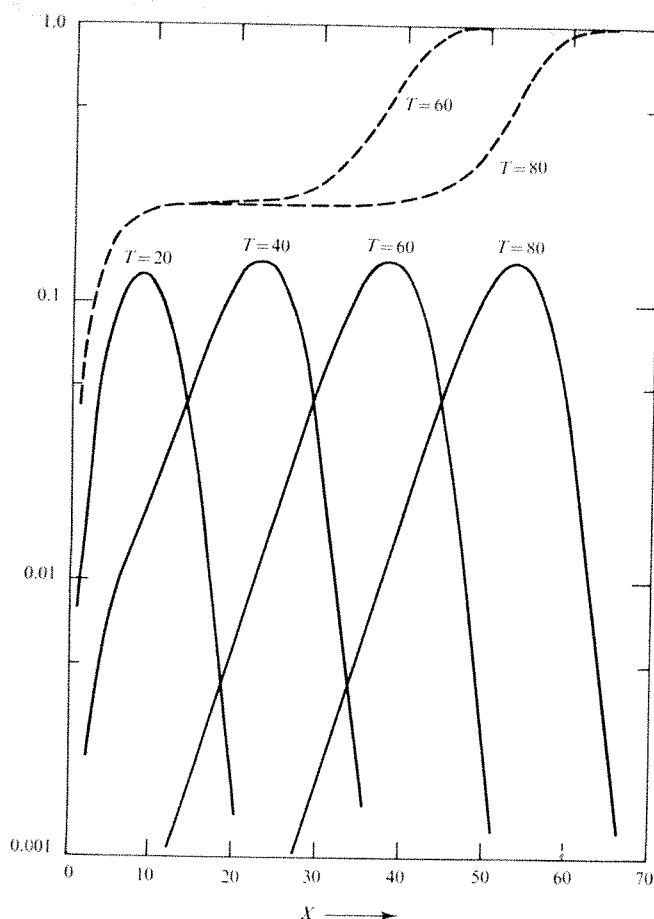


Fig. 2 A typical numerical solution of the (one-dimensional) equations (11a) and (11b) which shows clearly the pulse-like character of the plague after the introduction of the initial infection. The equations were solved on the spatial interval $0 < x < 100$ in unit steps and with time-steps of 0.5. The distributions of susceptibles, $S(x, t)$, are indicated by dashed lines after 60 and 80 time steps. The distributions of infectives after 20, 40, 60, and 80 time steps are indicated by the solid lines. The initial distribution of susceptibles was uniform and equal to unity, whereas that of the infectives was zero for $x > 5$, and consisted of 5 random numbers lying between 0 and 0.01, for $1 < x < 5$. Repeated runs indicate that after 20 time steps the distributions are independent of the initial ones, for a very wide range of initial infectives introduced in the vicinity of $x=0$. (Note also that the precipitous drop in $S(x, t)$ near $x=0$ is an artefact of the boundary conditions, which remains fixed in space and which does not reflect the true number of remaining susceptibles after the plague wave has passed.) $\lambda=0.5$.

critical (minimum) population density necessary for the outbreak of epidemics:

$$U > U_{crit} = \mu/K. \quad (12)$$

This relation may have some use as a historical tool, since if K and μ are known for a given type of epidemic, and if D can be estimated more accurately than was possible herein, for a given terrain and cultural level, then the local value of U can be extracted from the observed advance of plague fronts. Moreover, if we consider the maximum value of I in the wave, we see that it decreases monotonically with λ , and so gives an independent estimate of μ/KU . Finally, if we apply a reasonable model of the inheritance of susceptibility to estimate the relative frequency of susceptibles and immunes after a given period of time, starting from some initial relative frequency, and if we take into account the recurrence time for major historical epidemics of a given kind, we should be able to at least check for self consistency estimates of population and of plague severity such as those quoted by Langer³.

It is also interesting to extract from the solutions of this model the appearance of the plague wave to a stationary observer. He would see an extremely sudden onset of the

disease, during which some fraction of the susceptibles catch the disease, followed by the afflicted dying. Note that in these calculations, not all of the susceptibles become infected and die. The survivors of high-mortality plagues $\mu/KU > 0.5$ are therefore a mixture of the immune and the merely fortunate. Paradoxically, the more rapidly fatal the disease is on an individual basis, the better it is for the population. (This is, of course, well known from the theory of localised epidemics.) Because of the existence of a critical value, μ/K , of the susceptible density, we expect the actual development of a plague to follow the sequence: (1) increase of U by general population growth or in-migration past U_{crit} and thereby the creation of a substantial instability toward a plague wave; (2) introduction of the plague agent above the minimum threshold; (3) extremely rapid development of plague. The present situation of many industrialised nations is such that the density of susceptible individuals is at least a factor of 10 greater than that which obtained in mediaeval Europe. The present calculations suggest that any serious outbreak of bubonic plague, such as might be expected to follow a lengthy disruption of medical services, would result in the death of essentially all the susceptibles. What fraction of the population might be so described is not clear, especially in the face of possible mutations of the responsible microorganisms, but it probably is at least 25% and might exceed 75%.

Table 1 Parameters of plague waves for several values of the damping parameter $\lambda = \mu/KU$

μ/KU	v (in units of \sqrt{KUD})	I (max)	S_i
0.10	2.0	0.7	~ 0
0.25	1.9	0.4	~ 0.025
0.50	1.5	0.14	0.22
0.75	0.8	0.03	0.6
0.90	0.55	0.004	0.8

There are several grounds on which to criticise the present model. First, the usual density distribution of human populations is clumped, not uniform (because people tend to live in villages and cities); and second, the model takes only the most elementary account of chance. As long as we do not attempt too detailed a simulation, averaging over the clumps will doubtless give the same gross predictions as the uniform distribution. On the other hand, the major divergence of the results of the deterministic model from those of the more fundamental stochastic approach is most likely to be that the latter would also predict a minimum threshold for the number of initial cases of the disease, below which propagating solutions are improbable. (We would expect this effect because statistical fluctuations would tend to damp out a small outbreak with near certainty.)

It is worth reiterating that models such as that presented here could be applied directly to the study of epizootics and should therefore be useful in ecology, wildlife management and veterinary medicine. Further, the spread of certain mass sociopsychological phenomena (such as new religions) might be sufficiently similar to that of plagues as to be worth investigating by similar methods.

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1. Bailey, N. T. J. *The mathematical theory of epidemics* (Hafner, New York, 1957).
2. Bailey, N. T. J. *The mathematical approach of biology and medicine* (Wiley, New York, 1967).
3. Langer, W., *Scient. Am.* **210**, 2, 114 (1964).
4. Rashevsky, N., *Looking at history through mathematics* (MIT Press, Cambridge, 1968).

- ⁵ Keller, E. F., and Segel, L. A., *J. theor. Biol.* **30**, 225 (1970).
⁶ Hodgkin, A. L., and Huxley, A. F., *J. Physiol. Lond.*, **117**, 500 (1952).
⁷ Lefever, R., and Nicolis, G., *J. theor. Biol.* **30**, 267 (1971).
⁸ Zhabotinski, A. M., *Biofizika*, **2**, 306 (1964).
⁹ Moran, P. A. P., *The statistical processes of evolutionary theory*, 178 (Oxford University Press, London, 1962).
¹⁰ Fisher, R. A., *Ann. Eugen.*, **7**, 355 (1937).
¹¹ Skellam, J. G., *Biometrika* **38**, 196 (1951).

Identification and follow-up of infants at risk of sudden death in infancy

In the past 10 yr infant deaths in the age group 1 week up to 1 yr have levelled off at about 7.5 per thousand. About half these deaths are sudden and unexpected and are consequently referred to a coroner for certification; subsequent *post mortem* investigation reveals evidence of disease likely to cause death in 30% of sudden deaths: in the remainder the cause of death is obscure and such a case may be described as sudden infant death syndrome, SIDS, or more conveniently as cot death. In 1972 a panel, convened by the National Institute of Child Health and Human Development, Washington, to discuss the status of epidemiological research in SIDS considered a prospective study of high risk babies of primary importance, but doubt was expressed as to whether a sufficiently high risk group could be identified to make such a study feasible (P. Froggatt, personal communication). We report the results of the first year of such a prospective study.

To determine means of identifying high risk babies at or soon after birth, detailed obstetric and perinatal histories were abstracted for 119 cases of sudden infant death and 135 live controls born in the same hospitals¹. In this study we excluded cases of sudden infant death associated with serious congenital anomaly, for example, congenital heart defect or Down's syndrome. We intended that the high risk group should include both explained and unexplained deaths. The former group, comprising 29% of the 119 cases, are potentially preventable and their identifying characteristics were found to differ little from those of the unexplained deaths.

From univariate tabulations a set of 40 variables were selected which could be ascertained at or soon after birth and which gave some indication of being of prognostic value. Stepwise discriminant analysis showed that the variables, shown in Table 1 in order of importance, were statistically significant. The sign of the coefficient indicates whether estimated risk increases as the variable increases. For example, the risk decreases as the age of the mother increases and also if her blood group is A and if she intends to breast feed the baby. Conversely, the risk increases as the birth order of the child increases and if the blood group is O, B or AB. Using these variables Mahalanobis' generalised squared distance between the groups is 1.62 and the observed

Table 1 Variables included in the best linear discriminant function and the sign of the coefficient

Sign of coefficient	Variable	
—	Mother's Age	} Together the most powerful discriminators
+	Birth Order	
—	*A	} Blood group of mother*
+	*O	
+	*B or AB	
—	*Intention to breast feed	
—	Duration of 2nd stage of labour	
+	*Urinary infection	} during pregnancy
+	*Polyhydramnios	
+	*Infant premature (< 2500 g and (or) < 37 week gestation)	

* Scale: yes = 1, no = 0.

Table 2 Number of infants, sudden deaths and hospital admissions in various groups of the prospective study

Group	No. in group	No. deaths	Risk relative to B	Hospital admissions	
				No.	% of group
B	5,077	7	1.0	180	3.5
A not selected	477	4	6.1*	42	8.9
A followed up	354	—	—	25	7.1
A did not participate	80	1	9.1†	9	11.3
Excluded congenital anomaly	15	—	—	—	—

* $0.005 > P > 0.001$. † $0.05 > P > 0.01$.

discriminant scores were approximately normally distributed. This implied that a high risk group comprising 15% of the population with the highest scores was expected to include 60% of sudden infant deaths and hence have a relative risk 8.6 times the remainder. A similar analysis by Kraus *et al.*² using linked birth and death registration data for white children in California apparently gave a generalised squared distance of 0.8 which would imply that the relative risk of the corresponding high risk group would be 4.5. Shrinkage³ may be expected to reduce the relative risks in practice.

A prospective study was set up as follows. The discriminant scores of all babies born in a defined area to be evaluated within 24 h of birth; scores larger than 85% quantile defined group A and the remainder group B. Half group A, selected at random, together with samples of group B were invited to participate in the study. This comprised an initial clinical examination made within 48 h of birth, a second at 5 weeks, together with 10 visits to the home spread over the first 20 weeks of life by specially appointed health visitors. The study started in January 1973.

During 1973, 6,003 births were screened, 19 more than the number of births registered in the area for the year. The results were as shown in Table 2. The observed relative risk for those in group A not selected for study is 6.1 which is significantly greater than 1.0, $0.005 > P$. The risk of the group who did not participate is higher and also significant. There are encouraging indications that the study may be preventing some deaths. This agrees with case reports. Hospital admission rates confirm that group A is a high risk group.

We conclude that a large scale study of all forms of post-neonatal infant mortality along these lines is feasible, and offers the best method of reducing these deaths. Meanwhile our study continues.

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¹ Protestos, C. D., Carpenter, R. G., McWeeny, P. M., and Emery, J. L., *Archs Dis. Childh.*, **48**, 835 (1973).

² Kraus, J. F., Franti, C. E., and Borhani, N. O., *Am J. Epidemiol.*, **96**, 328 (1972).

³ Lachenbruch, P. A., *Biometrics*, **24**, 823 (1968).

Thegosis in herbivorous dinosaurs

The name thegosis was coined by Every and Kühne¹ for the short and powerful process of tooth sharpening which is accomplished in mammals by the grinding of one tooth against another. Thegosis produces sharp-edged, planar (or curved planar) wear surfaces on the teeth. The thegosis wear

facets cut straight across boundaries between dentine and enamel and are typically marked with deep parallel striations (not necessarily coincident with the maximum slope of the wear facet). In some mammals thegosis keeps certain teeth sharply honed for use as weapons (as with the canines of suids and hippopotamids); in others thegosis sharpens the teeth which deal with food (as with the carnassial apparatus in felids).

In mammals thegosis wear is accompanied by a second type of tooth wear, dental abrasion, which occurs at food/tooth interfaces during mastication. Areas worn by abrasion are not sharply defined and have an irregular topography which more or less follows the original form of the tooth. In abraded areas the dentine is excavated to leave the more resistant enamel as a rounded wall and there are deep and shallow scratches without preferred orientation. During mastication the thegosis wear facets suffer abrasion; eventually they need to be recut by thegosis.

Tooth sharpening is not exclusive to mammals. Some herbivorous reptiles (notably ornithischian dinosaurs) seem to have sharpened the teeth by thegosis-like grinding, in spite of the fact that there was rarely any consistent pattern of occlusion. Two types of tooth wear in ornithischians are counterparts of thegosis wear and abrasion wear in mammals. Though the thegosis wear facets of ornithischians seem to differ from those of mammals in lacking deep parallel striations, it is evident that a process of tooth sharpening which closely resembled mammalian thegosis did operate in ornithischians and in some other herbivorous reptiles. Lack of striations from the thegosis wear facets of reptiles might be explained by assuming that active thegosis was a relatively infrequent occurrence. Then, in the long intervals between episodes of tooth sharpening, any striations would have been obliterated by abrasion wear. This is exactly the system of tooth wear which Every and Kühne describe¹ in the Eocene tapiroid *Lophiodon*. Tooth sharpening in herbivorous reptiles may not have been exactly comparable with tooth sharpening in mammals (particularly in view of reptilian polyphyodonty), but the mechanisms produce such similar patterns of tooth wear that I feel justified in referring to both as thegosis.

Before examining dental abrasion and thegosis in reptiles it is necessary to mention a third type of tooth wear which occurs in some ornithischians. This is wear caused by interdental pressure (Fig. 1a). Wear facets produced by interdental pressure are sharply defined, small in area, rounded in shape, and have flat or slightly irregular surfaces. These facets of this type are developed where the teeth in juxtaposition of the tooth crown, though they may sometimes be found on the lateral or medial surface. Wear facets of this type are developed where the teeth in adjacent alveoli are in contact and they are commonest where the teeth are crowded into the jaw bones and have an overlapping arrangement¹. Wear facets produced by interdental pressure are concealed in the intact dentition and can be seen only when the teeth are separated.

Blunting of the tips, edges, denticles, cingula and wear facets of the teeth in ornithischians is evidently the product of abrasion (Fig. 1b). Comparable tooth wear is doubtless universal among reptiles.

The literature dealing with the teeth in living and fossil reptiles gives the impression that thegosis wear is best developed (or at least best documented) in the ornithischian dinosaurs. Thegosis wear (though not identified as such) definitely occurs in some other herbivorous reptiles—in sauropods³ and in certain lizards⁴—and might well be expected to occur in some of the prosauropods, therapsids and pareiasaurs. I can find no evidence that the teeth were ever sharpened by thegosis-like grinding in omnivorous and carnivorous reptiles.

Few herbivorous dinosaurs used the teeth as sabre-like slashing weapons, analogous to the canine teeth of suids,

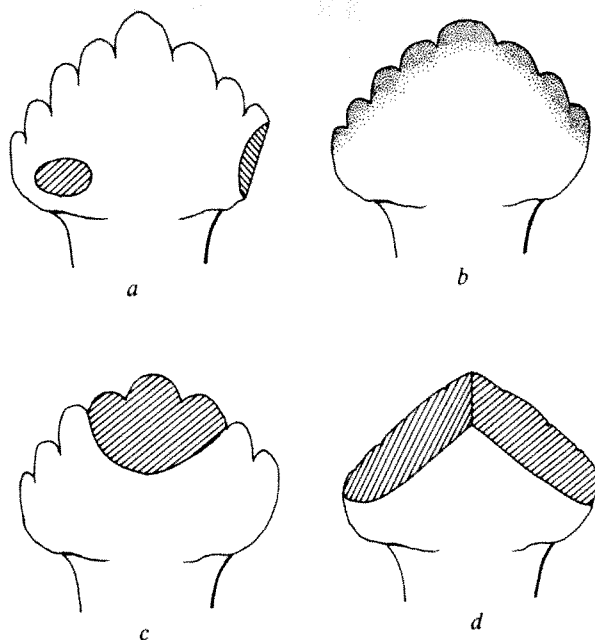


Fig. 1 Outline sketches of ornithischian teeth to show different types of tooth wear. a, Small, rounded and sharply-defined facets produced by interdental pressure; b, ill-defined areas of abrasion wear (stippled); c, single, large and sharply-defined facet produced by thegosis; d, pair of large sharply-defined facets produced by thegosis. Loosely adapted from illustrations in Thulborn^{2,13}.

tayassuids and tragulids, but this is almost certainly the function of the caniniform tusks in heterodontosaurid ornithopods⁵. In heterodontosaurids the tusks could not have been honed by tooth-on-tooth wear⁵ and their slashing edges are finely serrated instead⁶⁻⁸. No reptiles, living or extinct, seem to have employed thegosis to maintain dental weapons in fighting trim, and I conclude that herbivorous dinosaurs used thegosis solely to sharpen the edges of those teeth which dealt with food materials.

In some ornithischians thegosis wear (or a very similar type of tooth wear) was developed on a large scale: planar wear surfaces were formed along entire sections of the cheek dentitions in heterodontosaurids⁵⁻⁸, hadrosaurs⁹, ceratopsians¹⁰⁻¹² and some protoceratopsians¹²⁻¹⁴. In most ornithischians, however, thegosis wear facets were developed on individual teeth: single or paired facets (Figs 1c, d) are found in the teeth of fabrosaurids¹⁵, hypsilophodontids^{2,12,16}, pachycephalosaurids^{13,17}, some protoceratopsians^{13,18}, iguanodontids (*sensu lato*)^{13,19-21}, psittacosaurids^{13,22}, ankylosaurs^{13,23} and stegosaurs^{13,24}. A single wear facet (Fig. 1c) indicates that the tooth was sharpened directly against a single counterpart tooth in the opposing jaw; paired wear facets (Fig. 1d) were produced when the tooth interlocked with a pair of teeth in the opposing jaw¹³. Both single and paired facets may occur in members of a single species¹³.

Recognition of thegosis (or, at least, of a process closely akin to mammalian thegosis) in herbivorous dinosaurs warrants some reconsideration of tooth function in these animals. Evidently the sharp tooth edges and the planar wear surfaces produced by thegosis played important parts in mastication. The planar wear surfaces effectively reduced the tooth crowns to wedges, and as these were driven into the food they splayed it open and, eventually, divided it into two pieces. By sharpening and re-sharpening the leading edges of the wedge-like teeth thegosis enhanced and maintained the food-cutting action of the dentition. Any grinding of food between the thegosis wear facets of opposing teeth would have been incidental to this food-cutting process. It cannot be emphasised too strongly that thegosis was not directly involved in mastication: the process of thegosis merely shaped the teeth for use as tools

in mastication. In short, the cheek teeth of many herbivorous dinosaurs might best be envisaged as chopping or cutting tools, rather than as grinders.

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- ¹ Every, R. G., and Kühne, W. G., in *Early Mammals* (suppl. 1 to *Zool. J. Linn. Soc.*, **50**), edit by Kermack, D. M., and Kermack, K. A., (Academic, London and New York, 1971).
- ² Thulborn, R. A., *Mems Servs. geol. Port.* (in the press).
- ³ Wiman, C., *Palaeont. sin.*, **C6** (n.s.), 1 (1929).
- ⁴ Cooper, J. S., and Poole, D. F. G., *J. Zool.*, **169**, 85 (1973).
- ⁵ Thulborn, R. A., *Zool. J. Linn. Soc.* (in the press).
- ⁶ Haughton, S. H., *Ann. S. Afr. Mus.*, **12**, 343 (1924).
- ⁷ Crompton, A. W., and Charig, A. J., *Nature*, **196**, 1074 (1962).
- ⁸ Thulborn, R. A., *Zool. J. Linn. Soc.*, **49**, 235 (1970).
- ⁹ Ostrom, J. H., *Bull. Am. Mus. nat. Hist.*, **122**, 39 (1961).
- ¹⁰ Hatcher, J. B., Marsh, O. C., and Lull, R. S., *Monogr. U.S. geol. Surv.*, **49**, 1 (1907).
- ¹¹ Ostrom, J. H., *Postilla*, No. 88, 1 (1964).
- ¹² Ostrom, J. H., *Evolution*, **20**, 290 (1966).
- ¹³ Galton, P. M., *Lethaia*, **6**, 67 (1973).
- ¹⁴ Sternberg, C. M., *Bull. natn. Mus. Can.*, **123**, 225 (1951).
- ¹⁵ Thulborn, R. A., *J. Zool.*, **164**, 165 (1971).
- ¹⁶ Janensch, W., *Palaeontographica*, suppl. 7 (3), 105 (1955).
- ¹⁷ Gilmore, C. W., *Bull. Dep. Geol. Univ. Alberta*, **1**, 1 (1924).
- ¹⁸ Brown, B., and Schlaikjer, E. M., *Ann. N.Y. Acad. Sci.*, **40**, 133 (1940).
- ¹⁹ Owen, R., *Odontography* (Hippolyte Baillière, London, 1840–1845).
- ²⁰ Gilmore, C. W., *Proc. U.S. natn. Mus.*, **36**, 197 (1909).
- ²¹ Sternberg, C. M., *J. Paleont.*, **14**, 481 (1940).
- ²² Osborn, H. F., *Am. Mus. Novit.*, No. 144, 1 (1924).
- ²³ Russell, L. S., *Univ. Toronto Stud. geol. Ser.*, **43**, 1 (1940).
- ²⁴ Gilmore, C. W., *Bull. U.S. natn. Mus.*, **89**, 1 (1914).

Echolocation of insects by horseshoe bats

MOST bats of the Suborder Microchiroptera use echolocation during the pursuit of insect prey¹. This conclusion was originally based on observation that bats emitted high frequency orientation sounds during pursuit of insects and that their repetition rate increased sharply during close pursuit manoeuvres. Experiments in controlled laboratory conditions later demonstrated that little brown bats (*Myotis lucifugus*) could capture fruit flies (*Drosophila* sp.) by echolocation when vision and passive hearing of insect flight sounds were not possible².

This does not mean, however, that all insectivorous bats necessarily employ echolocation to the exclusion of other methods for locating insect prey. Indeed, Moehres³ observed that bats were attracted by the buzzing sound of insects which were not visible and probably not detectable by echolocation. Kolb⁴ has demonstrated experimentally that the European *Myotis myotis* commonly detects non-flying insects by passive hearing. Recently, Kolb⁵ suggests that olfaction plays an important role in food location, and that pulses of high frequency sound are accompanied by air currents which stir up odours. But the data Kolb presents do not conclusively show whether the bats located the food by scent or by echolocation.

Airapetjantz and Konstantinov⁶ have recently reported that in a large outdoor flight cage horseshoe bats of the genus *Rhinolophus* used echolocation to detect only stationary insects suspended by threads. The bats seemed to cease emitting high frequency orientation sounds when a tethered moth moved its wings and made limited flights within the cage. This report is so sharply at variance with extensive observations of many species of bats in both temperate and tropical latitudes that further investigation is clearly necessary.

Although horseshoe bats have been intensively studied in the laboratory, their insect pursuit behaviour has not been observed in natural conditions with appropriate acoustic apparatus. We were able, however, to study a small colony of *Rhinolophus ferrum-equinum* which has roosted for many years in the principal buildings of the Cimiterio Suburbano in Pisa. Thanks to special permission from the municipal authorities we were able to observe and record these bats during many evenings in June 1973 as they flew out of one building and hunted insects along the pathways of the cemetery and close to the walls of several other structures.

Our portable, battery-operated apparatus consisted of plastic dielectric microphones and the amplifier-detector circuit described by McCue and Bertolini⁷. The amplified ultrasonic signals were recorded on a Pemco Model 110A instrumentation tape recorder and monitored with a small battery-operated oscilloscope (Tektronix type 323). Almost all of the horseshoe bats disappeared after the first 20–30 min of each evening's foraging flights. We could not ascertain whether they flew away from the cemetery or landed, possibly with full stomachs as is frequently the case with insectivorous bats during the summer months when food is plentiful. Most flew along a predictable flight path each evening between vertical stone walls and rows of cypress trees whose branches were about 0.5–2 m from the walls. On most evenings we placed a Holgate ultrasonic detector⁸ 20–30 m 'upstream' from the position we had selected for tape recording. The audible beat notes from this heterodyne detector alerted us as horseshoe bats approached and allowed us to concentrate attention on them rather than the numerous vespertilionid bats (probably *Pipistrellus pipistrellus*) which were also abundant in the vicinity.

While many horseshoe bats flew rapidly past our position without slowing or turning, and thus were probably not hunting, many others seemed clearly to be pursuing insects such as moths of 1 to 2 cm wingspread which were often present. On several occasions a bat would circle very rapidly around a cypress tree, flying within a few centimetres of its outer branches, and we strongly suspect that at these times they were pursuing and sometimes capturing insects that had been resting on the outer twigs. These trees were 0.5–1.5 m in diameter at the altitudes where this circling behaviour occurred.

On some evenings we attempted to elicit more insect pursuit manoeuvres within range of our microphones by presenting small tethered insects attached by light threads to a fishing pole. These were manoeuvred into the customary flight path of the bats and caused to move as nearly as possible in a normal fashion. Two or three were repeatedly attacked.

On several dozen occasions during the last two weeks of June we were able to observe flight manoeuvres that resembled insect pursuit, although it was not possible to be certain in all cases that it was an insect rather than the cypress twigs or our poles and thread that was attracting the bats' attention. Many of these were spontaneous manoeuvres when no pole or tethered insect was present while others seem directed at our tethered insects. In none of these cases was there the slightest sign that emission of orientation sounds ceased. On the contrary, their repetition rate increased as has been repeatedly observed with other insectivorous bats both in the laboratory and in natural conditions. Every time a horseshoe bat flew within reasonable range of the microphone, typical orientation sounds were emitted at characteristic repetition rates. When the bats made rapid turns, dodged our poles, or seemed interested in insects, there was always a marked increase in repetition rate.

Preliminary analysis of our tape recordings shows approximately the same physical properties of the orientation sounds as have been described for the same species dodging

wires in a relatively small laboratory room. Whenever the signal-to-noise ratio was reasonably adequate both rising and falling frequencies were distinctly visible at the beginning and end of each pulse when the output of a period meter was displayed on a cathode ray oscilloscope^{9,10}.

It seemed worthwhile to analyse in some detail pulse durations and interpulse intervals in four cases when a single horseshoe bat yielded good recordings continuously for a few seconds and during that time engaged in what seemed to be an insect pursuit. Typical pulse durations when flying reasonably straight without apparent pursuit manoeuvres were 50–75 ms, but occasional pulses had clearly defined durations as long as 85 or 90 ms. These were single pulses at reasonably high signal-to-noise ratios without any interruptions. During 'buzzes' on close approach to an insect or other small object pulse durations dropped to about 10 ms and in one case to 7–8 ms. The short series of pulses, having durations of 10–15 ms, lasted for only about 0.1–0.2 s. Interpulse intervals were almost always shorter than pulse durations, and they decreased to approximately 5 ms during the buzzes. Occasionally relatively long pulses were interrupted by intervals as short as 2 or 3 ms. Similar patterns have often been observed in the laboratory.

Thus all the greater horseshoe bats we were able to observe closely while apparently hunting insects under natural conditions emitted intense and rapidly repeated orientation sounds and gave every evidence that they were employing echolocation to locate and intercept their insect prey.

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¹ Griffin, D. R., *J. exp. Zool.*, **123**, 435–466 (1953); *Listening in the dark* (Yale University Press, New Haven, Connecticut, 1958); in *Basic Mechanisms of Hearing* (edit. by Møller, A. R.) (Academic Press, New York, 1973).

² Griffin, D. R., Webster, F. A., and Michael, C., *Anim. Behav.*, **8**, 141–154 (1960).

³ Moehres, F. P., *Z. vergl. Physiol.*, **34**, 547–588 (1953).

⁴ Kolb, A., *Z. vergl. Physiol.*, **44**, 550–564 (1961).

⁵ Kolb, A., *Z. f. Säugetierkunde*, **38**, 277–284 (1973).

⁶ Airapetjantz, E. Sh., and Konstantinov, A. I., *Acta Physiol. Acad. Sci. Hung.*, **35**, 1–17 (1969); *Echolocation in nature* (Russian) (Nauka, Leningrad, 1970); *Echolocation in animals* (English translation by N. Kaner) (Jerusalem, Israel Program of Scientific Translations, 1973).

⁷ McCue, J. J. G., and Bertolini, A., *Trans. Sonics Ultrasonics Gr. Inst. Elec. Electron. Engng*, SU-11, 41–49 (1964).

⁸ Pye, J. D., and Flinn, M., *Ultrasonics*, **2**, 23–28 (1964).

⁹ Griffin, D. R., *Symp. Zool. Soc. Lond.*, **7**, 61–72 (1962).

¹⁰ Patridge, G. R., *J. Audio Engng Soc.*, **15**, 171–175 (1967).

Olfactory imprinting resulting from brief exposure in *Acomys cahirinus*

RECENT experimental studies of olfactory 'imprinting' in infant mammals have typically utilised relatively long term exposure to the training odour, during which that odour could become associated with such conventional reinforcers as food and warmth^{1–6}. Here we report an instance of mammalian olfactory imprinting which is truly analogous to classical avian imprinting, that is, the imprinting exposure being of a relatively short duration with no readily identifiable conventional reinforcer being present at the same time with the imprinted odour. The subject species, *Acomys*

cahirinus (spiny mouse), is a murid rodent whose precocial infants possess functional motor and sensory capabilities within hours of birth^{7,8}.

An initial experiment was conducted to ascertain whether infant *Acomys* would become attached to artificial olfactory cues associated with their cage—their home environment. As soon as a newly-born litter was discovered (about 1–12 h after birth), the parents and infants were placed together in a clean cage containing a shallow layer of bedding material overlying a level teaspoon of either ground cinnamon (Cin) or ground cumin (Cu) which had been evenly sprinkled over the bottom of the cage before the introduction of the bedding material. The animals remained in this cage in an isolation room until testing 24–26 h later—separate rooms being used for the two exposure odours. A total of 12 litters (34 young) were randomly assigned to the two exposure conditions; with seven litters (18 young) exposed to Cu, and five litters (16 young) exposed to Cin. An earlier control study had indicated that naive 1-d-old *Acomys* young show no inborn preferential response to either of the test odours.

After the 24–26 h exposure period (at 26–36 h old), the young were individually tested in a series of two-choice preference tests with Cin and Cu soiled bedding simultaneously present at opposite ends of the test cage. A young mouse was placed on a clean starting strip centred between the two areas of odourised bedding material and observed until moving entirely onto one of the areas of bedding, or until a maximum of 300 s had elapsed without a choice being made. Each young was given 10 successive two-choice preference tests in this manner.

A preference score was calculated for each subject by subtracting the number of positive responses to Cu from the number of responses to Cin. Thus, a positive score would indicate a preference for Cin over the 10 consecutive choice tests, while a negative score would indicate a preference for Cu. The 18 young that had been exposed to Cu had a mean preference score of –2.61. A Wilcoxon matched-pairs signed-ranks test on the individual preference scores indicated that the preference for Cu (the training odour) was significant $P < 0.02$ (two-tailed test). Likewise, the 16 young exposed to Cin showed a subsequent preference for that odour during the choice tests—mean preference score = +4.50 ($P < 0.01$, two-tailed Wilcoxon test).

Having ascertained that infant *Acomys* young prefer a chemical stimulus to which they had been exposed for 24 h (in their home cage) over a novel chemical stimulus, we next wished to determine whether conspecific young would show similar preferences as a function of a relatively brief exposure period during which the chemical stimulus would not be associated with any conventional reinforcer. This was investigated by exposing *Acomys* young 2–12-h-old to the odour of either Cin or Cu for a single period of 1 h, and individually testing them in a series of two-choice preference tests (as above) 24 h after the exposure period.

During the exposure session, all of the young from a given litter were removed from the home cage and parents and placed together into an acrylic cylinder (exposure chamber). A second cylinder (odourant chamber) containing a teaspoonful of the appropriate training substance (either Cin or Cu) was linked to the exposure cylinder by neoprene tubing (18 inches long) with a filter placed over the end entering the odourant chamber. Air was forced from the odourant chamber, through the filter and tubing, and into the exposure chamber; thereby exposing the young to the airborne odour of either Cin or Cu. The young were returned to the home cage immediately following the exposure period, where they remained until testing 24 h later. A total of 29 young mice (from 12 litters) were used in this experiment, with 15 being exposed to Cin and 14 to Cu.

A statistical analysis of the preference scores for all 29

young indicated that the exposure odour was preferred significantly over the novel odour during the choice tests ($P < 0.01$, two-tailed Wilcoxon test). Further analyses indicated that the mean preference score of the 15 young exposed to Cin was $+2.13$ ($P < 0.025$, one-tailed test) and -1.86 for the 14 young exposed to Cu ($P < 0.025$, one-tailed test). Thus, the exposure odour was preferred over the novel odour—regardless of whether the young mice had been exposed to Cu or Cin.

The survival value of such rapidly acquired stimulus preferences in this precocial murid rodent would seem to be similar to that of imprinting in avian species. In each instance, early attachment to a specific stimulus complex would serve to decrease the likelihood of the neonate wandering away from the parental figure or home area, and thereby decrease the possible risks of predation, starvation, or exposure to the elements. While precocial avian species tend to rely primarily upon visual cues for early stimulus attachments⁹ precocial species of macrosomatic mammals (such as *Acomys*) might be expected to rely primarily upon olfactory cues to mediate early attachments^{1,6,10,11}. There is, however, some evidence to suggest that other sensory modalities may function to some extent in attachment formation of both precocial avian and macrosomatic mammalian species. Gottlieb¹², and Porter and Stettner¹³, for example, report that hatchlings of several species of precocial birds become attached to specific auditory signals as well as visual cues; while Sluckin¹⁴ has found that visual imprinting may occur in guinea pigs. Thus, even during the first few days of life, stimulus attachments and preferences in these species are possible in other than the most salient sensory modality.

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- ¹ Carter, C. S., *Anim. Behav.*, **20**, 54–60 (1972).
- ² Carter, C. S., and Marr, J. N., *Anim. Behav.*, **18**, 238–244 (1970).
- ³ Fox, M. W., and Himwich, W. A., *Fedn Proc.*, **24**, 522 (1965).
- ⁴ Mainardi, D., Marsan, M., and Pasquali, A., *Atti. Soc. ital. Sci. nat.*, **104**, 325–338 (1965).
- ⁵ Marr, J. N., and Gardner, L. E., jun., *J. Genet. Psychol.*, **107**, 167–174 (1965).
- ⁶ Müller-Schwarze, D., and Müller-Schwarze, C., *Nature*, **229**, 55–56 (1971).
- ⁷ Ewer, R. F., *Ethology of Mammals* (Logos Press, London, 1968).
- ⁸ Ruch, W., *Rev. Suisse Zool.*, **74** (3), 566–569 (1967).
- ⁹ Sluckin, W., *Imprinting and Early Learning* (2nd ed.), (Methuen, London, 1972).
- ¹⁰ Fullerton, C., Berryman, J. C., and Porter, R. H., *Behaviour*, **XLVIII**, 145–156 (1974).
- ¹¹ Leon, M., and Moltz, H., *Physiol. Behav.*, **8**, 683–686 (1972).
- ¹² Gottlieb, G., *Development of Species Identification in Birds* (University of Chicago Press, Chicago, 1971).
- ¹³ Porter, R. H., and Stettner, L. J., *J. comp. Physiol. Psychol.*, **66**, 808–811 (1968).
- ¹⁴ Sluckin, W., *Nature*, **220**, 1148 (1968).

Evidence for a dual central role for angiotensin in water and sodium intake

ANGIOTENSIN II induces short-latency water intake when infused intravascularly or injected into certain brain regions of mammals^{1,2}. But conceptual difficulties arise if one asks why this should be so, and whether the data reflect real physiological actions. For example, angiotensin is a pressor hormone formed from kidney-based renin in response

to challenge such as hypotension and hypovolaemia^{3–7}. Water ingestion, in itself, is not an adaptive response to such challenges, since most ingested water is distributed intracellularly and will not relieve a vascular crisis. An adaptive response to hypotension or hypovolaemia would involve ingestion of an isotonic mix of fluids, since this would most rapidly and effectively increase vascular volume. In fact, hypovolaemia does result in an immediate increase in preference for isotonic saline over water and a delayed (6–10 h) acceptance of unpalatable hypertonic salt solutions^{8,9}. But, a possible involvement of angiotensin as a rapid and direct facilitator of sodium intake (apart from a slow and indirect role through stimulation of aldosterone release) has not yet been reported and some evidence suggests that angiotensin may not play any significant role in regulating sodium intake in the rat^{10–12}.

We now present data indicating that angiotensin can rapidly facilitate Na^+ intake, resulting in selection and ingestion of a mix of water and electrolytes best suited to overcome deficits in circulatory volume or pressure.

Adult male rats of the Long-Evans strain, weighing 225 to 300 g, were anaesthetised with Nembutal and stereotactically implanted with 23 gauge cannulae in lateral preoptic nucleus, nucleus accumbens, or lateral cerebral ventricles. One week was allowed for recovery from surgery; during this time animals were individually housed with free access to food pellets and deionised water as well as the saline solution with which they would later be tested. Testing was conducted in the home cages with the positions of the water and saline drinking bottles kept constant. Angiotensin and carbachol (another central dipsogen¹³) were prepared with isotonic saline vehicle to a concentration of $500 \text{ ng } \mu\text{l}^{-1}$ and $1 \text{ } \mu\text{g } \mu\text{l}^{-1}$ respectively. Intracranial injections were of $1 \text{ } \mu\text{l}$ volume and ventricular infusions of angiotensin were carried out at a rate of $500 \text{ ng } \mu\text{l}^{-1}$ per 8 min over an 8 h period.

The effects of several thirst-inducing stimuli on intake of water and 1.8% saline (two-bottle choice tests) were observed in rats in normal water and sodium balance during a 1 h test period. In 32 rats, intracranial injection of angiotensin resulted in an average intake of 9.6 ml of water and 6.8 ml of 1.8% saline. Following carbachol, 15 animals ingested an average of 9.5 ml of water and only 0.5 ml of 1.8% saline. In 14 other rats, thirst was induced by subcutaneous injection of 5 ml of 1.5 M NaCl. Average fluid intakes after this intracellular dehydration were 21.7 ml of water and 0.9 ml of 1.8% saline. Thus, thirst induced by intracellular dehydration or by central cholinergic stimulation resulted in substantial intake of water only, with minimal intake of the normally non-preferred 1.8% saline, whereas central injections of angiotensin resulted in substantial intakes of both water and 1.8% saline.

If a potentiation of sodium as well as water intake is characteristic of angiotensin action, this should remain evident when animals that are deprived of water or sodium are stimulated with angiotensin. Intake of water and 1.8% saline was observed in 13 rats (1 h tests) when deprived of fluid for 24 h and when deprived of fluid for 24 h plus receiving central injections of angiotensin. Following deprivation alone, the rats consumed an average of 17.1 ml of water and 3.0 ml of 1.8% saline; following deprivation plus angiotensin, the rats consumed an average of 23.9 ml of water and 11.7 ml of 1.8% saline. Increased intake of water and saline noted with angiotensin was significant ($P < 0.01$, paired sample t test). A similar study was carried out on 10 animals, but with a 48 h period of fluid deprivation and a choice test between water and 2.7% saline. After 48 h of fluid deprivation, the average intake of water was 22.4 ml, and the average intake of 2.7% saline was 2.2 ml. After deprivation plus angiotensin, intake of water increased to 25.7 ml, while intake of 2.7% saline

suggest that angiotensin is one of the factors directly influencing the intake of sodium as well as water.

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- ¹ Epstein, A. N., Fitzsimons, J. T., and Simons, B. J., *J. Physiol. Lond.*, **210**, 457 (1970).
- ² Fitzsimons, J. T., and Simons, B. J., *J. Physiol. Lond.*, **203**, 45 (1969).
- ³ Peart, W. S., *Pharmac. Rev.*, **17**, 143 (1965).
- ⁴ Vander, A. J., *Physiol. Rev.*, **47**, 359 (1967).
- ⁵ Pitcock, J. A., Hartroft, P. M., and Newmark, L. N., *Proc. Soc. exp. Biol. Med.*, **100**, 686 (1959).
- ⁶ Brown, J. J., Davies, D. L., Lever, A. F., Robertson, J. I. S., and Vernion, A., *J. Physiol. Lond.*, **182**, 649 (1966).
- ⁷ Meyer, D. K., Peskar, B., Tauchmann, U., and Hartting, G., *Eur. J. Pharm.*, **16**, 278 (1971).
- ⁸ Stricker, E. M., and Jalowiec, J. E., *Am. J. Physiol.*, **218**, 191 (1970).
- ⁹ Stricker, E. M., and Wolf, G., *J. comp. physiol. Psych.*, **62**, 275 (1966).
- ¹⁰ Fitzsimons, J. T., and Stricker, E. M., *Nature new Biol.*, **231**, 58 (1971).
- ¹¹ Radio, G. J., Summy-Long, J., Daniels-Severs, A., and Severs, W. B., *Am. J. Physiol.*, **223**, 1221 (1972).
- ¹² Stricker, E. M., *Physiol. Behav.*, **6**, 299 (1971).
- ¹³ Fisher, A. E., and Coury, J. N., *Science*, **138**, 691 (1962).
- ¹⁴ Fisher, A. E., in *The Neurophysiology of Thirst* (edit. by Epstein, A., Kissileff, H. R., and Stellar, E.) (Winston, in the press).
- ¹⁵ Block, M. L., and Fisher, A. E., *Physiol. Behav.*, **5**, 515 (1970).
- ¹⁶ Chiarvaglio, E., and Taleisnik, S., *Am. J. Physiol.*, **216**, 1418 (1969).
- ¹⁷ Wolf, G., and Stricker, E. M., *J. comp. Physiol. Psych.*, **63**, 252 (1967).

Exchange of neurotransmitter amino acid at nerve endings can simulate high affinity uptake

AXELROD¹ found that released noradrenaline is recaptured by presynaptic nerve terminals, and proposed reuptake as a mechanism for rapid neurotransmitter inactivation. Subsequent studies led to the identification of high and low-affinity components in the uptake of several putative neurotransmitters by nerve terminals²⁻⁷. Although the apparent K_m values reported for the high-affinity uptake of some neurotransmitters (notably amino acids) are comparable to those reported for the low-affinity uptake of other neurotransmitters, it is generally thought that the inactivation of most neurotransmitter amino acids is obtained through high-affinity uptake systems having a K_m of the order of 10^{-5} M. Indeed, the existence of a high-affinity uptake for a given substance is often considered to favour its being a neurotransmitter.

High-affinity uptake systems for neurotransmitter amino acids have been demonstrated by methods in which high tissue: medium ratios of radioactivity, simulating net uptake, might have been obtained by homoexchange^{8,9}. So far this possibility has been investigated, by ourselves, amongst others¹⁰⁻¹², by methods that could not give a satisfactory answer.

We have now explored the possibility that GABA, at concentrations used for studies on high-affinity uptake, can release GABA from the endogenous pool.

It seems that exchange can largely account for what has previously been interpreted as uptake. Purified synaptosomes¹³ were incubated in the presence of a very low concentration of ³H-GABA to label the endogenous pool, without altering its size significantly. Aliquots were then superfused with regular medium, which was replaced after few minutes by identical medium containing varying concentrations of GABA. The superfusion apparatus, which largely

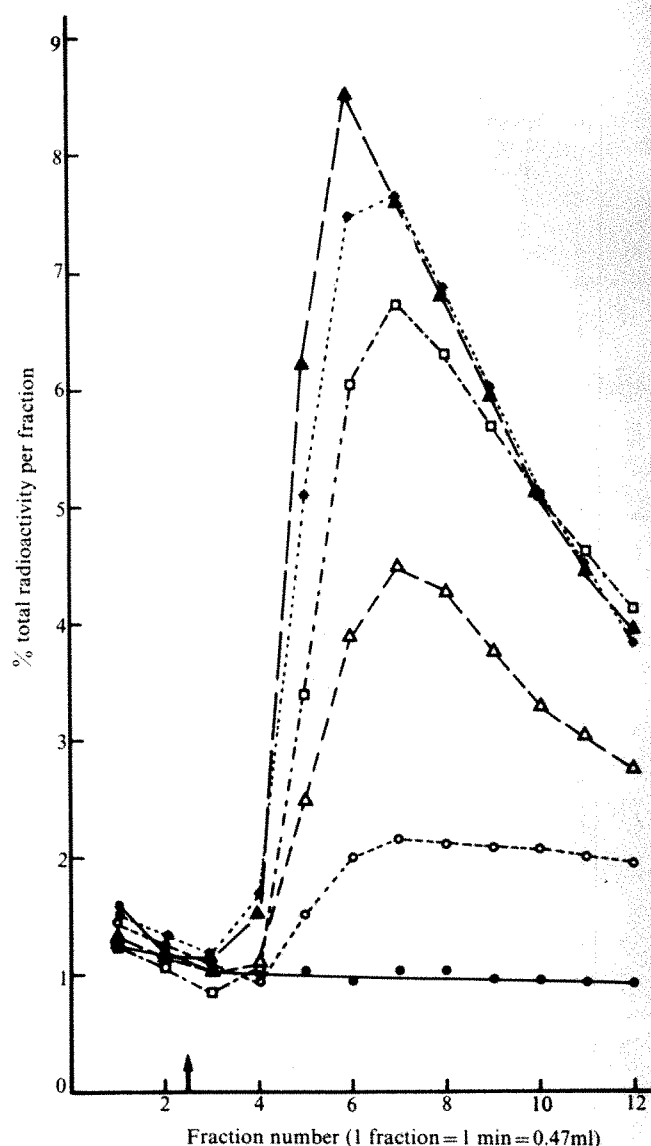


Fig. 1 Stimulation of ³H-GABA release from synaptosomes by different concentrations of unlabelled GABA. Purified synaptosomes¹³ from adult rat cerebrum were resuspended in 0.32 M glucose, at a protein concentration of 5 mg ml⁻¹. Aliquots were diluted 1 : 10 in Krebs-Ringer medium²⁶; after 15 min equilibration at 37° C in a rotary waterbath, a small volume (1/100 of the final volume) of a solution containing 50 μM 2, 3-³H-GABA (New England Nuclear Corporation, specific activity 10 Ci mmol⁻¹) was added to the incubation flasks. After an additional 10 min, 1 ml aliquots of the suspension were placed on Millipore filters (0.65 μm pore) lying at the bottom of six parallel superfusion chambers¹⁴, the filters were washed, 3 ml of prewarmed, oxygenated, glucose-containing medium were added to each chamber, and the superfusion was started, at a rate of 0.47 ml min⁻¹ (ref. 14). After 5.5 min, 20 ml of medium containing varying concentrations of GABA were added to the chambers (arrow) and superfusion was stopped at 15 min. Fractions were collected every minute; the radioactivity of the effluent and that remaining on the filters was measured by liquid scintillation. All the solutions contained 0.1 mM amino-oxycetic acid to prevent GABA metabolism. In these conditions, over 90% of the radioactivity was recovered as unchanged ³H-GABA. These and subsequent experiments run without amino-oxycetic acid gave qualitatively similar results, but a significant amount of ³H-GABA metabolites was recovered. The radioactivity recovered in the first three fractions was at times more erratic, and is not presented. The radioactivity found in each fraction is given as a percentage of the total radioactivity recovered (filter plus total fractions). Each curve is the average of three experiments. ▲, 1 mM GABA; △, 100 μM GABA; □, 50 μM GABA; △, 10 μM GABA; ○, 1 μM GABA; ●, control.

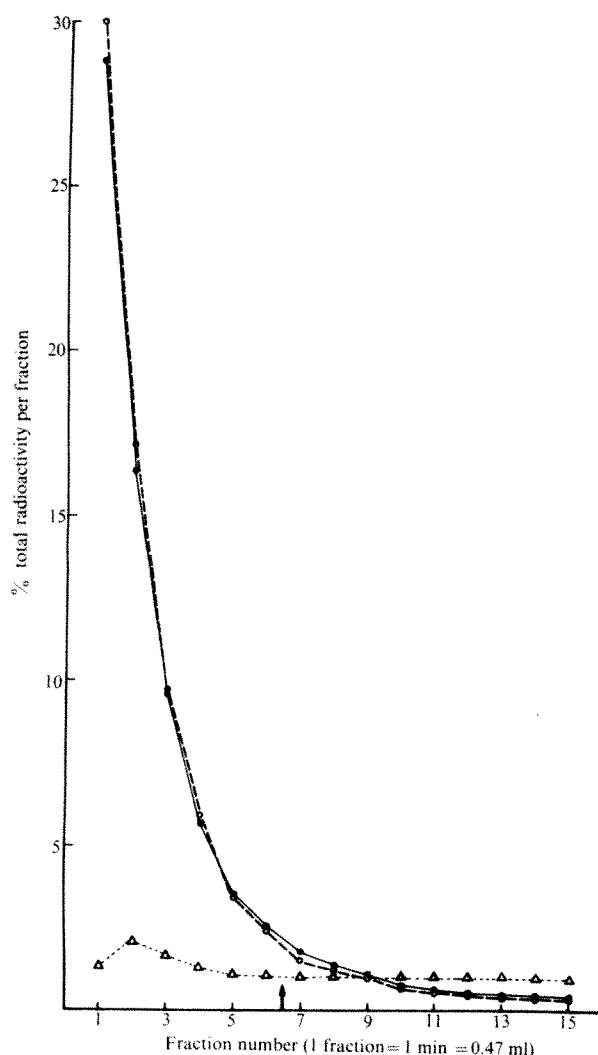


Fig. 2 Effect of sodium deprivation on the release of ^3H -GABA from synaptosomes. Experimental conditions as described in the legend for Fig. 1, except that the medium used for superfusion contained 0.256 M sucrose instead of 0.128 M NaCl. The figure shows the radioactivity recovered in all the 15 fractions collected. The addition of 0.5 mM GABA to the Na^+ -free superfusion fluid had no appreciable effect on the release of ^3H -GABA. Other concentrations of GABA that were tried (10, 100 and 1,000 μM) were also ineffective. Each curve is the average of two experiments. \circ , Na^+ -free, 0.5 mM GABA; \bullet , Na^+ -free; \triangle , control.

prevents reuptake, is described elsewhere¹⁴. Superfusion with unlabelled GABA caused an immediate, dose-dependent increase in the efflux of ^3H -GABA (Fig. 1). The release was about doubled by 1 μM GABA and was increased several fold by a concentration of 10 μM ; both concentrations are in the range of the high-affinity uptake system^{4,6}. The radioactivity released reached a peak after a few minutes and then

decreased, because of dilution of the synaptosomal ^3H -GABA by unlabelled GABA entering the tissue. The stimulation of ^3H -GABA release showed a substrate specificity similar to that described for uptake. Among the various compounds tested, only γ -amino- β -hydroxybutyric acid was a strong stimulator of ^3H -GABA release (Raiteri *et al.*, unpublished). Homoexchange and heteroexchange of amino acids at millimolar concentrations has already been shown in brain slices^{15,16}.

On the basis of an endogenous GABA level of 19 ± 4 nmol per mg synaptosomal protein (G. L., and M. R., unpublished) and assuming that the ^3H -GABA is homogeneously mixed with the endogenous pool, the rates of ^3H -GABA release can be calculated from the percentage of radioactivity released at the peak of the curves (taking the immediate prestimulation level of radioactivity as 100%). These rates are comparable with the initial rates of GABA high-affinity uptake obtained in a previous study⁶. In particular, the release rate at a saturating GABA concentration (1 mM) was similar to the apparent V_{max} of the high-affinity uptake system. In some experiments, in which ^3H -GABA release was stimulated by adding 1 or 10 μM ^{14}C -GABA to the superfusion fluid, to measure the uptake of ^{14}C and the release of ^3H simultaneously, the calculated ^3H -GABA efflux was similar to the influx of ^{14}C -GABA. Even considering the approximation introduced by the calculation, these data suggest that homoexchange accounts for at least a large part of the radioactive GABA entering synaptosomes by the so called high-affinity uptake system. In the concentration range of the low-affinity system, however, the influx of ^3H -GABA (ref. 6) was greater than its calculated efflux, indicating that a net uptake of the amino acid took place.

It is known that GABA uptake is sodium-dependent¹⁷⁻¹⁹. We previously showed that the high-affinity uptake of GABA detectable in embryonic chick brain slices is more sodium-dependent than the low affinity uptake¹⁹. Sodium deprivation also seems to inhibit the high-affinity more than the low-affinity synaptosomal uptake of glutamate, aspartate, glycine⁷ and GABA (G. L., and M. R., unpublished). Figure 2 shows a rapid depletion of synaptosomal GABA content upon superfusion with sodium-free medium. Over 50% of ^3H -GABA present was released in 2-3 min and the addition of GABA (10-1,000 μM) to the superfusion fluid did not appreciably alter the release pattern. Extensive release and absence of any detectable homoexchange could well account for what looks like an almost complete inhibition of the high-affinity uptake of GABA.

If glycine behaved like GABA, then the exchange of glycine at low concentrations should be detectable in synaptosomes from the spinal cord, medulla and pons (where a high-affinity uptake system for glycine was described^{5,7} and where this amino acid acts as a neurotransmitter^{20,21}), but not in synaptosomes from the cerebral cortex (where only a low-affinity uptake system for glycine was detected^{5,7}). Figure 3 shows that synaptosomes from spinal cord and brain stem do indeed exhibit a dose-dependent increase in the release of ^3H -glycine upon superfusion with 10, 25 and 100 μM glycine. In contrast, cortical synaptosomes did not respond to the addition of glycine at concentrations of 10 and 25 μM , which are both in

Table 1 Changes in the levels of GABA and of radioactivity in media after incubation

Original concentration (μM)	0	1	5	10
% change in radioactivity		-49 (2)	-46 (2)	-36 \pm 5 (6)
Concentration expected (μM)		0.5	2.7	6.4
Concentration found (μM)	1.3 \pm 0.3 (6)	2.1 \pm 0.3 (5)	5.5 \pm 0.3 (6)	9.6 \pm 0.9 (12)

Synaptosomes from rat cerebrum, resuspended in 0.32 M glucose at a protein concentration of 4 mg ml⁻¹ were diluted 1 : 10 in Krebs-Ringer medium and equilibrated at 37°C for 15 min in a rotary waterbath. Then, a small volume (1/100 of the final volume) of a solution of ^3H -GABA was added, to give the final concentration reported in the first line. After 10 min incubation, the suspension was either filtered through 0.45 μm Millipore filters or centrifuged for 10 min at 10,000g. The radioactivity was measured in the filters (or pellets) and in the filtered medium (or supernatant). The concentration of GABA present in the medium after incubation was measured by an assay to be described elsewhere. Some data obtained by this assay were checked by a double isotope dansyl derivative method²², which gave essentially identical results.

Means \pm s.d. are presented. The number of experiments is given in parentheses.

the range of the high affinity uptake system^{5,7}, and responded only weakly to a concentration of 100 μ M.

In a set of typical uptake experiments run at low amino acid concentrations, we measured concentration and radioactivity of ³H-GABA in incubation media before and after incubation. In the presence of exchange, the decrease of concentration observed after incubation should be less than that of radioactivity. Table 1 shows that a large part of the radioactivity originally present in media containing 1, 5 or 10 μ M ³H-GABA was taken up by synaptosomes. Changes in concentration of GABA showed a different pattern. With 1 or 5 μ M GABA there was an actual gain in concentration, and with 10 μ M GABA a barely detectable, not significant decrease in concentration was observed. If one takes into account the 1.3 nmol ml⁻¹ of GABA appearing in the medium in the absence of added GABA, the net removal of GABA which seems to take place is much lower than that expected from radioactivity measurements.

In conclusion, homoexchange seems to be a major mechanism by which radioactive GABA is transported into synaptosomes when low substrate concentrations are used. A net uptake, which may or may not be of high affinity component, could be computed only after subtracting the large contribution of homoexchange; therefore the problem of the existence and function of the high-affinity net uptake of GABA and, possibly, of other neurotransmitter amino acids in synaptosomes should be re-evaluated.

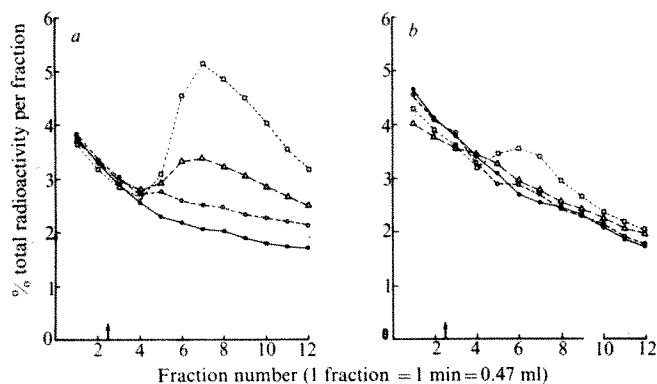


Fig. 3 Effect of different concentrations of unlabelled glycine on the release of ³H-glycine from synaptosomes. Experimental conditions as described in the legend for Fig. 1, except that 0.5 μ M 2-³H-glycine (New England Nuclear Corporation, specific activity 11.1 Ci mmol⁻¹) was used to label the synaptosomal glycine pool. *a*, Synaptosomes from spinal cord, medulla and pons; *b*, synaptosomes from cerebral cortex. \square , 100 μ M Gly; \triangle , 25 μ M Gly; \circ , 10 μ M Gly; \bullet , control.

Interestingly, several properties (such as saturability and sodium-dependence) thought to be typical of GABA high-affinity uptake seem also characteristic of exchange. The utility of sodium-free media to characterise high-affinity uptake is questionable, because of the large loss of endogenous amino acid determined by this condition.

In view of our findings, it is possible that the specific labelling of certain populations of nerve endings by a given amino acid, observed in various experimental conditions^{7,24}, may occur through an exchange process. The data obtained with glycine seem to support this interpretation.

It should be noted, finally, that if reuptake by presynaptic terminals were the main mechanism of inactivation of the liberated neurotransmitter, an uptake system with a K_m of the order of 10⁻³ M (low affinity) might be as effective as one with a K_m of about 10⁻⁵ M. The possible contribution of post-synaptic areas and of glial cells^{22,23} to the reuptake process should also be considered.

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- ¹ Axelrod, J., *Harvey Lect.*, **67**, 175 (1973).
- ² Iversen, L. L., in *Biochemistry of simple neuronal models* (edit. by Costa, E., and Giacobini, E.) 109 (Raven Press, New York, 1970).
- ³ Snyder, S. H., Kuhar, M. J., Green, A. I., Coyle, J. T., and Shaskan, E. G., *Int. Rev. Neurobiol.*, **13**, 127 (1970).
- ⁴ Iversen, L. L., and Johnston, G. A. R., *J. Neurochem.*, **18**, 1939 (1971).
- ⁵ Johnston, G. A. R., and Iversen, L. L., *J. Neurochem.*, **18**, 1951 (1971).
- ⁶ Levi, G., and Raiteri, M., *Life Sci.* (part I) **12**, 81 (1973).
- ⁷ Snyder, S. H., Young, A. B., Bennett, J. P., and Mulder, A. H., *Fedn. Proc.*, **32**, 2039 (1973).
- ⁸ Le Fevre, P. G., and McGinnis, G. F., *J. gen. Physiol.*, **44**, 87 (1960).
- ⁹ Heinz, E., *A. Rev. Physiol.*, **29**, 21 (1967).
- ¹⁰ Iversen, L. L., and Neal, M. J., *J. Neurochem.*, **15**, 1141 (1968).
- ¹¹ Aprison, M. H., and McBride, W. J., *Life Sci.* (Part I) **12**, 449 (1973).
- ¹² Levi, G., Bertolini, A., Chen, J., and Raiteri, M., *J. Pharmac. exp. Ther.*, **188**, 429 (1974).
- ¹³ Gray, E. G., and Whittaker, V. P., *J. Anat.*, **96**, 79 (1962).
- ¹⁴ Raiteri, M., Angelini, F., and Levi, G., *Eur. J. Pharmac.*, **25**, 411 (1974).
- ¹⁵ Levi, G., Blasberg, R., and Lajtha, A., *Archs Biochem. Biophys.*, **144**, 339 (1966).
- ¹⁶ Crnic, D. M., Hammerstad, J. P., and Cutler, R. W. P., *J. Neurochem.*, **20**, 203 (1973).
- ¹⁷ Weinstein, H., Varon, S., Muhleman, D., and Roberts, E., *Biochem. Pharmac.*, **14**, 273 (1965).
- ¹⁸ Martin, D. L., *J. Neurochem.*, **21**, 345 (1973).
- ¹⁹ Levi, G., *Archs Biochem. Biophys.*, **151**, 8 (1972).
- ²⁰ Aprison, M. H., Davidoff, R. A., and Werman, R., in *Handbook of Neurochemistry* (edit. by Lajtha, A.) **III**, 281 (Plenum Press, New York, 1970).
- ²¹ Curtis, D. R., and Johnson, G. A. R., in *Handbook of Neurochemistry* (edit. by Lajtha, A.) **IV**, 115 (Plenum Press, New York, 1970).
- ²² Henn, F., and Hamberger, A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2686 (1971).
- ²³ Schon, F., and Kelly, J. S., *Brain Res.*, **66**, 289 (1974).
- ²⁴ Iversen, L. L., and Bloom, F. E., *Brain Res.*, **41**, 131 (1972).
- ²⁵ Braun, J. P., and Perham, R. N., *Eur. J. Biochem.*, **39**, 69 (1973).
- ²⁶ Levi, G., and Raiteri, M., *Brain Res.*, **57**, 165 (1973).

Stimulation of synaptosomal dopamine synthesis by veratridine

ISOLATED synaptosomal preparations maintain many of the functional properties of intact neurones¹ and the uptake of putative biogenic amine neurotransmitters such as noradrenaline, dopamine and serotonin, is well documented²⁻⁵. In addition, stimulation of synaptosomal catecholamine release has been demonstrated in response to depolarising concentrations of potassium^{4,6} or veratridine⁶, an alkaloid able to produce depolarisation in intact neurones by increasing sodium permeability^{7,8}. In intact adrenergic neurones stimulation of transmitter release is often accompanied by a concurrent increase in the rate of transmitter synthesis, possibly through a reduction in feedback inhibition of tyrosine hydroxylase⁹⁻¹³. The demonstration of a link between stimulated neurotransmitter release and stimulated synthesis in synaptosomes would be an important indication of the validity of studies of synaptosomal processes in order to gain insight into the functioning of intact neurones. We report here that striatal synaptosomes maintain the ability to respond to depolarising concentrations of veratridine with a calcium-dependent tetrodotoxin-sensitive increase in dopamine synthesis.

Male Sprague-Dawley rats (200–250 g) were decapitated and the striatum was dissected out and placed in polyethylene tubes in ice. The P_2 fraction (containing synaptosomes, mitochondria and myelin) was prepared essentially as described by Gray and Whitaker¹⁴. Most of the tyrosine hydroxylase activity in the P_2 fraction has been shown to be associated with the synaptosomal of this fraction after sucrose density gradient centrifugation¹⁵. The P_2 fraction was resuspended in a Tris-buffered incubation medium, with 15%–20% of the P_2 from one rat used for each incubation. Dopamine synthesis was measured by incubating the tissue with L-1-¹⁴C-tyrosine and monitoring the production of ¹⁴CO₂, as previously described¹⁶. Essentially all newly formed dihydroxyphenylalanine is converted to dopamine under these conditions¹⁶. Synthesis was proportional to tissue and time for at least 30 min. The uptake and efflux of L-3,5-³H-tyrosine was measured by separating the tissue from the medium on a Millipore filter, as previously described¹⁶.

To measure the effect of stimulated catecholamine release on the rate of dopamine formation, synthesis was measured during exposure to veratridine. Blaustein *et al.*⁶ have demonstrated that synaptosomal catecholamine release is increased by exposure to veratridine. As Table 1 shows, veratridine produced a 50% increase in synthesis of dopamine. In intact neurones veratridine-induced depolarisation is prevented by treatment with tetrodotoxin, which seems to block sodium entry¹⁷. Blaustein *et al.*⁶ have shown that tetrodotoxin blocks the veratridine-induced increase in synaptosomal amine release. Similarly, Table 1 shows that the veratridine-induced increase in synthesis is prevented by earlier treatment with tetrodotoxin. Tetrodotoxin alone has no effect on dopamine synthesis.

The uptake of labelled tyrosine was inhibited by veratridine—the percentage control uptake 2 and 5 min after exposure to veratridine was 67%±2% and 60%±2% respectively ($N=6$). Veratridine also stimulated the efflux of labelled tyrosine after *in vitro* labelling. The amount of labelled tyrosine remaining in the tissue 2.5 and 5 min after exposure to veratridine was 70%±3% and 65%±4% of controls, respectively ($N=6$). That both the decrease in uptake and increase in efflux of tyrosine seem to be equally affected by veratridine suggests that the specific activity of tyrosine in the tissue is not increased by veratridine; but since this preparation consists of synaptosomes from several different transmitter systems (as well as free mitochondria), measurements of labelled tyrosine uptake and efflux in the total preparation may not reflect alterations that might occur in tyrosine-specific activity in the dopaminergic synaptosomes.

It has been shown repeatedly that for transmitter release to occur from nerve endings, the presence of extracellular calcium is necessary¹⁸. This is also true for veratridine-induced synap-

Table 2 Calcium-dependence of the veratridine-induced increase in dopamine synthesis

	–Ca ²⁺ _o	Dopamine synthesis (nmol L ⁻¹ g ⁻¹) –Ca ²⁺ + EGTA	–Ca ₃₃
Controls	13.8±0.22 (18)	13.8±0.41 (18)	12.8±0.72 (10)
Veratridine	18.1±0.30 (18)*	14.4±0.44 (18)	12.0±0.82 (10)

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37°C either in control medium, Ca²⁺-free medium containing 1 mM EGTA, or simply Ca²⁺-free medium, followed by the simultaneous addition of veratridine (7.5×10^{-5} M) plus L-1-¹⁴C-tyrosine (1×10^{-5} M) or by the addition of tyrosine alone, and incubated for an additional 5 min. Values represent the mean ± s.e.m. The number of observations is in parentheses.

* $P < 0.001$ controls.

somal amine release⁶. Table 2 shows that the increase in dopamine synthesis that usually occurs in response to veratridine was prevented when calcium is omitted, with or without the chelating agent ethyleneglycol-bis- $[\beta$ -aminoethylether]NN'-tetracetic acid (EGTA). It is important to note that in order to demonstrate a consistent increase in synthesis, it was necessary to measure synthesis during the initial 5-min exposure to veratridine. For example, if the tissue was preincubated for 10 min with veratridine and then labelled tyrosine was added and synthesis measured during the next 10 min, a significant increase was not observed. A similar time-dependency has been observed with the increase in synthesis produced by increased potassium¹⁶. Whether or not this time-dependency of the increase in synthesis is related to a similar time-dependency of transmitter release has yet to be determined.

These results demonstrate that isolated synaptosomes can respond to depolarising concentrations of veratridine with a calcium-dependent increase in the rate of catecholamine synthesis. These findings show that the interact neurone is not necessary for the production of a stimulation-induced increase in neuro-transmitter synthesis, and are consistent with the suggestion that synaptosomes are capable of a depolarisation-induced transmitter release⁶. These observations may be relevant to studies of neurotransmitter synthesis not only in adrenergic, but also in serotonergic and cholinergic synaptosomes.

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Table 1 Effect of tetrodotoxin on the veratridine-induced increase in dopamine synthesis

	Dopamine synthesis (nmol h ⁻² g ⁻¹)
Controls	9.54±0.53 (12)
Tetrodotoxin	9.50±0.57 (12)
Veratridine	14.7±0.46 (12)*
Tetrodotoxin + veratridine	10.5±0.43 (12)

Aliquots of the striatal P_2 fraction were incubated for 5 min at 37°C either without further additions or in the presence of tetrodotoxin (2×10^{-7} M), followed either by the simultaneous addition of veratridine (7.5×10^{-5} M) plus L-1-¹⁴C-tyrosine (1×10^{-5} M) or by the addition of tyrosine alone, and incubated for an additional 5 min. The apparent rate of synthesis was calculated by dividing the d.p.m. of product formed per hour per gram of original tissue by the specific activity of the tyrosine added to the medium. The normal incubation medium had the following composition: NaCl, 125 mM; KCl, 5 mM; CaCl₂, 1 mM; MgCl₂, 1 mM; glucose, 10 mM; ascorbic acid, 1 mM (made fresh daily); and Tris-HCl, 50 mM pH 7.4. Values represent the mean ± s.e.m. The number of observations is in parentheses.

* $P < 0.001$ controls, $P < 0.001$ tetrodotoxin plus veratridine (t test).

- DeBellerroche, J. S., and Bradford, H. F., *Progr. Neurobiol.*, **1**, 275–298 (1973).
- Colburn, R. W., Goodwin, F. K., Murphy, D. L., Bunney, W. E. jun., and Davis, J. M., *Biochem. Pharmacol.*, **17**, 957–964 (1968).
- Snyder, S. H., Coyle, J. T., *J. pharmac. exp. Ther.*, **165**, 78–86 (1969).
- Baldessarini, R. J., and Vogt, M., *J. Neurochem.*, **18**, 951–962 (1971).
- Tissari, A. H., and Bogdanski, D. F., *Pharmacology*, **5**, 225–234 (1971).
- Blaustein, M. P., Johnson, E. M., and Needleman, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2237–2240 (1972).
- Straub, R., *Helv. Physiol. Acta*, **14**, 1–28 (1956).
- Ulbricht, W., *Ergb. physiol. biol. Chem.*, **61**, 18–71 (1969).
- Weiner, N., *A. Rev. Pharmacol.*, **10**, 273–290 (1970).
- Spector, S., Gordon, R., Sjoerdsma, A., and Udenfriend, S., *Molec. Pharmacol.*, **3**, 549–555 (1967).
- Thoa, N. G., Johnson, D. G., Kopin, I. J., and Weiner, N., *J. pharmac. exp. Ther.*, **178**, 442–449 (1971).
- Boadle-Biber, M. C., Hughes, J., and Roth, R. H., *Br. J. Pharmacol.*, **40**, 702–720 (1970).
- Harris, J. E., and Roth, R. H., *Molec. Pharmacol.*, **7**, 593–604 (1971).
- Gray, F. G., and Whittaker, V. P., *J. Anat.*, **96**, 79–88 (1962).
- Coyle, J. T., *Biochem. Pharmacol.*, **21**, 1935–1944 (1972).
- Patrick, R. L., and Barchas, J. D., *J. Neurochem.* (in the press).

¹⁷ Narahashi, T., Moore, J. W., and Scott, W. R., *J. gen. Physiol.*, **47**, 965-974 (1964).

¹⁸ Katz, B., and Miledi, R., *Proc. R. Soc. London*, **B161**, 496-503 (1965).

Suppression of fibrinolysin T activity fails to restore density-dependent growth inhibition to SV3T3 cells

SMALL quantities of proteolytic enzymes added to cultures can cause untransformed fibroblastic cells to manifest transiently the altered growth potential^{1,2}, enhanced lectin-mediated agglutinability³ and enhanced glucose transport⁴ characteristic of fibroblast cultures transformed by oncogenic viruses. Thus, the enhanced proteolytic activity of cells transformed by both oncogenic DNA viruses^{5,6} and RNA viruses⁷ may play an important role in the loss of density-dependent growth inhibition exhibited by virus-transformed cells. In particular, Reich *et al.* have shown that cells transformed by simian virus 40 (SV40) or Rous or murine sarcoma virus produce increased levels of a proteolytic activity (fibrinolysin T) which hydrolyses ¹²⁵I-fibrin^{8,9}. This fibrinolysin T activity is the result of the conversion of serum plasminogen to plasmin by a plasminogen activator (cell factor)^{10,11}. In addition, using plasminogen-deficient serum prepared by affinity chromatography, Reich *et al.* have demonstrated plasminogen dependence of several phenotypic parameters associated with viral transformation¹². We have used ε-aminocaproic acid (EACA), an inhibitor of plasminogen activation¹³ and fibrinolysin T activity⁸, and found that suppression of the fibrinolysin T activity of SV40-transformed 3T3 (SV3T3) cell cultures does not restore density-dependent growth inhibition to these cells. This suggests that the enhanced plasmin level in SV3T3 cell cultures is not responsible for loss of density-dependent growth inhibition in this cell line.

We added medium containing 10 mg ml⁻¹ (76.3 mM) EACA (Sigma) to Swiss SV3T3 cells after plating them on ¹²⁵I-fibrin coated dishes⁸, and simultaneously determined the effects of EACA on cell growth and release of ¹²⁵I-fibrinopeptides. The results (Fig. 1 and Table 1) show that fibrinolysin T activity was relatively low on the first and second days after plating the SV3T3 cells, but by the end of the third day in culture extensive hydrolysis of the ¹²⁵I-fibrin was apparent. (Because the cell sheet peeled off the Petri dish at cell densities greater than 12 × 10⁶ per dish, fibrinolysin T could not be assayed on untreated SV3T3 cells after day 4.) In contrast, fibrinolysin T activity in EACA-treated cultures remained low and relatively constant for at least 7 d after plating. The inhibitory effect of EACA on fibrinolysin T activity was rever-

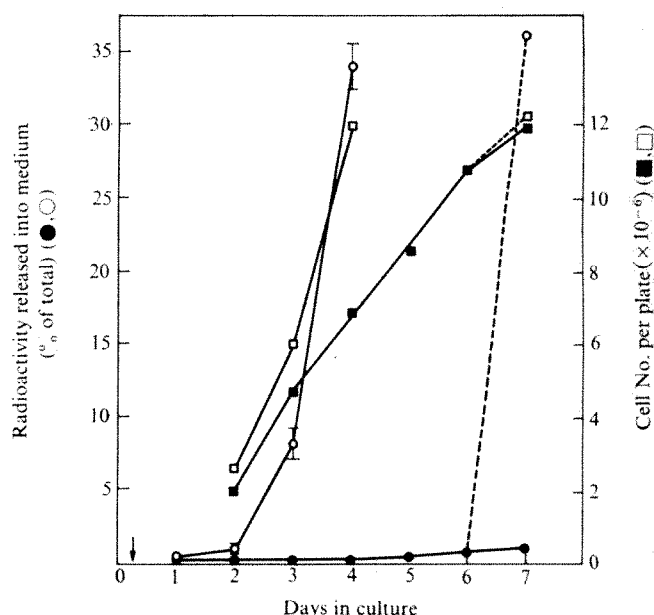


Fig. 1 Growth and fibrinolytic activity of Swiss SV3T3 cells treated with ε-aminocaproic acid. At time zero, 5×10^6 Swiss SV3T3 cells in 5 ml of Dulbecco's medium + 10% foetal calf serum were seeded into 60-mm Falcon plastic Petri dishes coated with ¹²⁵I-human fibrinogen ($10 \mu\text{g cm}^{-2}$; total radioactivity 1.17×10^6 c.p.m. per dish) which was converted to fibrin before plating cells according to Unkeless *et al.*⁸. Five hours later, the medium was removed and 5 ml of fresh Dulbecco's medium + 10% foetal calf serum with or without 10 mg ml^{-1} EACA was added (↓). At 24-h intervals thereafter, the cell culture medium was removed, aliquots were assayed for released ¹²⁵I-fibrinopeptides as described⁸, and replaced with 5 ml fresh medium with or without EACA. The amount of released ¹²⁵I-fibrinopeptides on appropriate control plates without cells and with or without EACA has been subtracted. The activity detected represents the accumulated radioactivity during a 24-h incubation period. Each point represents the mean value (\pm s.d.) of the average of duplicate counts of replicate determinations on separate plates. Cell growth was determined on ¹²⁵I-fibrin-containing plates which were seeded in parallel with those used for enzyme assays. In each case the medium was changed at 24-h intervals. Cell counts were determined on trypsinised cell suspensions using a laser beam cell counter (Cytograph). Fibrinolysin activity: ○, control; ●, EACA-treated. Cell number: □, control; ■, EACA-treated. After removal of EACA, both fibrinolysin activity (●, ○) and cell number (■, □) increased during the next 24 h.

sible, since changing to medium without EACA on day 6 resulted in extensive release of ¹²⁵I-fibrinopeptides during the next 24 h (Fig. 1). Most important, under conditions where fibrinolysin T activity had been suppressed 83-98% by EACA, Swiss SV3T3 cells continued to grow and eventually reached the same cell density as the untreated control culture. Thus, essentially complete suppression of the fibrinolysin T activity of SV3T3 cell cultures failed to restore density-dependent growth inhibition to these cells.

As Fig. 2 shows, the fibrinolytic activity of untreated Swiss 3T3 cells was also relatively low for the first 2 d after plating, increased sharply on days 3 and 4, and then decreased on days 5 and 6. We have evidence from other experiments (unpublished data) that elaboration of cell factor depends on the growth state of the culture and is decreased when 3T3 cells become confluent. This may account for the diminution of fibrinolytic activity on days 5 and 6 (Fig. 2), although depletion of the ¹²⁵I-fibrin substrate may also be responsible in part. EACA (10 mg ml^{-1}) treatment of 3T3 cultures leads to 92-100% inhibition of their fibrinolysin T activity (Table 1). In addition, EACA treatment caused 3T3 cells to cease increasing in cell number about 2 d earlier than untreated cells and at a lower final cell density (EACA-treated: 8.7×10^5 cells per dish; untreated: 1.5×10^6

Table 1 ε-aminocaproic acid inhibition of the fibrinolytic activities of Swiss 3T3 and SV3T3 cells

Days	SV3T3			3T3		
	-EACA	+EACA	% Inhibition	-EACA	+EACA	% Inhibition
1	0.40*	0.05*	88	0.60*	0.05*	92
2	0.35	0.06	83	2.65	0.05	98
3	1.36	0.05	96	15.42	0	100
4	2.83	0.05	98	13.92	0	100
5	—	0.06	—	4.96	0	100
6	—	0.08	—	0.29	0	100
7	(2.96)†	0.10	97	(1.33)†	—	—

* Activities (% of total) without correction for cell number. Total = 1.17×10^6 c.p.m. per dish.

† Activity of cells 24 h after removal of EACA from growth medium.

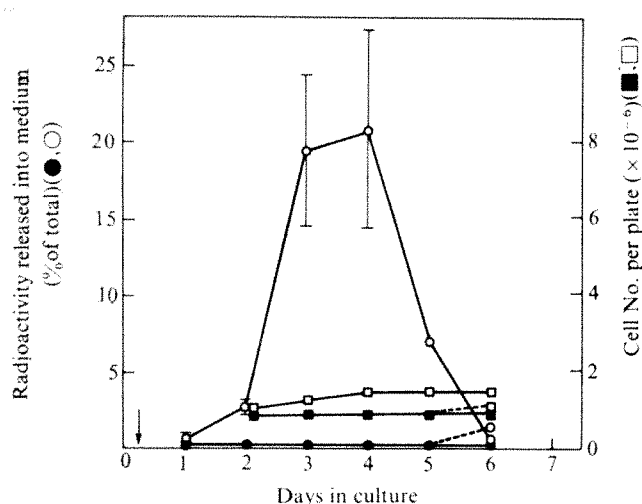


Fig. 2 Growth and fibrinolytic activity of Swiss 3T3 cells treated with ϵ -aminocaproic acid. Experimental details and symbols are as described for Fig. 1. Swiss 3T3 cells were seeded at 5×10^5 cells per dish.

cells per dish). The inhibitory effects of EACA on the final cell density and fibrinolytic activity of 3T3 cells were also partially reversed within 24 h after removal of EACA (Fig. 2).

Treatment of 3T3 and SV3T3 cells with 10 mg ml^{-1} EACA for 45–48 h inhibited incorporation (c.p.m. mg^{-1} protein) of a mixture of radioactive amino acids into acid-precipitable material by 40% and 45% respectively. Similarly, incorporation of ^3H -uridine into acid-precipitable material was inhibited 21% (SV3T3) and 15% (3T3), and incorporation of ^3H -thymidine was depressed 10% (SV3T3) and 40% (3T3). Since we have previously shown that treatment of these SV3T3 cells with low doses of cycloheximide depresses protein synthesis and leads to a dose-dependent growth inhibition¹⁴, the decreased growth rate of EACA-treated SV3T3 cells was probably a consequence of the inhibition of protein synthesis in EACA-treated cultures. Inhibition of protein synthesis by EACA may also have been responsible for reducing the final cell density of EACA-treated 3T3 cells (Fig. 2)¹⁴.

Studies on the metabolism of ^{14}C -EACA (New England Nuclear Corp.) have demonstrated (1) that EACA is taken up into a trichloroacetic acid (TCA)-extractable pool by both Swiss 3T3 and SV3T3 cells to approximately the same extent on a per cell basis and (2) that EACA is quite stable in cultures of both cell types since less than 0.2% of the input ^{14}C -EACA was broken down and reincorporated into TCA-insoluble cellular material during a 24 h incubation (R.O.R., B.Ash and P.H.B., unpublished data). The high anti-fibrinolytic activity and metabolic stability of EACA, coupled with its low cytotoxicity, thus make it a useful agent with which to probe the involvement of fibrinolysis in viral transformation.

Our results demonstrate that extensive fibrinolysis takes place under normal growth conditions in cultures of both untransformed 3T3 and transformed SV3T3 cells. This is so, even though the cells were cultured in medium containing foetal calf serum, which contains relatively high levels of inhibitors of fibrinolytic activity^{8,9}. Our assay for fibrinolytic activity differs from others, however, in that it measures cumulative proteolytic hits over a prolonged period. Prolonged incubation may be required to detect low levels of fibrinolytic activity since the ^{125}I -fibrin substrate is a complex, multi-stranded, cross-linked molecule and more than one proteolytic cleavage may be required to liberate ^{125}I -fibrinopeptides into the medium.

It is also interesting that the untransformed 3T3 cell line exhibited greater fibrinolytic activity per 10^6 cells during the growing phase than did its SV3T3 counterpart (Table 1). Thus, SV40 transformation of 3T3 cells did not appear to lead to increased levels of intrinsic fibrinolytic activity in SV3T3 cells.

Removal of EACA from SV3T3 cultures, however, resulted in considerably more fibrinolytic activity per 10^6 cells than was observed when EACA was removed from 3T3 cell cultures. Thus, there appeared to be a relative reduction of fibrinolytic activity in dense cultures of 3T3, but not SV3T3 cells. The nature of this density-dependent reduction of fibrinolytic activity is under investigation.

At the concentration used in these experiments (0.076 M), EACA probably interferes with fibrinolytic activity in several ways. It can inhibit activation of plasminogen to plasmin by cell factor^{8,13}, probably by binding to and altering the conformation of plasminogen¹⁵. EACA may also interact with fibrin and thus directly inhibit the hydrolysis of fibrin by plasmin¹⁶. Finally, EACA may reduce the amount of available cell factor through its inhibition of protein synthesis. Ambrus *et al.*¹⁷, however, have reported circumstances under which EACA almost completely inhibited the fibrinolytic activity of plasmin while it failed to reduce the caseinolytic activity of similar plasmin preparations. Thus, in our experiments, although the fibrinolytic activity of the EACA-treated cultures was almost eliminated, residual plasmin activity for other substrates may have remained. We are investigating whether such residual plasmin activity exists and whether it plays a role in loss of density-dependent growth inhibition.

Our results show that essentially complete suppression of fibrinolysis T activity failed to restore density-dependent growth inhibition to SV3T3 cells. In addition, we observed high levels of fibrinolytic activity in growing cultures of both 3T3 and SV3T3 cells and only when confluent 3T3 cells were compared with growing SV3T3 cells did the SV3T3 cell cultures exhibit higher fibrinolytic activity per 10^6 cells than the 3T3 cell cultures. Thus, the enhanced fibrinolysis T activity of SV3T3 cells is unlikely to be the cause, but may rather be a consequence of unrestrained cell growth. Our results are consistent with those of Ossowski *et al.*¹², who showed that while complete expression of several phenotypic properties of transformed cells (such as growth in agar, and morphology) required the presence of a serum plasminogen fraction, SV40-transformed hamster cells grew to high cell densities in plasminogen-depleted serum.

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- ¹ Burger, M. M., *Nature*, **227**, 170–171 (1970).
- ² Sefton, B. M., and Rubin, H., *Nature*, **227**, 893–895 (1970).
- ³ Inbar, M., and Sachs, L., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 1418–1425 (1969).
- ⁴ Sefton, B. M., and Rubin, H., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3154–3157 (1971).
- ⁵ Bosmann, H. B., *Expl. Cell Res.*, **54**, 217–221 (1967).
- ⁶ Schnebli, H. P., *Schweiz. Med. Wochenschr.*, **102**, 1194–1197 (1972).
- ⁷ Bosmann, H. B., *Biochim. biophys. Acta*, **264**, 339–343 (1971).
- ⁸ Unkeless, J. C., Tobia, A., Ossowski, L., Quigley, J. P., Rifkin, D. B., and Reich, E., *J. exp. Med.*, **137**, 85–111 (1973).
- ⁹ Ossowski, L., Unkeless, J. C., Tobia, A., Quigley, J. P., Rifkin, D. B., and Reich, E., *J. exp. Med.*, **137**, 112–125 (1973).
- ¹⁰ Quigley, J., and Unkeless, J., *Fedn Proc.*, **32**, 851 (1973).
- ¹¹ Unkeless, J., Tobia, A., Ossowski, L., Quigley, J., Rifkin, D., and Reich, E., *Fedn Proc.*, **32**, 851 (1973).

- ¹² Ossowski, L., Quigley, J. P., Kellerman, G. M., and Reich, E., *J. exp. Med.*, **138**, 1056-1064 (1973).
¹³ Alkjaersig, N., Fletcher, A. P., and Sherry, S., *J. biol. Chem.*, **234**, 832-837 (1959).
¹⁴ Chou, I-N., Black, P. H., and Roblin, R. O., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1748-1752 (1974).
¹⁵ Castellino, F. J., Brockway, W. J., Thomas, J. K., Liao, H-T., and Rawitch, A. B., *Biochemistry*, **12**, 2787-2791 (1973).
¹⁶ Maxwell, R. E., Nawrocki, J. W., and Nickel, V. S., *Thromb. Diath. Haemorrh.*, **19**, 117-128 (1968).
¹⁷ Ambrus, L. M., Ambrus, J. L., Lassman, H. B., and Mink, I. B., *Ann. N.Y. Acad. Sci.*, **146**, 430-446 (1968).

Activation of guanyl cyclase and intracellular cyclic GMP by fibroblast growth factor

THE induction of cell growth by animal serum in quiescent cultured fibroblasts is preceded by a sequence of regulated steps¹. These steps include stimulation of cellular transport systems¹, protein synthesis¹, ribosomal and tRNA synthesis² and eventually the induction of DNA synthesis followed by cell division^{3,4}. Two of the earliest changes observed after growth induction by serum are a transient increase in intracellular cyclic GMP⁵ (10-fold) and a decrease in cyclic AMP (two-to-threefold)^{5,6,7}. It has been suggested that cyclic GMP acts as a positive intracellular signal for cell growth since intracellular cyclic GMP concentrations showed an early transient increase upon growth induction by phytohaemagglutinin whereas no changes were observed in cyclic AMP concentrations⁸, and additions of high, non-physiological (10^{-6} to 10^{-4} M) concentrations of cyclic GMP can induce substantial increases in DNA synthesis in resting fibroblasts⁵. Recently a new polypeptide hormone, fibroblast growth factor (FGF), was isolated from bovine pituitary glands⁹. FGF in combination with the glucocorticoid, hydrocortisone, and a nonspecific carrier protein, bovine serum albumin (BSA), can completely replace exogenously added serum in bringing about all the steps leading to the initiation of DNA synthesis and cell division in some lines of BALB/c 3T3 cells¹⁰. Hydrocortisone, as it fails to initiate DNA synthesis alone in the absence of serum¹¹ is considered to potentiate the action of FGF⁹ (permissive effect).

Here we show that physiological concentrations of FGF with hydrocortisone cause the same transient increase in intracellular cyclic GMP concentrations of quiescent cultures as those caused by serum. Little or no alteration, however, is observed in cyclic AMP concentrations. Furthermore FGF specifically activates the membrane-bound guanyl cyclase, but not the adenylyl cyclase system. We suggest that growth-initiating polypeptide hormones interact with a guanyl cyclase system bound to the plasma membrane, in a way similar to that of the well known interaction between polypeptide hormones and the adenylyl cyclase system¹².

When FGF was added to resting BALB/c 3T3 cells maintained in the presence of serum² the concentrations of cyclic GMP rose after 10 to 20 min by a factor of 10- to 15-fold over the value in unstimulated cultures. Thereafter the concentration rapidly declined reaching approximately twice the value in resting cultures within 1 h (Fig. 1a). Cyclic AMP concentrations showed a reduction of only 20%, much less than the two-to-threefold decrease observed after addition of serum⁵. The concentrations of cyclic GMP approached those of cyclic AMP for a short time—a result similar to that obtained by adding serum to these cultures⁵ and by adding phytohaemagglutinin to human peripheral blood lymphocytes⁸. It was not possible to maintain non-degenerating cultures of BALB/c 3T3 cells with only BSA in the absence of serum, but addition of low concentrations (0.1 ng ml^{-1}) of FGF could prevent deterioration of the cultures for at least 7 d (ref. 5). Addition of

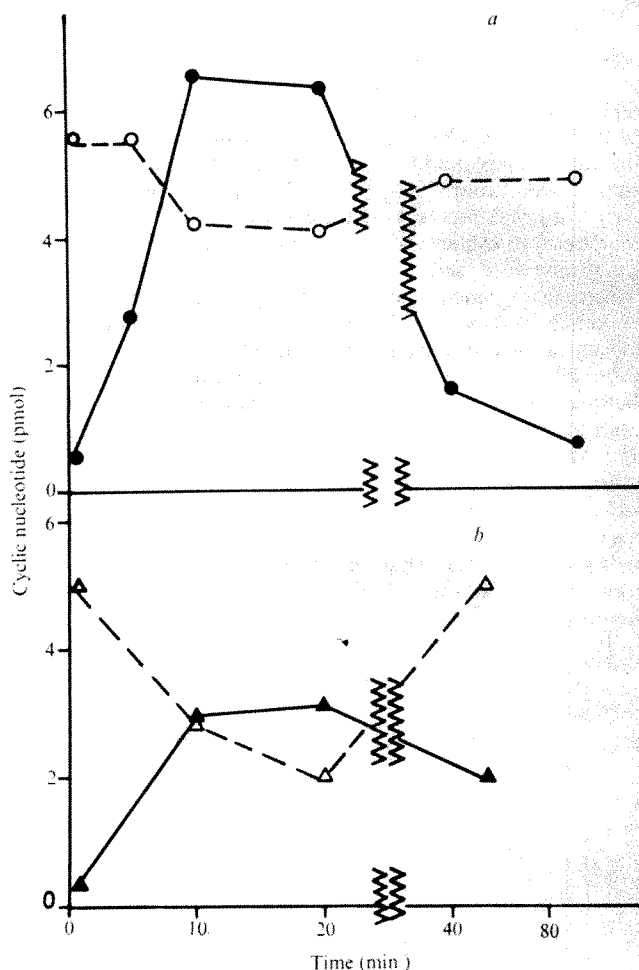


Fig. 1 Kinetics of cyclic nucleotide changes. BALB/c 3T3a cells¹¹ were grown as previously described⁵. For *a*, (FGF) cultures were allowed to become quiescent at a final cell density of 1.2×10^6 per plate before addition of 50 ng ml^{-1} FGF. For *b*, (insulin) BALB/c 3T3b cells², 6-d cell monolayers, washed with DEM, were incubated for 16 h in Dalbec's modified Eagle's Medium containing $250 \mu\text{g ml}^{-1}$ BSA and $0.5 \mu\text{g ml}^{-1}$ hydrocortisone (final cell density as *a* before addition of $5 \mu\text{g ml}^{-1}$ insulin (bovine pancreas, Calbiochem B grade). At the times indicated 10 plates were isolated as previously described⁵ and the cyclic GMP (—●—, —▲—) and cyclic AMP (—○—, —△—) were determined by a radioimmuno assay¹⁸. After centrifugation and ether extraction half the samples were digested with 3' - 5' cyclic nucleotide phosphodiesterase and all the samples were purified over Dowex Columns⁹ prior to assay. Results for digested samples (0.1 and 0.5 pmol for cyclic AMP and cyclic GMP respectively) were deducted to yield the final results expressed in $\text{pmol per } 10^6$ cells. Duplicate experiments agreed to within 15%. Parallel cultures radioactively labelled with ^3H -thymidine (Fig. 2) from 10 to 28 h after addition of FGF, insulin, or nothing incorporated 22, 10, or 0.6×10^3 c.p.m. into DNA, and 89%, 44% or 1.5% of the cell nuclei became labelled with radioactivity.

FGF was purified from bovine pituitary glands as previously described⁹. The possibility that FGF was acting on the cells to potentiate the action of trace amounts of other growth factors adsorbed to BSA was excluded by obtaining the same results as in Fig. 2 after replacing BSA by gelatin and completely synthetic polypeptides. It was also unlikely that minor growth-promoting impurities in the insulin preparations²⁴ were responsible since similar results were obtained from commercial preparations of insulin which were further purified by column chromatography.

increasing concentrations of FGF in the presence of hydrocortisone caused concomitant increases in both the cyclic GMP concentrations measured at 20 min and the eventual induction of DNA synthesis (Fig. 2) and cell division (not shown). Virtually no changes were observed in cyclic AMP, and the small changes observed in Fig. 1 may be due to other components in serum. Resting cultures were maintained with hydrocortisone long enough for the glucocorticoid to exert

its permissive effect¹³. Omission of hydrocortisone caused a four-fold drop in both DNA synthesis and cyclic GMP concentration and hydrocortisone alone did not induce early changes in cyclic nucleotide concentrations (Fig. 2).

High, non-physiological concentrations of insulin (10^{-7} M to 10^{-6} M) can also increase DNA synthesis and cell multiplication¹⁴ after addition to quiescent fibroblasts. At these high concentrations (100 to $1,000 \times$ physiological) both the early transient increase in intracellular cyclic GMP and the depression in cyclic AMP were observed in cultures resting in the presence of serum (not shown), or in medium free of exogenously added serum with hydrocortisone (Fig. 1b). Maximal increases in DNA synthesis and in cyclic GMP concentrations were only approximately 35% to 40% of those observed for FGF in Fig. 2. The concentration dependence for the increases in cyclic GMP concentrations and for the stimulation of DNA synthesis were roughly parallel (maximal from 10^{-7} M to 10^{-6} M; not shown), perhaps suggesting a correlation. Conversely, the initial depressions in cyclic AMP occurred at much lower insulin concentrations, in the normal physiological range (10^{-10} M to 10^{-9} M; not shown). Without hydrocortisone the changes in cyclic nucleotides and the eventual stimulation of DNA synthesis were reduced two- to threefold.

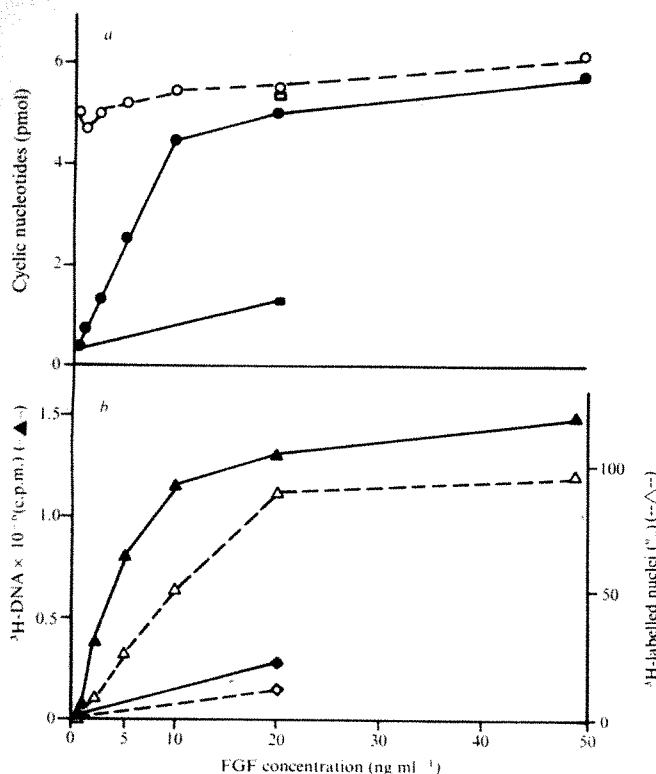


Fig. 2 Variations of cyclic GMP with FGF concentrations. Cell cultures were grown as in Fig. 1 except that 6 d after plating (confluent cultures) cell monolayers, washed with DEM containing 0.12 ng ml^{-1} FGF and $250 \text{ } \mu\text{g ml}^{-1}$ BSA, were then incubated for 2 d in this medium. Then $0.5 \text{ } \mu\text{g ml}^{-1}$ cortisol (hydrocortisone, Calbiochem) was either added (circles, triangles) or omitted (squares, lozenges). After 10 h (final cell density 1.1×10^6 per plate) varying amounts of FGF were added. *a*, Ten Petri dish cultures for each concentration were isolated 20 min later for cyclic nucleotide determinations (Fig. 1). Results are expressed in pmoles of cyclic AMP (open circles, squares) or cyclic GMP (black circles, squares) per 10^6 cells. *b*, Parallel cultures were also radioactively labelled with $3 \text{ } \mu\text{Ci ml}^{-1}$ ³H-methyl-thymidine at $3 \text{ } \mu\text{M}$ (for DNA synthesis) or $1 \text{ } \mu\text{M}$ (for autoradiography) from 8 to 26 h after additions. The c.p.m. of ³H-thymidine incorporated into DNA per culture (black triangles, lozenges) or the percentage of radioactively labelled cell nuclei (open triangles, lozenges) was recorded as before². Control additions of cortisol alone showed no changes in the intracellular cyclic nucleotide concentrations after 20 min or detectable increases in DNA synthesis.

Hitherto guanyl cyclase in a variety of tissues was thought to occur primarily in the membrane-free cytoplasm of the cell^{15,16}, unlike the adenylyl cyclase which is attached to the plasma membrane¹². Recently, however, a large increase in guanyl cyclase activity was obtained after treatment of particulate (membrane-containing) cell fractions with non-ionic detergents¹⁷. This finding was confirmed for 3T3 fibroblasts by the results in Table 1, where a ten-fold stimulation of guanyl cyclase activity in the particulate (microsomal) cell fraction was observed after Triton treatment, little or no increase being seen in the membrane-free cytoplasmic activity. The guanyl cyclase in the particulate fraction was also stimulated nearly three-fold by additions of FGF. No activation of the soluble cytoplasmic activity was observed, and the greatly enhanced activity in the particulate fraction after Triton treatment could not be increased by subsequent addition of FGF. Isolation of the plasma membrane fraction also confirmed these results: FGF stimulated the guanyl cyclase activity approximately sixfold whereas insulin had little or no effect (Fig. 3a).

The production of cyclic GMP during this reaction was measured by a radioimmune assay¹⁸. Even larger stimulations (eight- to tenfold) were achieved when radioactive cyclic GMP was isolated by chromatographic procedures from reaction mixtures containing ³²Pα-GTP, at higher GTP concentrations (0.001 M) (not shown). FGF, unlike insulin^{19,20} failed however to reduce the adenylyl cyclase activity of the plasma membrane (Fig. 3b). All incubation mixtures contained 3'-5' nucleotide phosphodiesterase inhibitors (theophylline and caffeine) to minimise phosphodiesterase cleavage of the newly synthesised cyclic nucleotides, as production of small quantities of one cyclic nucleotide could possibly affect rates of breakdown of the other²¹.

In a system uncoupled from other intracellular events FGF directly stimulates the guanyl but not the adenylyl cyclase of plasma membrane; presumably through a similar mechanism to that of the known hormonal stimulation of the adenylyl cyclase system¹². This is manifested in intact cells maintained in culture without added serum by a rapid, transient increase in intracellular cyclic GMP shortly after addition of FGF, little or no early changes being observed in cyclic AMP. Physiological

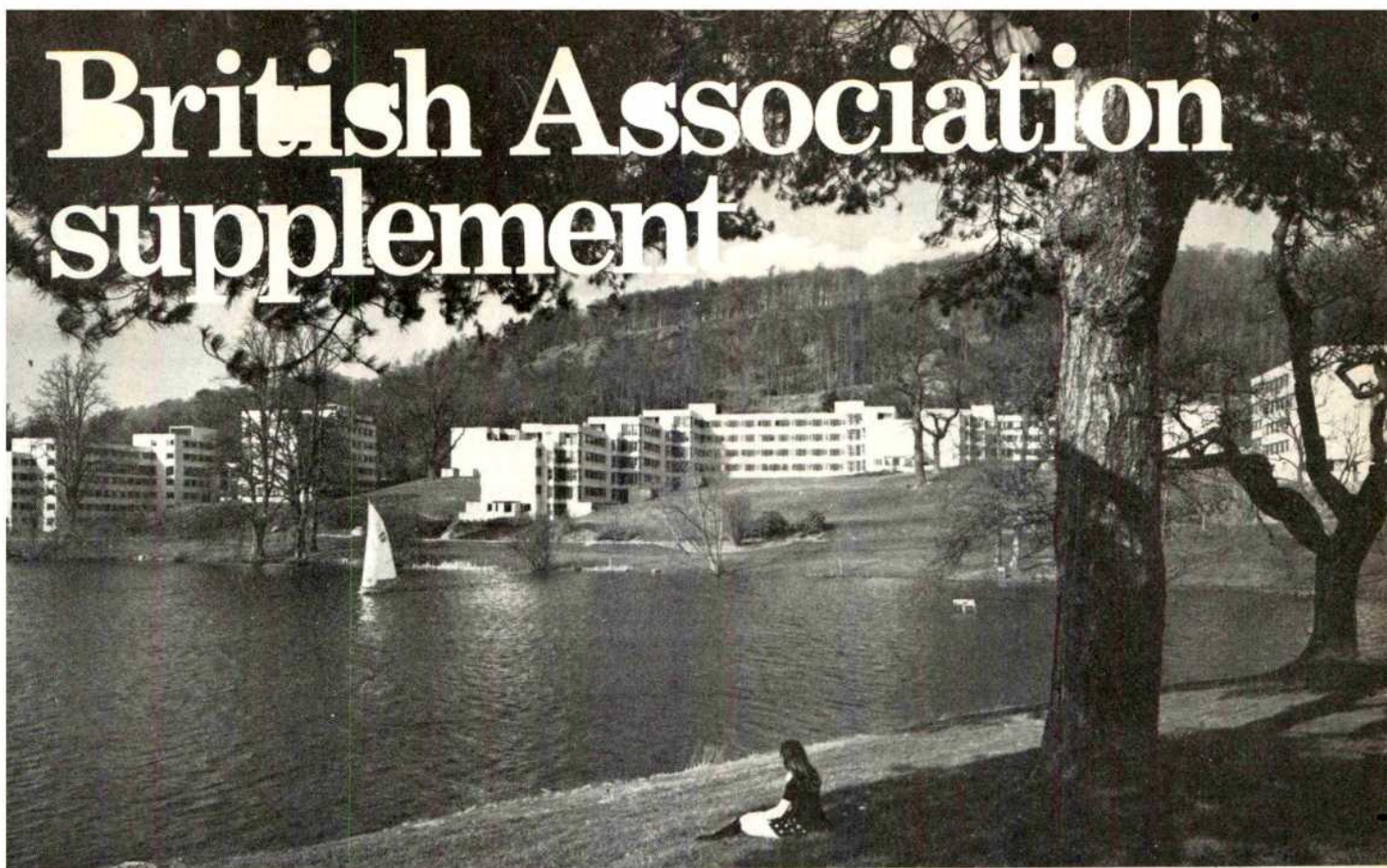
Table 1 Subcellular distribution of guanyl cyclase

Cell fraction	Cyclic GMP synthesised (pmol/min/mg extract)				
	Control	+ FGF	+ Triton	+ FGF	+ Triton
Supernatant	23	20	27	31	
Microsomal	9.4	27	97	108	

BALB/c 3T3a cells were grown in 10% serum as described (Fig. 1a). Cell monolayers were washed with Tris-buffered saline detached and centrifuged. This and subsequent operations were at 4°C . Cells, resuspended at 10% to 15% v/v in Buffer A (0.005 M Tris-HCl ($\text{pH } 7.4$), 0.0002 M MgSO_4 , 0.25 M sucrose) plus 0.00025 M dithiothreitol (DDT) were homogenised by douncing. The homogenate was made 0.001 M in EDTA Na_2 and centrifuged at $2,000g$ for 5 min. Then the supernatant was made 0.005 M in MgCl_2 and centrifuged at $150,000g$ for 2 h. The microsomal pellet was resuspended in Buffer A + 0.00025 M DTT and stored with the microsomal-free supernatant at 4°C . Reaction mixtures (0.5 ml) contained 0.04 M HEPES ($\text{pH } 7.6$) (N-hydroxyethylpiperazine-N'-2-ethane sulphonic acid), 0.009 M MgCl_2 , 0.0001 M GTP, 0.0005 M EDTA Na_2 , 0.0005 M DTT, 0.12 M sucrose, 0.01 M theophylline, 0.02 M caffeine $200 \text{ } \mu\text{g}$ of supernatant protein or $110 \text{ } \mu\text{g}$ of microsomal protein and where indicated 100 ng of FGF and 1% Triton X100. Reactions were incubated at 37°C for 15 min, terminated by boiling for 3 min, followed by centrifugation at $1,000g$ for 10 min, and the extracts stored frozen. Duplicate $20 \text{ } \mu\text{l}$ samples were assayed for cyclic nucleotides by the radioimmune assay (Fig. 1), and results are expressed as pmol cyclic GMP synthesised per min per mg protein. Blank values at zero time (5.3 and 2.7 pmol for supernatant and microsomal fractions) were deducted. The blank cyclic GMP values may be in part due to non-enzymic synthesis of cyclic GMP from GTP during the boiling step which nevertheless would be less than 2 pmol per reaction mixture, whereas non-enzymic synthesis at 37°C would be negligible²². In control reactions the cyclic GMP synthesised was fully digested with 3'-5' cyclic nucleotide phosphodiesterase, synthetic reactions were linear with time up to 30 min, and there was no interference of FGF or Triton at concentrations used in the radioimmune assay.

(Continued on p. 773)

British Association supplement



Architectural Review

Stirling campus • Fishlock: Science journalism • 1874 transit of Venus

Goldsmith: Popularising science • Science in Northern Ireland

Hall: Social responsibility • Murder solution by fluorescence

Cotgrove: Objections to science • Art preservation • Bullard: Rutherford's Cavendish

It has been possible in recent years to look at the British Association for the Advancement of Science from a slightly lofty viewpoint and wonder whether it is worth preserving. The annual meeting, the only occasion on which the association has surfaced, has also become a fairly regular occasion for pointed editorialising about an organisation that had outgrown its usefulness. No longer is this a reasonable line to take, and for that great credit above all to Magnus Pyke whose unique qualities have done much to breathe life into the body. If we talk here about the way ahead it is not without feeling admiration for the achievements of the recent past.

What, if anything, do scientists, those interested in science, and indeed those confronted with science need from a national organisation with a wide potential membership? Clearly not another forum for technical discussion; there are enough of these as it is, and why would a scientist take his latest results to Stirling when he could ride them to Stockholm, San Francisco or Sydney. Nor is it obvious that mere presentation and explanation of science to an enthusiastic audience will be other than an activity of moderate interest to scientists, particularly as the quality of science presentation by other media is often high.

There remains, however, a field which is as yet almost unexplored—the study of science itself. In this supplement we have tried to draw attention to some facets of science to which any scientist could contribute and from which he could draw some intellectual satisfaction. The history, sociol-

ogy, philosophy and way of going about science; the borderlands where science meets other disciplines; the morality of science; the way that science is presented to the public—there is much fertile ground here for regular discussion. To which we could add the deployment of scientists, science in defence, science in the Third World, education for scientists, government policy for science, nationalism in science. These are all subjects that scientists should have special knowledge of without needing to integrate a differential equation or operate a microscope. They emphasise common experiences, and may be the way towards bridging the gulf, both personal and professional, between teachers, academics, and scientists in government and industry. Here is also scope for the amateurs of science to make significant contributions to the world of learning.

The British Association is the ideal vehicle for all this, with its potentially very broadly based membership and its lack of political colour. It can point already to the increase in throughout-the-year activities both of its younger members and of its *ad hoc* committees. What is needed now is more nation-wide activity amongst its general membership and a vigorous campaign to persuade scientists that the study of science itself makes them a better practitioner. Cells of mutual instruction and discussion, rather like the Mechanics Institutes of the nineteenth century, could do the scientific community, in its broadest sense, much good and are well worth the serious attention of the association in the coming years. □

Stirling University, host to the British Association

Tom Willoughby

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In this article an architect examines how the university campus at Stirling was planned. He also describes a computer study of the site carried out at the University of Strathclyde as part of a project on computer-aided design of large building complexes.

THOSE lucky enough to go to the British Association conference at Stirling this September will find one of the most beautiful campuses in Britain, sited near the citadel of Stirling Castle, the anvil on which much of Scottish history was hammered out. For Stirling is a town over which England and Scots battled for centuries, and where, in the absence of the English, the Scots fought amongst themselves. The almost unscaleable crag on which Stirling Castle stands is the gateway and key to the Highlands. Stirling had the lowest dry crossing of the Forth at the 'auld Brig' and with the resulting trade and patronage of the Scots royal house, the town grew to a prosperous centre.

The Romans under Agricola occupied the rock as a natural defence in 82 AD, legend has it that King Arthur fought the Saxons over the castle, and from 1194, in the reign of King Alexander I, the town became the 'favourite resort for the royal house'. The victorious Edward I of England pushed into Stirling in 1296, routing the locals, but Wallace replied by defeating the invaders. The English were down but not out and Edward returned; after a furious siege, they retook the castle. By 1313 the indomitable Scotsman Bruce had the castle under siege, and the following year Edward II marched to its relief but was blocked fair and square at Bannockburn where Bruce won a much hailed victory. Bruce then dismantled the castle to prevent its misuses by the dreadful English but Edward III was soon back and so the battles continued. The Scottish kings from Richard III to James VI resided with the court at Stirling. The uncertain form of the Scottish state, with its bloody tribal or clan wars, litters the royal history with assassinations, torture, plots and counter plots but, by the time of James VI, the framework of laws and Parliament were established, although the Western Isles chiefs were never cowed in the same way as the English Barony. The battle of Sheriffmuir in 1715 saw the highlanders and lowlanders fighting it out. In 1745 the castle was strongly held for the protestant crown, but catholic Prince Charles took the town in triumph. The town Stirling reflects the royal patronage with the castle and the magnificent store of buildings such as the 'Argyll Ludging' one of the finest examples of Scottish renaissance extant, built in 1630 by the Earl of Stirling.

New university

Returning to the present day, the 1950s and early 1960s saw the change of government policy towards an expansion of higher education, culminating in the long awaited Robbins report which recommended six new universities of which at least one, if not two, should be located in Scotland. The new university was to be the first for 300 years on Scottish soil, which, interestingly, boasts four ancient universities whereas England has only two.

The burgh of Stirling decided in October 1960 to do all in its power to attract the new university. Its competitors

were six other Scottish towns—Ayr, Cumbernauld, Dumfries, Falkirk, Inverness and Perth—which simultaneously entered the fray to attract the project.

The University Grants Committee (UGC) felt that it wished to found universities in medium sized towns, where people welcomed a new university, where land was cheaply available and the existing town offered prospects for the development of town-grown facilities. An investment in terms of capital, expanded job opportunities and cultural pump priming, provided by a university, would act as a regenerative force, possibly attracting specialised light industry into the bargain.

The lobbying was enthusiastic if not downright aggressive, Inverness arguing the benefits to the Highland economy, Stirling and Falkirk their nearness to the main centres of industry and population. Cumbernauld was felt to be too new, Perth to be near the university towns of Dundee and St Andrews, Dumfries and Ayr to be too isolated. The argument was really between Inverness, Falkirk and Stirling, with leanings towards the latter two.

The Falkirk-Grangemouth area is one of the most rapidly expanding in Europe, even more so with the advent of North Sea Oil. Falkirk could accommodate a university with both technical and humanities faculties and the town was large enough to offer a choice of 'digs' or rented flats. But middle class nonindustrial Stirling offered a magnificent site.

When looking at university planning in the stringent financial climate of 1974, one should not forget those heady days of the 1960s when governments jealously viewed each other's 'graduate count' as an international virility symbol. The euphoric days saw the UGC dispensing rapidly expanding resources and the choice of Stirling related to the general pattern of UGC policy. In Britain, as in many other parts of the world, university planning is as dominated by the Oxbridge ethos as vicars are by Gothic. Universities are in stone towns, in 'nice places'; thus historic York, although surrounded by existing universities, gets a new

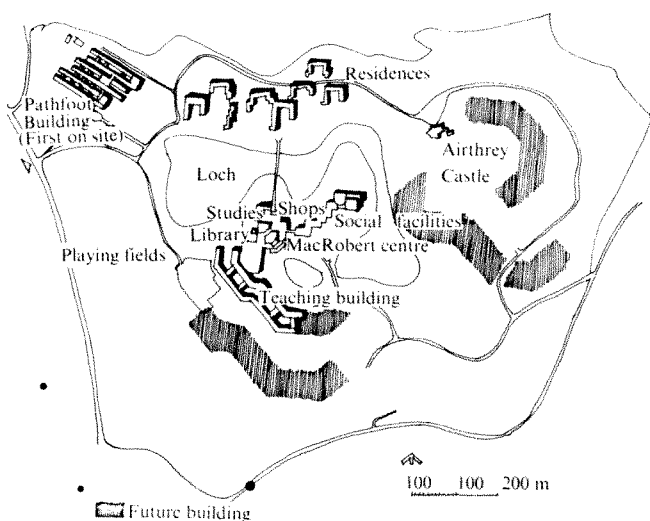
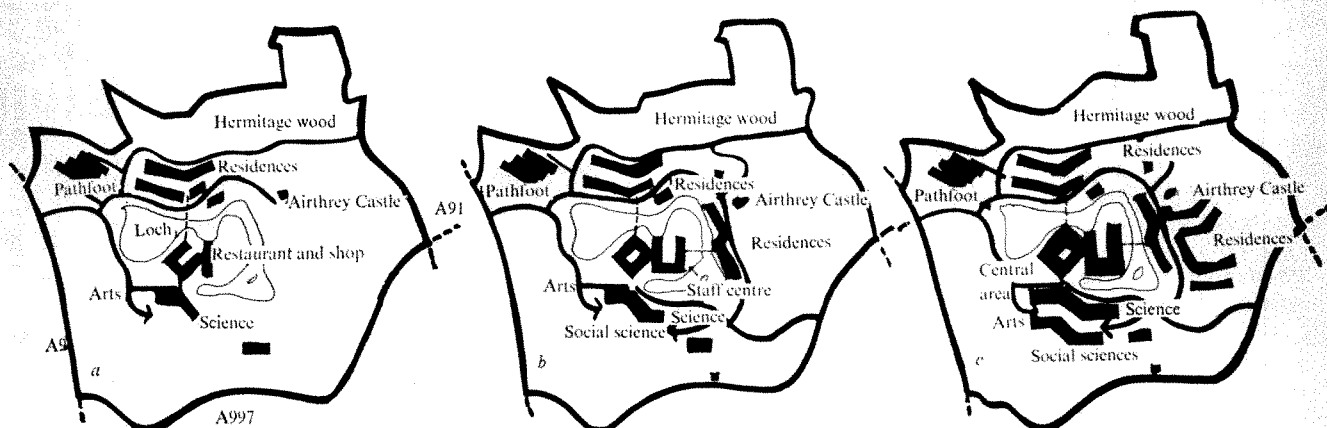


Fig. 1 Present site plan for a campus for 3,000 students.



university, whereas industrial Teesside just to the north, an industrial conurbation three times the size, does not. Almost incredibly, prosperous Bath, a mere 12 miles from the existing and famous University of Bristol, gets a new university; the town, although stone, pretty and small like Stirling, being almost incapable of supporting facilities that allow the word 'universities' to have much meaning. In their more lunatic moments, people were even suggesting Stamford, a town of 10,000 but oh! such a pretty place. The ivory tower concept dominated the pattern of thinking and in this way Stirling was selected while Scotland's fastest growing population centre was passed by.

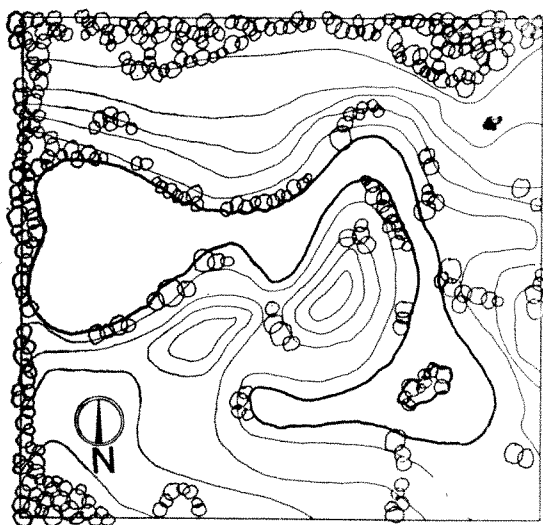


Fig. 3 An area of the site around the lake.

The decision in favour of Stirling, when announced, was rightly made final, but of course there were howls of anguish from Inverness. In Falkirk where the committee chairman, Andrew Duncan, had made getting the university his life's work, bewildered and bitter comments about 'the snob town' of Stirling were heard.

Stirling is on the new A80 from Glasgow and the M9 for Falkirk and Edinburgh, and this and the sheer magnificence of the site were major factors in its selection. The site of over 300 acres on the Airthrey Estate lies between the west slopes of the Ochills and the wooded area of Abbey Craig on which stands the splendid Wallace memorial, a flamboyant victorian bombastitude from which the view is well worth the climb. The site had an existing maternity hospital in the old castle and of course this had to be closed, the town council thus neatly securing a new university and guaranteeing the building of a new £1 million maternity hospital.

The first vice-chancellor, Tom Cottrell, was appointed in 1965 and set out the main objectives: there were to be three main areas of study, arts or humanities, basic sciences and social sciences. From the start it was intended that there would be minimal boundaries between these, thus continuing the valuable traditional assets of a general education given within the Scottish schools system. In 1967, 1968 and 1969 the university was to take 130 to 200 students, in 1971 a further 603. In 1972 the intake was 640, the intention being to have a steady 3,000-5,000 students by 1975. Although there is room on the university site for further expansion, the present budget will not allow this for the time being.

Buildings

The design and building programme was the responsibility of the architects Robert Matthews, Johnson Marshall and Partners. In 1966, the development plan was published, and has, in its essentials, been followed.

The first building, 'Pathfoot', was rapidly erected and positioned so as not to inhibit future development; it served to get the academic programme off the ground. Temporary buildings were wisely avoided as they have to be upgraded or demolished later, or more likely are still in heavy use 30 years on. Pathfoot, a simple set of parallel blocks following the contours of the site and linked by crossing corridors down the slope, is one of the least obtrusive and most successful buildings; it has won several design awards (Fig. 1).

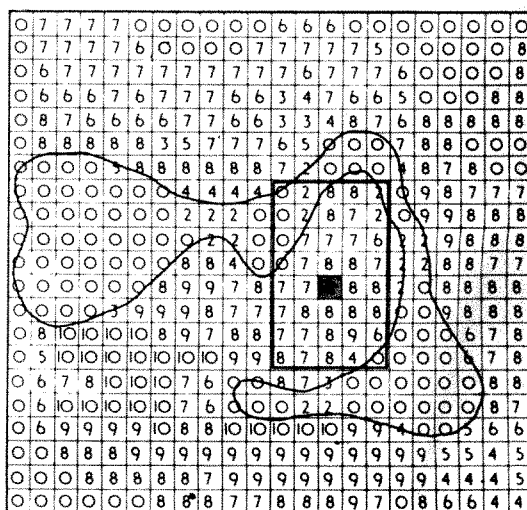


Fig. 4 Each cell has a value showing ease of building.

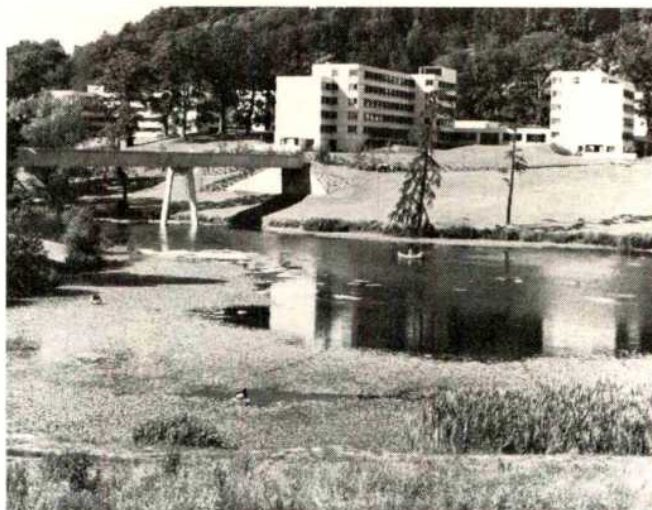
The architects took the artificial lake at the centre of the site as the natural focus. The main non-residential buildings to the south are the teaching, research, library and private study buildings. The object, as can be seen on development plans, is to extend southwards as student numbers grow. The level areas of the site are reserved for playing fields. The general proposed expansion can be seen in Fig. 2a, b and c. At the centre of the campus the MacRobert Centre has been built to provide an arts centre for both town and gown. The southernmost of the nonresidential buildings is the teaching block which is designed as a continuous form along the contours, expanding eastwards and southwards. A student may be involved in up to five departments within the broad based course structure and the housing of all teaching under the same roof makes mobility simple.

Although priority has always been given to the academic building programme, several coffee bars and club rooms have now been added or are under construction. The residences are built to the north of the lake and provide accommodation for two-thirds of the students. The UGC does not offer finance for residences and it is quite an achievement for the university to have raised the money by appeal and borrowing to build these blocks. The budget for the architect has obviously been very tight but the spartan interior has merit and is quite adequate. The rooms are arranged in flats or in halls of residence, the flats being generally the more popular though the halls give more seclusion for those wishing to work.

Computer study

The plan for Stirling University by Robert Matthews has made a great deal of the site. The consistency of the external cladding materials (some students say monotony) does not detract from the natural beauty of the setting. As a result of interest in university planning, a computer-aided study of the best development strategy for Stirling University was carried out at the University of Strathclyde. It was suggested that both the residential and teaching blocks should be south of the lake initially and the northern areas developed later. In addition, it was contended that with a compact development the site could accommodate a university of 12,000 students with all necessary facilities excepting some sports fields. The study attempted to develop a computer-aided design tool to assist in the laying out of large complexes of buildings. For illustrative purposes an area of the site was taken around the lake (Fig. 3).

To develop a layout plan or zoning for functions on this



The University of Stirling. With the lake as a centrepiece the site is an unusual one for a University.

site the strength of the relationships between different functions of the university was expressed on a scale from 1 to 10, a relationship of 10 being the essential need for immediate contact. Thus each department of the community, such as administration, arts, chemistry, was assigned a number describing how strongly it was connected with each of the others.

In addition, to allow for the difficulties of building on the site, a grid was drawn on the plan (Fig. 4) and in each square a number was assigned which reflected how 'buildable' that part of the site was. Plainly the lake has zeros whereas the areas to the south with gentle slopes and good foundation conditions are easily and cheaply built on and score highly.

In this way a numerical picture of what the site was like and how the university functions were interrelated could be presented to the computer. In principle, the function, faculty or department needing to have access to all others is placed on the site by choice of the designer and the others are located after this by a simple computer algorithm, to minimise the dislocation expressed in the need for proximity on the 1 to 10 scale. Rather than an actual building layout, the result is a series of zones on the plan indicating in which area a new building should be located from the point of view of easy communication and planning efficiency (Fig. 5). Various experimental computer routines were developed for three-dimensional zones and graphic outputs. The study, although simple, does provide another point of view and certainly the suggestion of a compact development, leaving larger areas of the site unspoilt, would have produced a completely different character on campus; whether it would have been better is a matter for argument.

Relationships

The University of Stirling, like any other new institution in a town, needs time to get to know its environment and to win over the town people. Its position on an isolated campus, without any substantial residence in the town, does not help. Certainly the MacRobert Theatre has been used by local dramatic societies and visiting theatre companies to the benefit of Stirling and the whole region but, as yet, there seems little use by the town of the university sports facilities; recently the university refused to go part way with the town on financing a joint swimming pool, preferring to build a small one of its own while the town built another.

It was hoped that the new employment brought by the university would encourage industry but this has not, as yet, occurred. Of course the university now employs over

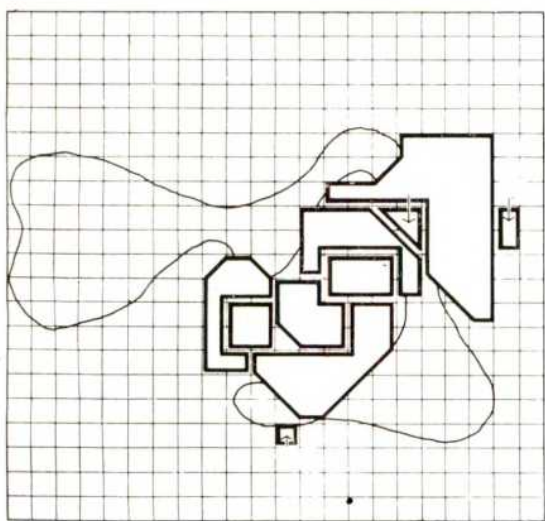
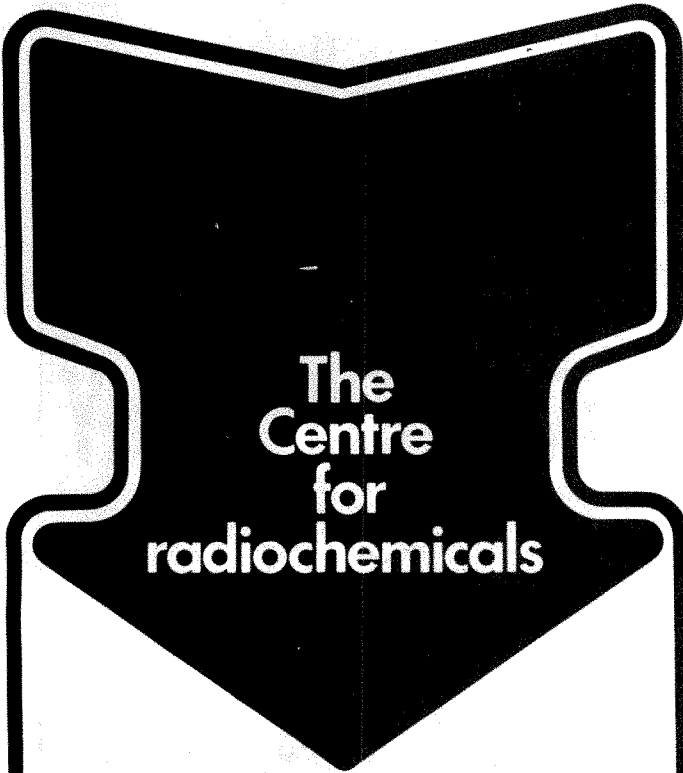


Fig. 5 The zones for a student population of 3,000.



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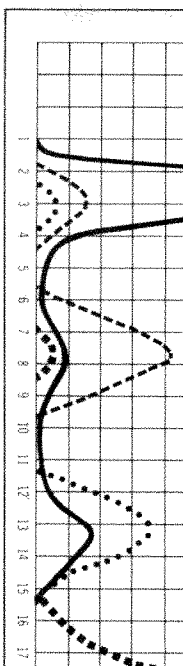
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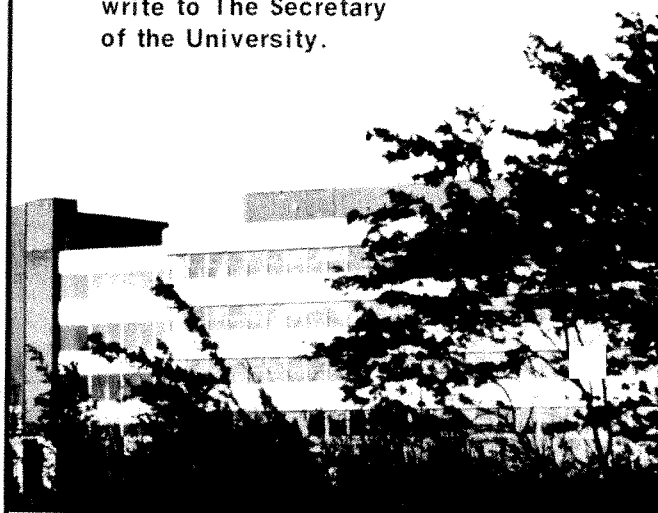
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1,000 people, which is in itself a boon; yet conversely, while more clerical and other jobs have been created so the number of people coming to Stirling expecting to find such work has increased and the availability of jobs for the local people does not seem to have markedly improved. Perhaps the most difficult point with a separate campus is the provision of all facilities on the spot—bookshops, banks and supermarkets. This is a great pity, for surely a university bookshop located in the town would provide an invaluable specialist service a small population cannot support. Perhaps if residences can be built in town in future these things may come about. A good example of how a university can generate new shops can be seen in the streets around the University of Glasgow.

The University of Stirling and the town should start thinking now about the effect of future growth of the campus; after all, a student population of 6,000 would give the campus a daytime population of about 1,000, which in a town of 28,000 will have a significant impact. The road programme and problems of general access may well have to be reassessed, as well as a monumental parking problem.

The university authorities, students and architects have done well to achieve a rapid build-up to the present almost complete campus. This has been done despite problems both logistic and academic. The courses offered are already diverse and include MSc and PhD courses in the pioneering aquatic pathology department, a unique educational degree that combines supervised experience with academic study allowing students to teach immediately on qualifying, and an Institute of Finance and Investment which offers one-year courses.

Stirling University, famous for its student outbursts in the past, now gives the impression of consciously trying to achieve a real community spirit while determined to develop a town-gown understanding. There is undoubtedly an underlying pride and mutual confidence developing between the town of Stirling and its university.

¹ Willoughby, T., Paterson, W., and Drummond, G., *Architects' Journal* (March 25, 1970).

² Matthews, R., *University of Stirling Development Plan 1960* (Johnson-Marshall and Partners, 1960).

³ *Architects' Journal* (June 20, 1973).

⁴ *University of Stirling Prospectus 1975-76*.

What makes a good science writer?

David Fishlock

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David Fishlock is Science Editor of the Financial Times.

SOME years ago a friend of mine mentioned to another research scientist that he intended to become a science writer. His colleague commented drily that it meant he would need to know ten times as much as he ever wrote on any aspect of science. That advice sometimes comes to mind when I am writing against a daily newspaper deadline in the late afternoon or early evening, aware that I am stretching my own knowledge of the subject to the limit, with no time to seek further advice. More often, however, I recall it when I am reading reports of other science writers who, whether for lack of knowledge or because their own prejudices have insulated them from the other side of the story, reveal all too plainly that their writing has outdistanced their knowledge.

Accuracy and indignation

The professional scientist's constant complaint against science writers is that they get it wrong; that they imbue with all the authority of Science some observation, opinion or conclusion which at best is open to argument and at worst is factually incorrect or misleading. It is a grievance I hear in many laboratories I visit, as well as in Whitehall and corporate headquarters. It is one of which I was made very firmly aware soon after I joined the *Financial Times* in 1967. A scientist who has since risen to become one of the government corps of scientific advisors told me bluntly that he didn't waste time reading newspaper reporting of science.

More recently the indifference this physicist then showed has changed into a genuine concern at the top of the profession for the damage that misunderstandings, inaccurate reporting and biased comment can do when published by newspapers under the authoritative sounding by-line "Our Science Correspondent". Examples range from an interruption, on wholly spurious grounds, to efforts to clear up

the oil leaking from the Torrey Canyon, to fears unnecessarily aroused by hypothetical representations of the 'hazards' of pesticides, herbicides, drugs, toiletries, nuclear reactors and nuclear by-products, supersonic airliners, and many other products of applied science. Reports that readers might reasonably expect to be written from a standpoint of some knowledge and expertise are all-too-often characterised largely by indignation and prejudice.

Indignation has tended to be the keynote of far too much science writing in the past few years. When, in the second half of the 1960s, it dawned on the public that science and technology did not have pat solutions to man's social ills and if exploited too greedily might even exacerbate them, many science writers joined enthusiastically in the assault on scientists' motives. They competed for space in some journals to the point where it was hard to distinguish their writing from that of a new breed of specialists which emerged at that time, called Environment Correspondents, created by many newspapers to provide 'gloom and doom' stories forecasting the imminence of mankind's destruction. For editors it was a fresh angle on the insatiable public thirst for bad news.

Also deeply infected by indignation at this time was the weekly journal *New Scientist*. Soon after it started in 1956 its founder-editor Percy Cudlipp, who had been one of the most eminent of Fleet Street editors, wrote that his journal was to be a

"link between science and industry. It would report technological innovations and promising lines of research. It would pay careful attention to the scientifically progressive sections of industry and would help, by describing their activities, to create a growing interest in scientific applications among industry as a whole."

In the early years its highly authoritative explanations of every facet of technology and science, written wherever possible by the foremost practitioners in a particular field, yet painstakingly edited—when necessary—for assimilation by the inexpert, were eagerly seized upon by other writers as well as by scientists anxious to keep abreast of progress

in science, if only to see that their interests were not being neglected in the goldrush.

Lest I leave the impression that early *New Scientist* editors hung on to every word, each prediction of the wise men of science and engineering, let me hasten to say that by the start of the 1960s the journal was making its own critical appraisals of projects and plans. Increasingly it was finding flaws in the proposals for harnessing science. In short it was attempting—sometimes with conspicuous success, as in the case of an ill-conceived scheme for large cross-Channel hovercraft-ferries¹—what later became dignified as the new discipline of 'technology assessment'.

Influence

The *New Scientist* has had an important influence on science writing in Britain, and probably elsewhere too. Although I do not think it has bridged the gap between the 'two cultures'—by this I mean that few people who are not professionally concerned with science trouble to read the journal—it has bridged many narrower gaps between the scientists themselves. It has also been invaluable to science correspondents on other journals, as the first stage of breaking down a complex piece of research or 'emerging opportunity' into a simple but lucid newspaper story.

Unfortunately, the influence of *New Scientist* began to wane sharply in the late 1960s. The downturn coincided with a change of publisher, from a small but dedicated company to a large and remote empire. It also coincided with two changes of editor, both of whom interpreted their role not as helping scientists to adapt to the changing attitudes of society towards science and technology, but as lending their weight to the attack. What characterised many of its editorial assaults at this time was not the cool, dispassionate and knowledgeable writing which distinguished so many of its early articles and leaders in the first decade of the journal's life, but the irrational, emotion-laden tirade of the writer who is composing for effect and not going to allow himself to be deflected or confused by facts.

A low threshold of indignation is the *sine qua non* for the successful investigative reporter, of the kind that exposed the Watergate debacle in the *Washington Post*, for example. But as the two Pulitzer prize-winning reporters in this case make so plain in their book *All the President's men*² they also exercise uncommon care in sifting and double-checking their facts and deductions before publication. (The rigorousness of this process may owe much to the fact that Howard Simons, their managing editor, who launched the paper's investigations into Watergate, was for many years its highly respected science correspondent.)

Too many science correspondents in the last few years have assumed that a low threshold in indignation and an input of information from someone, somewhere in science, however obscure, was enough to add up to a story. Too many have been ready to select facts and sources regardless of their intrinsic merit and to overlook a much greater weight of contrary evidence. Too many, in short, have flouted the basic principles of the profession they are being paid to write about, in quest of a story to meet the supposed public taste for gloom and doom.

A science writer who accepted without question claims for perpetual motion would be laughed out of court. But no less improbable assertions are often implied about the explodability of nuclear reactors, the hazards from radioactivity or the damage left in the wake of supersonic transports. The referee system of exposing a man's observations and conclusions to the scrutiny of his peers, who then approve or reject them for publication in an accredited journal, is the science writer's best safeguard against the dubious claim. It may not be perfect but it is much healthier than the activities of those scientists who have tried—sometimes with devastating success—to bypass the

journals and appeal directly to a general public in no way equipped to assess the merits of their claims.

Wit and grace

Scientists, understandably, have been at a loss to know how to respond to assaults from science writers. As that formidable United States physicist, the late Dr Edward Condon, once said ruefully, when assailed by a Congressman for allegedly being a security risk, "If you say I've got a wart on my nose, I can deny it. But if you just say I'm one of the ugliest men in town, all I can do is argue that I'm really quite pretty"³. Aggrieved scientists and technologists, attacked for the 'ugliness' of their discoveries, projects and propositions, sometimes turn for help to a science writer who may lend a more sympathetic ear. It is unfortunate but the impact of a dispassionate appraisal can rarely counterbalance the damage done by the preceding highly dramatised story. One instance where the counter-attack was very successful, however, came after lurid newspaper accounts of the mercury found in fish landed in Britain, when the science correspondent of *The Observer* reported that fish from the Smithsonian Museum, caught in the 1920s, had been shown to contain at least as much mercury when assayed by the same method.

The one real source of redress a scientist has who feels the facts have been ignored or distorted—and I can assure readers that it can be a very effective form of redress—is a brief letter to the Editor. Few newspaper editors will ignore a letter from, say, a professor or research director who says bluntly "your science correspondent was wrong". Fewer still will decline to publish it prominently if the letter puts the facts straight with some leavening of wit or grace.

If I have a general literary complaint about science writing and the reporting of science, technology and medicine, it is that so much of it is so humourless, so lacking in wit and charm. Nowhere is this more evident than in the stories that reek of indignation. Yet science itself is not at all like that. Names that come easily to mind of scientists who can be very witty about the failings and faults of their own profession include Victor Rothschild (as in his 1971 Royal Society of Arts lecture on the need for reorganisation of government science in Britain), Kenneth Mellanby and Magnus Pyke. Most recently, in the pages of *Nature*, we have the splendid example of Erwin Chargaff with a delightfully ironic essay, criticising the validity of much present-day biology and the honesty of such euphemistic evasions as "the results that I reported last year are based on facts that are no longer available"⁴.

Far too often the facts science writers reported last year are 'no longer available', either. Sometimes, inevitably, this is unavoidable, and no fault of the writer, who is simply reporting results, conclusions or claims, formally or publicly stated, that are later overtaken or invalidated by more research or other events. Sometimes it is the result of injudicious selection of 'facts', as was the case in a protracted (but quite unsuccessful) assault on a well known domestic pesticide by a journalist who, when I challenged his motives, simply said "Well, what's wrong with flies?" Sometimes, unfortunately, it is the result of misunderstanding, misinterpretation, or simply miscalculation—as when a prominent story in *The Guardian* of alleged pollution of the English Channel turned out to be based on calculations assuming that the channel had but two dimensions⁵.

Lessons

Seven years of reporting and appraising science, technology and medicine for the *Financial Times* has taught me some harsh lessons about the scope and limitations of newspaper reporting of science, and the pitfalls of taking too freely the word of its practitioners. A background which

includes experience of industrial science and of reporting industrial practice has encouraged, I think, more sympathy for the formidable difficulties of translating the discoveries of science into practice, often dismissed impatiently by science writers as mere parsimony or 'engineering detail'.

But it has also taught that the scope is much greater than the limitations; that given editors with a genuine interest and curiosity about science—its discoveries and opportunities and the people who make them and who take the decisions—the scope is immense. The *Financial Times* enjoys such editors, and has done so certainly since the post-war years when the late Professor Sir Francis Simon, head of the Clarendon Laboratory, Oxford, was also its science correspondent. It is a paper of industry and commerce, specialising in facts and fair interpretation and not dependent for sales on polemics, propaganda or unabashed entertainment.

As Science Editor I am one of about a score of specialised feature writers, mainly writing regular articles of up to 2,000 words—much longer than most newspapers normally accept—sometimes at the Editor's instigation but most of the time as a result of my own quarrying among the papers or people of science. In the past twelve months, for example, it has published over 70 feature-length articles under the by-line of its Science Editor, and many more on such technologies as aerospace and computers by other staff feature writers.

What I cannot claim is that those articles represent a complete spread of science and technology. The reader will find little on astronomy, genetics or sex-change surgery, for example. But he will find a more complete (and I would venture more accurate) coverage of the science, technology and politics of nuclear energy than any other newspaper has carried. He will also find many articles discussing the organisation, management and funding of science, rare indeed outside the pages of specialised journals on research management.

No two national newspapers in Britain produce a similar science coverage. You can take your pick from a wide variety of interests and levels of treatment, of preferences and prejudices. At its best the reporting of science compares favourably with that of such countries as the United States, West Germany or Sweden.

It has recently been proposed quite seriously to me that science correspondents should exercise as much responsibility as the referees of scientific papers. In principle, I agree and would like to see the suggestion developed more fully; but in practice I cannot imagine how such standards could be enforced, when science correspondents must work constantly under constraints called deadlines that no referee would tolerate.

What I think scientists are entitled to ask of those who write regularly about them is that they will exercise the basic tenets of the scientific approach; that they will reject illogical argument, that they will reject stories where the facts do not fit, that they will not suppress facts that conflict with their arguments. Newspapers nowadays employ very bright people as journalists, many of them university graduates. Most of them are perfectly capable of handling in an interesting and accurate way most of the science stories that come to light. If the science correspondent has any claim at all to distinction as a specialist writer it is surely that he is versed in the scientific method—the way scientists approach a situation—and thus better equipped to spot the fallacies for his readers.

¹ Fishlock, D., *New Scientist*, 590–591 (March 5, 1964).

² Bernstein, C., and Woodward, B., *All the President's men* (Simon and Schuster, New York, 1974).

³ Branscomb, L. M., *Physics Today*, 27, 68 (June, 1974).

⁴ Chargaff, E., *Nature*, 248, 776 (1974).

⁵ Lord Zuckerman, *Times Literary Supplement*, 1419–1422 (November 12, 1971).

The transit of Venus in 1874

A. J. Meadows

Department of Astronomy and History of Science, University of Leicester, Leicester LE1 7RH, UK

Observations of the 1874 transit of Venus seem to have been based on a misguided belief that nineteenth century techniques were superior to those of the previous century. Here A. J. Meadows explains, with the benefit of hindsight, how the expectations of the nineteenth century astronomers were not fulfilled.

THE average distance from the Earth to the Sun is a fundamental astronomical constant: it forms the basis of the scale of distance used both outside and within the Solar System. Outside the Solar System, the Earth–Sun distance acts as the base line for the measurement of the distances of nearby stars, and so, ultimately, of the entire Universe. Within the Solar System, any single estimate of distance can be used to calibrate all remaining distances by using Kepler's third law. For example, if we know the Earth–Sun distance we can work out the Venus–Sun distance:

equally, however, we can derive the Earth–Sun distance from a knowledge of the Earth–Venus distance at any instant.

The classical method for the determination of distance in astronomy has always been by the use of parallax. This requires three things: a relatively nearby object, a distant background, and two observers at either end of as long a base line as possible. When the observers measure the position of the object relative to the background, it does not seem to be in exactly the same place for both. The difference—the parallactic shift—decreases with the distance of the object; so the parallax provides a direct measure of the object's distance. The current value of the solar parallax is 8.794", although in fact, it is known to much better accuracy. Thus, two observers situated at the ends of an equatorial radius of the Earth would see the Sun displaced against the background stars by that amount. Clearly, the direct detection from Earth of so small a displacement of the Sun is virtually impossible. The prob-

lem of determining the solar parallax has, therefore, been one of finding a closer and easier object to measure than the Sun.

Observing Venus to determine parallax

Of all the planets, Venus comes closest to the Earth: its minimum distance from the Earth at conjunction is, on average, only half that of Mars. Unfortunately, the value of parallax measurements for Venus is diminished by its juxtaposition to the Sun when at conjunction, which makes accurate visual observation extremely difficult. The way out of this impasse was suggested by Edmond Halley in 1716. Venus usually passes above, or below, the Sun as seen from the Earth; but on rare occasions, because of the mutual inclination of the orbits of both planets, they are so placed that observers on Earth actually see Venus transiting the solar disc. Under these circumstances, the brightness of the Sun becomes an advantage, rather than a disadvantage, for Venus appears as an obvious and easily measurable, black dot on the Sun's surface. Halley proposed that, during a transit, Venus should be observed from several widely spaced stations on Earth. Observers at points distant from each other would see the planet describe slightly different paths across the solar disc. Therefore, the duration of a transit would depend on the observer's position. Measurements of the times of the beginning and end of the transit could therefore be converted into a value for the parallax of Venus at conjunction and, thus, into a figure for the solar parallax.

At the time the main problem with Halley's proposals was the infrequent occurrence of transits of Venus. At present, the transits occur in pairs separated by a gap of 8 years, but with a delay of over a century between successive pairs. In Halley's time, the next pair were not due until 1761 and 1769: but, to eighteenth-century astronomers, the wait seemed worthwhile, for the method apparently provided much greater accuracy than any other then available. (From his observations of a transit of Mercury in 1677, Halley estimated that a transit of Venus should provide a value for the solar parallax which would be accurate to one part in six hundred—far better than other eighteenth century methods.)

Great preparations were made for the transit in 1761, with expeditions dispatched to distant parts of the world'. But the results were a major disappointment: the extreme values obtained for the solar parallax differed by as much as 20%. It was generally agreed that the main inaccuracy had been introduced by an unexpected 'black drop' effect

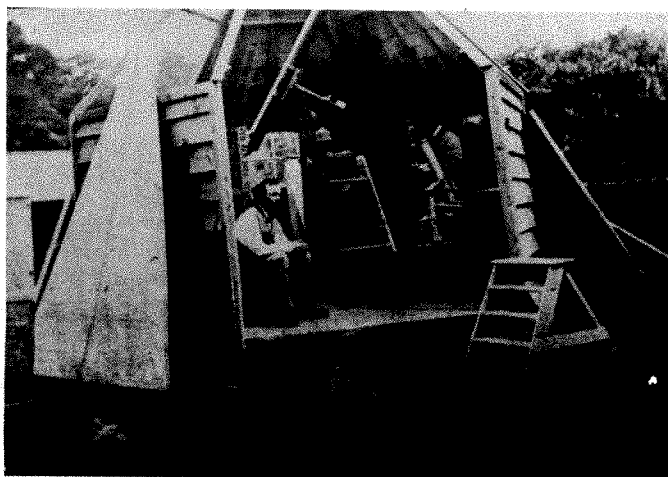


Fig. 1 One of the huts erected at Greenwich for testing prior to observations of the transit of Venus in 1874.



Fig. 2 Observers waiting for the transit to begin.

as Venus encroached on to the solar disk. (As the limbs of Venus and the Sun approached they seemed to flow together—an effect that made accurate timing of the beginning and end of the transit very difficult.) Forewarned, the observers at the following transit, in 1769, obtained rather better agreement; but their results were not properly reduced until the 1820s, when the work was undertaken by the German astronomer, Encke. The final figure he obtained for the solar parallax was $8.571''$, a value that was widely accepted for a quarter of a century. By the latter part of the 1850s, however, it became evident that this made the Earth too far away from the Sun. Solar gravitation acts differentially on the Earth-Moon system because the Moon is sometimes closer to the Sun than is the Earth, whereas at other times it is more distant. As a result, the motion of the Moon is dependent on a factor that involves the mean distance of the Sun. By the mid 1850s lunar theory had become sufficiently refined to show that Encke's result could not satisfy the observational data on lunar motion. It therefore became essential to re-determine the solar parallax.

A different approach

In 1857, G. B. Airy, the Astronomer Royal, proposed that arrangements should be made for detailed observations of the next transit of Venus, due on December 9, 1874, to obtain a better value for the solar parallax. Airy was confident that the technical advances since the eighteenth century would permit a much more accurate result to be obtained. His colleagues agreed with him, and he was put in charge of a major British campaign (to be linked ultimately with similar campaigns mounted by a number of other countries).

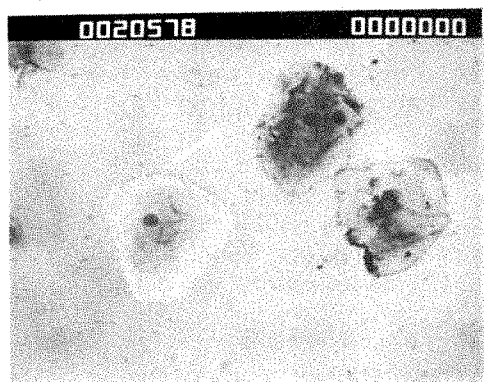
One of Airy's first decisions was to abandon the use of Halley's method of observation, and to substitute instead one proposed by the eighteenth century French astronomer, Delisle. Halley's method, which involved timing both the beginning and the end of the transit, required that the entire transit should be visible from each observing station. This meant that any change in weather during the course of the transit could vitiate the result. More importantly, it meant that large areas of the Earth's surface, from which only the ingress or the egress could be seen, were ruled out as places for observation. Delisle's method, on the other hand, only required the timing of one contact—either ingress, or egress. To compensate for this, it required—in contrast to Halley's method—a very precise determination of the longitude of the observing station. Which approach was preferable depended on the particular circumstances of the transit; Airy's analysis of the 1874 transit suggested that Delisle's method would be more suitable. He therefore began to search for a series of

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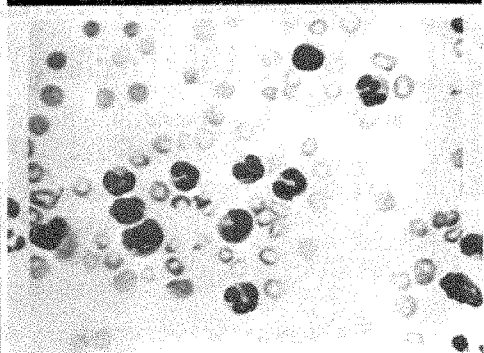
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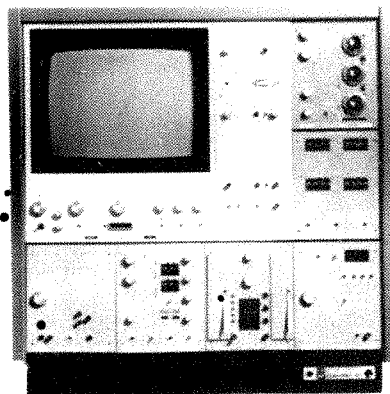
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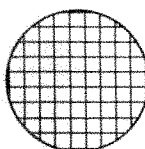
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widely spaced stations from which that type of observation of the transit could be made. As the zone of visibility for the 1874 transit was centred on the Pacific, suitable sites necessarily tended to be situated on islands. That forced Airy into extensive consultations with the Hydrographer for the Navy, Admiral Richards; for the charting of Pacific islands still left something to be desired at that time.

Airy outlined his proposals for observations of the transit at a meeting of the Royal Astronomical Society in 1868. In the following year, another prominent member of the Society, R. A. Proctor, attacked Airy's conclusions, claiming, in particular, that Halley's method might well be applied to the 1874 transit, and proposing that a somewhat different set of stations should be selected with that possibility in mind. Airy took no notice, but pushed ahead with his own plans, and during 1869 he obtained a sum of £10,500 from the Treasury to finance them. In 1873, however, Proctor (who had meanwhile become Secretary of the Royal Astronomical Society) once again took up the argument. This time it was pursued before the general public, especially through the columns of *The Times*. At first, Airy resisted any change in the arrangements, but eventually he agreed that additional stations should be sought. This, if anything, only served to enhance the dispute, for Proctor and his friends now entered on a debate with Admiral Richards to discuss which new stations might be occupied. Richards, in exasperation, finally observed²:

"Enthusiasts no doubt there are who, however accomplished they may be as astronomers, are wanting and cannot but be deficient on many subjects which it is as necessary to take into account as astronomy in a question of this kind; and hence we are told to send to the Antarctic Continent and to visit a variety of small rocky islets interspersed over the Southern Ocean at distances from each other varying from 1,000 to nearly 4,000 miles, many of which are actual myths, while on those which do exist it is certain that there is no anchorage for a ship, and that even landing would be generally impossible."

Proctor was not prepared to let Richards have the last word. In a subsequent supplementary issue of the

*Monthly Notices of the Royal Astronomical Society*³ he produced a chart of possible observing stations, together with the annotation:

"The chart requires no explanation beyond perhaps the remark that the islands in the less-known regions have been taken from ordinary atlases (after comparison of several), in preference to the Admiralty charts; because . . . one naturally feels doubtful about Admiralty statements which would appear to be variable according to official requirements. It did not seem well to insert any island, or group of islands, in the chart with some such note as 'Here, if convenient to those in authority, there is an island,' or 'this group of islands can be regarded as a reality or a myth as may be required,' and so on."

There, however, he had overstepped the mark; for, as Editor of the *Journal* at that time, he published without reference to other members of the Society. When Richards complained to the RAS Council, the result was virtually inevitable: by the end of 1873, Proctor had resigned as Secretary, and the controversy over the transit died down.

Airy had begun preparations for the transit on the assumption that the actual times of contacts would be determined visually in much the same manner as in the eighteenth century, although with better instrumentation. It was soon pressed on him, however, that the rapidly developing art of astronomical photography should also be used along with the visual method. Initially Airy was dubious, but was eventually persuaded, more especially because De la Rue, the leading British exponent of astronomical photography, agreed to take charge of preparing the photographic instrumentation. Airy therefore obtained a further £5,000 from the Treasury for this type of work, and British expeditions observing the transit carried out a mixture of visual and photographic measurements.

The raw data from the expeditions was collected together at the Royal Observatory, Greenwich, and Captain Tupman, an army officer, was put in general charge of the reductions. The work dragged on slowly until, finally, Parliamentary pressure began to build up for immediate publication: the expenditure on the 1874 transit made it, after all, one of the most expensive single scientific investigations supported by the state up to that time. When, partly as a result of the pressure, preliminary conclusions were published in 1877, the difficulty facing Tupman and Airy became apparent. The new measures of the solar parallax were no more accordant than the eighteenth century measures had been. Airy, himself, derived a final value for the solar parallax of 876"; but this represented an average from values for ingress and egress that differed by 0.11". Moreover, Stone, HM Astronomer at the Cape, obtained, from the same data a value of 8.88". The photographic results were even worse. From these, Tupman obtained a solar parallax of only 8.08"; and an independent re-examination of the material only improved this to 8.17". Part of the problem was that the type of photographic instrumentation used by most British parties was not particularly suitable for transit measurements. But even American measurements, which were better arranged, did not achieve the expected results⁴. Indeed, the deficiencies apparent in the British observations, both visual and photographic, were to varying degrees, found in the results obtained by all the participating countries.

Looking back, the observations of the 1874 transit seem to have stemmed from a misplaced belief in the superiority of nineteenth century methods and instrumentation over those of the eighteenth century. Certainly, the 1874 transit, together with the much less enthusiastically observed transit of 1882, finally delivered the quietus to this form of solar parallax measurement. Nobody will consider it worth observing the next transit of Venus, in 2004, for this purpose. Yet the idea that the solar parallax can best be

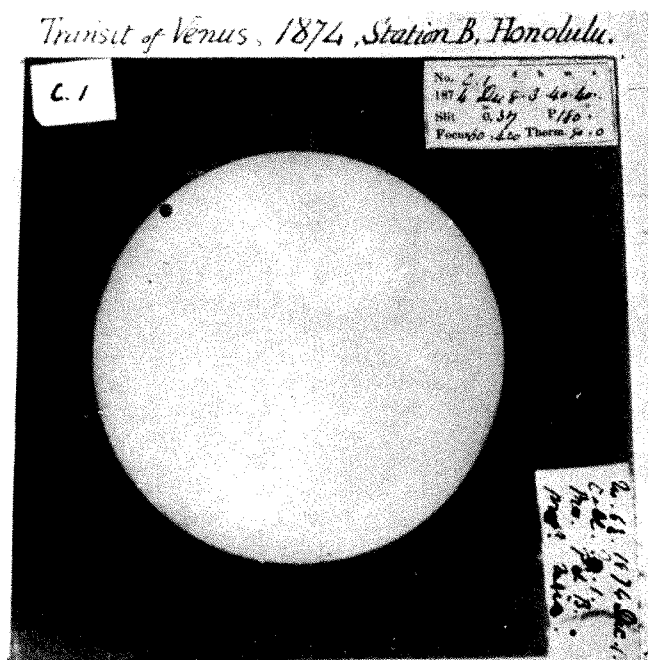


Fig. 3 Photoheliograph observation of Venus near the solar limb.

determined by observation of Venus at inferior conjunction remains valid. The present standard method involves radar observations of Venus near this point in its orbit.

All the photographs used in this article are from the Royal Observatory archives, and have been made available by Lt.-Cdr. H. D. Howse (National Maritime Museum).

¹ Woolf, H., *The Transits of Venus* (Princeton University Press, Princeton, 1959).

² *History of the Royal Astronomical Society*, 183 (Royal Astronomical Society, London, 1923).

³ *Ibid.*

⁴ Newcomb, S., *Popular Astronomy*, 2nd ed., 183–192 (Macmillan, London, 1883).

'Popularisation' of science

Maurice Goldsmith

Science Policy Foundation, Benjamin Franklin House, 36 Craven Street, London WC2N 5NG, UK

With the increasing dependence of the civilised world on science, the need is not so much for 'popularisation' as for understanding and critical appreciation of scientific advances if alienation from science is to be avoided.

ASTRAY one evening recently in an unfrequented cupboard at home, I came across a copy of that pioneer publication *Penguin Science News*: to be exact, issue number four, dated July 1947. And as I turned the pages to renew with delight this old acquaintance, the editor once again spoke to me—but with words of such innocence that I was obliged to shut him up. From this surprise reunion I came away with an embarrassed feeling of sophisticated guilt, not so much in disturbing the innocent but in realising that his simple views were still held so widely. Although techniques of communication of scientific ideas to the public have developed greatly in these past three decades the philosophy of the communicators has not.

In an editorial the editor claimed that "every scientific subject, without exception, can be made clear to the layman." He added, "All the ideas of science are basically simple and understandable by the average brain," and went on, "Provided an author really understands his subject himself and has enough space to write his expanded discussion in simple words, there is nothing under the sun which he cannot clarify and teach."

In those pioneer days of science writing, I too, had such innocent views. We did not understand then, and I am not sure that many of us have grasped even today, that communication means not only to send out messages but also to receive messages from the world about us. We were concerned then only with the communicator. We assumed that if he wrote simply and well—"Goldsmith," one famous editor said to me, "you must learn to write more compellingly, otherwise your readers will ignore you—and so shall I"—we would be read and understood. O, blessed paradisaical innocence! The final awakening came for me about 1950. I was puzzled because since 1945 we had exuded millions upon millions of words, written and spoken, on the nature of the atom, and I found that most people remained unhappily ignorant on this subject.

We were most successful in scaring them, however, so that fear and prejudice are impeding progress in, for example, the development of nuclear power plants. It is not sufficient to write clearly and well. Literate writing helps but it carries in itself no guarantee that understanding or even reading will follow. Although, I agree it helps.

This is in great part due to the primitive view of the popularisation of science which inspires so much of our thinking. It is as constricting to us as was the veneration of the circle to Galileo. He was neutral about Kepler's thesis that the planets moved in elliptical orbits because he

could not accept as significant any speculation which neglected the axiom that the circle is the perfect curved form. We are in bondage to a concept that I believe has been an important factor in alienating people from science. We have tended to use the term "popularisation of science" loosely to describe what we hope for rather than what actually happens. This is more than an exercise in semantics, for what we are concerned with is the definition of the social phenomenon which has blinded us into believing that we were enlightening the masses when the opposite was more true.

I believe it would be generally accepted that the popularisation of science is designed to make science popular (by which I mean intelligible) among non-scientists. The argument is that science is difficult to understand and that it requires to be translated into terms simple enough for the non-scientist to understand. This view has been expressed almost from the beginning of science itself.

But a certain knowledge of science spread among the public long before that. There have always been scholars and non-scholars, therefore always the need to interpret. We are aware that there was a weakness for the encyclopedic in the literature of the Middle Ages. The general cosmological system of Dante's *Divine Comedy* was based on Aristotelian, Ptolemaic and Christian ideas. The most widely read of mediaeval romances, the *Roman de la Rose* of 1270, contained statements on topics such as philosophy, medicine, physics, astronomy and theology.

In this way, general ideas about nature of the universe, the planetary system and the nature of matter were put into circulation.

The word 'science' began to enter the English language during the Middle Ages (the beginning of the 17th century.) It came from France, and was synonymous with knowledge. But the early Latin translators of Aristotle gave the adjective "scientificus" a technical meaning, and this was transferred to science to mean accurate and systematised knowledge. The Aristotelian theory of knowledge was the guide: you had "scientific knowledge" when you arrived at it demonstratively, not by experiment.

But with the beginning of modern science—first with the Copernican revolution in the mid-16th century, and then with the great age of science inspired by Galileo and Newton in the 17th century—the expression "scientific knowledge" came into use to distinguish this form of knowledge from common knowledge: that is, science and knowledge were coming to be regarded in England as no longer synonymous. Science came to stand for a particular kind of knowledge, whether derived by straight deductive logic (as in Euclid), or whether using observation and experiment (as in Bacon and Harvey.) But the full realisation of this did not come until almost the mid-19th century, as expressed, for example, in Herschel's *Discourse on the Study*

of *Natural Philosophy* in 1830.

In the 17th century, with the emergence of modern science, the new systems of the world began to be part of the education of the well-read person, and the new ideas were made available to the aristocracy and the middle class.

It was at this period that what has been described as "the first systematic written attempts at scientific popularisation" appeared. The first of the great popular expositors was Bernier le Bovier de Fontenelle (1657–1757). He must have been a delightful person. Voltaire considered him the most universal genius he had met. He was a rare person in his combination of scientific knowledge and love of literature. He was a nephew of the great Corneille, and a contributor to the *Mercure Galant*, a paper edited by another uncle, Thomas Corneille. He was appointed secretary of the Académie des Sciences in 1699, and like Oldenburg, the secretary of the Royal Society in London, he had wide contacts bringing him information on the new developments in science throughout Europe.

Fontenelle wrote particularly for the public of the "salons". His *Entretiens sur la pluralité des mondes* (1686) is in the form of a dialogue and is addressed to the needs of an imaginary marquise, "jeune, aimable et ignorante". His intention, he declared, "is to deal with philosophy (he means principally astronomy and physics) in the least philosophical manner possible; I have tried to develop it to a point where it shall be neither too dry for the gentry nor too superficial for the scientists . . ."

For Fontenelle, popularisation was a class matter. The plebs had no place in his dissemination. His works and those of the writers who followed him, such as the *Spectacle de la Nature* (1732) by the Abbé Pluche, were primarily for the aristocracy, the wealthy bourgeoisie, and the ladies of the Court. The story is told of Fontenelle that one day at the Café Procope, the famous intellectual centre of Paris in the 17th century, he declared: "If my hand were cram full of knowledge, I wouldn't open it for the people". And on the sixth evening of his *Entretiens* he advises the marquise: "Let us content ourselves with being a select little band and not disclose our mysteries to the people".

In fact, the popularising of science to a general public could not come about until public forms of education had made literacy more general, until the media of dissemination were more widespread, and until there was a demand. People needed to realise that there was something to know which they were missing.

The *Entretiens* may have been written for a need already expressed: for example, Molière's *Femmes Savantes* was 15 years before the *Entretiens*, and his middle-class women spent evenings in their attics trying with a telescope to see the people on the moon. I do not regard Fontenelle as a populariser of science. He was a gifted propagandist for scientific ideas.

If Fontenelle was not a populariser, then who was? For an answer we need to go to the end of the 19th century when science was becoming established as a full time occupation for professionals.

During the early 19th century there were efforts—following the impetus of the industrial revolution—to make science available to such as mechanics, but the movement led by Henry Brougham, founder of the Society for the Dissemination of Useful Knowledge in 1826, to give working men a scientific education was doomed to failure; scientific knowledge could not be secured by superimposing instruction in the sciences upon a highly inadequate system of State education.

Similarly, the Royal Institution founded in 1799 to train mechanics, within a few years became a centre of scientific "entertainment" for a select few. Thomas Webster, who was Clerk of the Works in the early days of the Royal Institution, wrote to Count Rumford in 1799 with a proposal to found a school for mechanics in the house of the

Royal Institution. Rumford was delighted with the scheme and it was accepted by him—and by the Managers after some hesitations. But, by 1802, Webster had been granted sick leave and the project was abandoned. Why? I shall quote Webster's own words, but we must bear in mind that these were written in 1837, 35 years after the events, and Webster was by then an old and rather frustrated man. He wrote: "But this project for improving mechanics, well intended as it was, which promised to be so useful, and which had already gained for the Institution 'golden opinions' was doomed to be crushed by the timidity (for I shall forbear to speak more harshly) of a few. I was asked rudely (by an individual I shall not name) what I meant by instructing the lower classes in science. I was told likewise it was resolved upon, that the plan must be dropped as quietly as possible. It was thought to have a dangerous political tendency. I was told that if I persisted I would become a marked man."

The popular presentation of science was still designed for a few and not for the masses. The widely popular lectures of men like Huxley and Tyndall were of significance only to those who could read and write, and thus had little effect on the working masses.

Until recently the popularisation of science has been a class phenomenon designed for a privileged minority. Today we must rid ourselves of this concept of "popularisation". It smacks of the condescending, of Victorian do-goodism, of a patronising aloofness irrelevant to today's needs. We do not need to make science popular in a world in which science is of key importance. A single experience of space technology is more educative in this regard than millions of words. Besides, we cannot make science popular in the sense that Huxley and Tyndall wished to. The atom bomb has put an end to that. The catastrophic power of modern science confronts each one of us with profound moral issues. Further, the specialist fragmentation of science and its speed of change renders invalid the view that scientific ideas can be made clear through popular presentation. It was surely easier to popularise science a century ago when physics was largely a study of pulleys, levers and electric circuits; when chemistry consisted of reactions and formulae; and when biology was concerned with classifying different genera and species of plants and animals.

The trouble with most science writing—and in this I include all the mass media—is that we seek still to present science as a collection of facts. The mathematician, Professor Hyman Levy, commented: "The trouble about science and scientific explanation is that it tends to be incomprehensible to anyone except the expert". We must abjure the concept of popularisation in a democratic and rapidly changing society.

What we need then is more public understanding of science, and more public appreciation of the impact of science. This can only be achieved through a continuing education programme. The *raison d'être* of such a programme should be to help us to escape from the shackles of the scientific past; how to deal with new situations and how to find new facts; how to assess facts critically; and how to be one's own 'science critic' as well as football, or jazz, or music critic.

As we become more dependent on science, we become more vulnerable as the thing that has not been, and whose consequence cannot be foreseen, becomes the thing that is. Speedy and planned adaptation is already our need. It will become more, and not less, urgent. The social scientist has a key part to play here. I suggest there may be a law of social vulnerability, that is, a particular stage beyond which people begin to demand action at what seem to them to be "the harmful consequences of the application of science", for example, the social problems of air pollution, land conservation, use of pesticides, and urban development. If such a law can be found, then we shall be able to prevent

much misery, by securing corrective action before consequences become so gross that great quantities of antibodies—pressure groups of various kinds—are generated by the body social.

The populariser of science, as he functions today, cannot disseminate the subtle ideas of science. These really cannot be understood without hard, disciplined effort. It may be said that my reaction against former naive ideas about popularisation has gone too far. Of course, I am not against popularisation. I want a redefinition, a re-examination of the philosophy of popularisation. I believe popularisation of science must give way to wide public understanding and appreciation of science.

Who should be responsible for the necessary programme of activity? Clearly, it should be in considerable part a government responsibility. Just as the nation ploughs back a certain percentage of the gross national product to do scientific research, so it should invest a certain percentage of the research and development budget in public understanding of science, to help society to contend with some of the social problems that the applications of research and development cause.

What I have said are subjective impressions, generalisations for which I apologise. We need desperately in this country a research programme into the communication of scientific ideas to the public. Many millions of pounds are being spent annually for this purpose through press, radio, television, films, books, exhibitions, and museums. I have guesstimated that this sum is at least £20 million a year. Our ignorance about the impact of these media is complete. We know damn all. We have no objective criteria for assessing the social usefulness or effectiveness of these

media. We have only box-office and subjective generalisations. These are just not good enough.

I am happy that Glaxo is awarding, in collaboration with the Association of British Science Writers, four annual prizes of £500 each for popular science writing. I had hoped that from its inception there would be built into it a research programme. I say this because the American Westinghouse Awards for Science Writing, which have been running since before the Second World War, are useless in this respect. Their records are incomplete, and no one can say whether the Awards do more than put dollars into the pocket of some science writers each year—and provide publicity for Westinghouse. I would like to know how science writing and the communication of scientific ideas have benefitted. I apply the same criticism to the annual Kalinga prize for science writing administered by UNESCO—incidentally, an award I was proud to help into being when I worked at UNESCO.

The communication of scientific ideas needs a new approach. We have somehow to cope with a situation in which although politicians, and the citizens who elect them, continue to think in idea-proof compartments, the beliefs, values and institutions which nourish them are subject to rapid change. We need a new type of communicator, a modern Fontenelle, a poet and a visionary, a scientist and a visionary. He can help us to meet the flight from reason originating from the false belief—one that the popular communicators have failed to counter—of the threat of a blind, self-motivating, all-possessing, juggernaut of science and technology. I expect that then the alienation of people from science—the confusion in a “horror of lost self” as Robert Lowell sees it—will cease.

Practising science in Northern Ireland

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The practice of science in Northern Ireland is no harder than elsewhere but unwillingness to come to Northern Ireland because of exaggerated fears of the risks causes concern.

WHEN I was invited to write this article I accepted without much hesitation, but my experience has taught me that the provision of plain answers is not enough. Some of these may leave the erroneous and distasteful impression that scientists are indifferent to suffering. The reason seems to be that in spite of, or perhaps because of, the exceptional intensity of news coverage which Northern Ireland has received there is a general lack of any real sense of proportion about the violence and about how it affects work. To reduce the likelihood of being misunderstood in this way, I shall first present data enabling the scale of the violence in Northern Ireland to be viewed other than in isolation.

Comparing violence

Death is only one of the consequences of violence but it is both the most terrible and the most clearly defined. For comparison, the annual violent death rate will therefore be taken as a relevant and convenient (though admittedly oversimplified) index of the distress caused. Following standard practice with mortality statistics it will be expressed as the rate per hundred thousand inhabitants.

Table 1 gives data on death rates due to the troubles¹. In compiling it no attempt was made to distinguish guerilla deaths from civilian deaths proper but the former are officially estimated to number about one third of the latter. The locally recruited security forces (Ulster Defence Regiment, Royal Ulster Constabulary and Royal Ulster Constabulary Reserve) are grouped together as they are an integral part of the community. The method of presenting the rates obscures the danger to which members of these forces and members of the Army are exposed but nevertheless is appropriate as the total of the rates R_{NI} shown in the last row provides some indication of the impact of the violence on the people of Northern Ireland. Scrutiny of the table, with the knowledge that the total mortality rate in 1969 was 1,080.2 (ref. 2), should be reassuring to a rational man but I own it does not prevent me from fretting if a

Table 1 Death rates (per hundred thousand inhabitants) arising from disturbances in Northern Ireland¹

Year	1969	1970	1971	1972	1973	1974*
Civilian	0.8	1.5	7.5	20.9	11.0	11.0
Local security forces	0.1	0.1	1.0	2.7	1.4	1.8
Army	0.0	0.0	2.8	6.7	3.7	1.5
Total R_{NI}	0.9	1.6	11.3	30.3	16.1	14.3

The more recent figures were supplied by the RUC Press and Information Officer.

* Entries in this column are based on first 6 months of year.

member of my family is late in arriving home when bombers have been active.

An obvious comparison to make is with the civilian death rates from air raids on the UK during the Second World War. These rates, R_{AR} , are given in table 2. Their mean value 21.7 is less than twice the mean value 12.4 of R_{NI} which will be designated \bar{R}_{NI} , but this is quite misleading because the best known descriptions of the effects of the air-raids refer to the Greater London area where approximately half the deaths occurred and where the death rates were extremely high. A clearer appreciation of the position can be gained by excluding the Greater London area from consideration. The mean value of R_{AR} for the rest of the United Kingdom and \bar{R}_{NI} are about equal. Destruction by a common enemy is of course easier to bear than destruction coming from within and arousing communal animosities.

Table 2 Civilian death rates, R_{AR} , (per hundred thousand) arising from air-raids on UK^a

1940	1941	1942	1943	1944	1945
50.0	44.4	7.0	5.4	19.3	4.2

In the United States the murder (including non-negligent manslaughter) rate, R_M , is correlated with the population of the town or city⁴. This provides a useful gauge for the troubles here. Referring to Table 3 it may be seen that on average R_M equals \bar{R}_{NI} in cities with a population of around a quarter of a million and equals the peak value of R_{NI} in the more notorious cities. As a garnish to the statistics I may mention that during the past 20 years, two members of the staff of my department have met with violence while in American cities (one shot at in Dallas because he witnessed a crime, another mugged in Los Angeles); neither of them has been injured as a result of the troubles—nor indeed as far as I can ascertain, has any member of the staff of the university.

Table 3 Murder rates, R_M , (per hundred thousand) in towns and cities of the United States (1970)⁴

No. of towns or cities in group	Population limits	Average population of member of group	Murder rate R_M
23,94	< 10,000	5,000	2.6
11,77	10,000–25,000	16,000	3.3
504	25,000–50,000	35,000	4.2
252	50,000–100,000	70,000	5.2
98	100,000–250,000	140,000	10.0
56	> 250,000	750,000	17.5
Selected cities			
	Philadelphia		18.1
	Houston		23.5
	Chicago		24.1
	Baltimore		25.5
	Dallas		28.7
	Washington DC		29.2
	Detroit		32.7
	Cleveland		36.1

The existence of very high murder rates in the United States causes much adverse comment but the existence of very high fatal accident rates R_A in many advanced countries passes almost unnoticed. Although fatal accidents are a greater hazard to life, they tend to be accepted apathetically as inevitable. A large number are undoubtedly inevitable because of human frailty but the width of the R_A range should kindle deep concern. Table 4 shows that the difference between R_A in England and Wales, where it is least, and R_A in most advanced countries is actually greater than \bar{R}_{NI} (12.4). Fatal accidents are so numerous (almost 50 d⁻¹ in England and Wales and over 100 d⁻¹ in France) that though all are tragic, only a very small fraction are widely or vividly reported. In contrast, almost any violence in Northern Ireland may be widely and vividly reported because of the concentrated attention on events there.

Reluctance to come

A melancholy way in which the troubles have affected scientists in Northern Ireland is that people tend to be deterred from coming because they (or more often their wives or parents) are worried by the risks. The worry is not based on an objective appraisal of the risks such as I have attempted in the preceding section. It arises from repeatedly seeing Northern Ireland on television screens and in news items. People are indeed aware that these are, quite properly, highly selective. Nevertheless it is difficult to avoid becoming partly conditioned by them.

There have been cases of service engineers refusing to travel from Great Britain to Belfast and of firms putting such travel on a volunteer basis and being loath to renew maintenance contracts. The inconvenience caused has been of only a minor nature and any accompanying irritation has been amply compensated by the good conceit of ourselves induced by this display of lack of discernment and faint heartedness.

Table 4 Fatal accident rates R_A (per hundred thousand) in some advanced countries (1969 or 1970)^a

Country	R_A
England and Wales (1970)	35.3
Northern Ireland (1970)	39.4
Japan (1970)	41.8
Republic of Ireland (1970)	42.9
Scotland (1970)	43.3
Sweden (1969)	43.3
Holland (1970)	49.4
Denmark (1969)	50.4
United States (1970)	54.1
Canada (1969)	55.4
Australia (1970)	55.6
New Zealand (1970)	56.4
Switzerland (1969)	58.2
Federal Germany (1969)	62.0
Belgium (1969)	66.0
France (1970)	74.3
Austria (1970)	78.5

Because of the troubles, a few university teachers have declined invitations to act as external examiners. The odd oral for a PhD candidate has had to be held outside Northern Ireland. Some external examiners have made enquiries about insurance cover while in Northern Ireland. Many have been apprehensive before their first visit. Feelings of apprehension, although unjustified, are entirely understandable and those who surmount them merit mild cossetting.

No difficulty has been found in getting visiting speakers for departmental research seminars. The practicality of holding major conferences here, however, has been uncertain. The Departments of Applied Mathematics and Theoretical Physics and of Pure and Applied Physics for example, obtained possession of attractive new buildings in September 1972. The five professors directly concerned had originally planned to mark the event by inviting the Institute of Physics to have its National Atomic and Molecular Physics Conference in the new buildings. But because of the disturbances they feared that attendance might be poor (though they did not doubt that distinguished invited speakers would come); and they decided unanimously to postpone the invitation. Perhaps they were over cautious. Certainly the Biochemical Society of London held a successful meeting here recently.

The Department of Zoology has observed a sad change in the willingness of groups to visit Northern Ireland. Before the troubles it established a Marine Biology Station near the entrance to Strangford Lough in County Down. A rich and varied fauna may be studied with the aid of the modern well-equipped fishing boat, the ecological diversity being unusually great because of the marked difference in exposure between the shores inside and outside the lough. Laboratory and residential accommodation for about 40 people is available and these excellent facilities were being enjoyed to an increasing extent by groups from other universities. That has virtually ceased.

Yet the Marine Biology Station is set amidst peaceful surroundings.

The most serious problem is presented by the number of students entering the Faculty of Science. In 1966 Queen's University, which from its opening in 1849 had remained an essentially local institution as far as its undergraduates were concerned, began to participate in the Universities Central Council on Admissions (UCCA) scheme in recognition of the fact that a considerable fraction of undergraduates both prefer, and are financially enabled to study away from home. An example illustrating the extent of the scholarly migration is that in October 1969 more than 25% of all entrants to the universities in the North-Western region of England (those in Lancaster, Liverpool, Manchester and Salford) were domiciled in the South-Eastern region⁶. Life in Northern Ireland had (and still has) much to offer, being engagingly (though alas now also agonisingly) different from life in England. In 1966 it did not seem unreasonable to expect that many students would elect to cross the Irish Sea and, indeed, by October 1968 the number entering the Faculty of Science from outside Northern Ireland had risen to 15% of the total. The troubles led to a drastic decrease in the inflow of students (and to some English students withdrawing at the insistence of their parents). Meanwhile the outflow, already substantial, continued to increase. Because of the imbalance the number entering Queen's Faculty of Science fell by 19% between October 1968 and 1973 whereas the number entering all faculties of science in the UK rose by 12% during the same period⁷. A turning may have been reached. Analyses of the UCCA application forms received by the end of June 1974 and June 1973, suggests that (against the national trend) the faculty may have more entrants in the coming academic year than it had this year.

The position regarding postgraduate students is happier. Between the academic years 1968-69 and 1973-74 the number in the Faculty of Science increased by 18%. This is actually 6% more than the corresponding increase in the total number of postgraduate students supported by the Science Research Council⁸ (which presumably provides a guide to the pattern in Great Britain).

Recruitment of staff is not easy. Fewer applications are received than for similar posts in other universities of the same standing and the practice of personally inviting applications from strong potential candidates has become less infrequent. The applicant who is offered an appointment occasionally states that he himself is eager to accept but that before doing so he must get the agreement of his wife who is worried at the prospect of living in Northern Ireland; and he may later have regretfully to write with the information that he has been unable to persuade her. The Board of Curators responsible naturally takes a long term view of the interests of the university and prefers to leave a temporary vacancy rather than make an appointment which is not fully satisfactory. A grave situation might have developed had it not been for the shortage of academic posts in Great Britain and the United States.

Carrying on

Some of the staff of the Faculty of Science have felt impelled to take part in communal or political activities—one (an applied mathematician) even ran successfully for election to the Northern Ireland Assembly. Their public spirited endeavours have inevitably been at the expense of their researches.

But what about the others, the great majority? I have asked many whether their researches have been affected by the troubles. All immediately replied that they have not and most seemed rather surprised by my question. A few expanded on their answer. Two added that any stress in life here is much less than the stress they had endured from commuting in London. Another, commenting introspectively on how little he was really disturbed by the sound of an explosion in spite of knowing that a tragedy may have occurred, said that except for a surge of anger his reaction was mainly transitory curiosity such as he

might experience on noticing a speeding fire engine anywhere.

An objective (if objectionable) check on the validity of the opinion expressed on research can be obtained by comparing the number of journal publications per faculty staff member which are based on research likely to have been done during the first three years of the present troubles (the latest years known) with the corresponding number for the preceding 3 years. The former is less than the latter by 5%, which is not significant, being within the possible percentage error in either of the two numbers. Again, the computer power used in Queen's University has risen through the years of the troubles at almost precisely the same rapid rate as the total computer power used in all universities in the UK.

The staff of the faculty work and live in relatively safe areas. It would be odd if they let their researches be impaired in view of the resilience shown by much less fortunate fellow citizens. Traders whose premises are blasted soon put up 'Business as Usual' signs; factory workers have helped make possible the 23% increase⁹ in the Index of Industrial Production for Northern Ireland between 1969 and 1973 (which is rather greater than the 15% increase for the UK as a whole).

Evidence on the extent to which life here continues much more normally than people elsewhere seem to think is provided by the fact that most of our graduates still prefer to take up employment in Northern Ireland: on average 77.5% of those graduating in the academic years 1968-69 to 1972-73 did so as compared with 73.1% of those graduating in the academic years 1964-65 to 1967-68.

There is little which need be said regarding teaching. Some changes have been made in field work; for instance the Department of Geology judged it imprudent to continue to use a mountainous area just across the border with the Republic of Ireland, which it favoured before the troubles, because guerillas are now reputed to train there. Other geologically suitable areas are readily accessible. The Department of Geography has also modified its field work pattern—not as a consequence of any incident but to ensure that the risk of one is remote.

Members of the staff in charge of field work have unexcelled opportunities of getting to know their students really well. Those I have spoken to have not noticed tensions between the students nourished by our two different traditions. The student body as a whole has displayed a remarkably high standard of responsibility.

The undergraduates seem to have become more serious and to be working harder. Table 5 shows the number of those in the Faculty of Science who in a particular academic year leave without a degree expressed as a normalised fraction f of the number of those who graduate (the normalisation factor being chosen to make f for 1968-69 have the value unity). As may be seen f has been declining steadily through the troubles.

Table 5 Number of undergraduates leaving the Faculty of Science without a degree expressed as a normalised fraction, f , of the number of those who graduate in the same academic year

Academic year	1968-69	1969-70	1970-71	1971-72	1972-73
f	1.00	0.85	0.78	0.76	0.72

Examinations are particularly vulnerable to deliberate or casual interference but the troubles have had only a minor effect on those in the university. In June 1972, a chemistry examination was disrupted by a bomb hoax. Another examination was quickly arranged. This was done easily because the question paper for the supplementary examination to be held in September (for the benefit of candidates who failed) was ready. Since the disruption, security precautions have been taken in and around examination halls. These are so thorough that any hoax warnings can be ignored with complete confidence.



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Ready Fall 1974, about 400 pp., illus.; hardbound, ISBN 0-201-06718-8, c. U.S. \$22.00; paperbound, ISBN 0-201-06719-6, c. U.S. \$13.50

The recent general strike organised by the Ulster Workers' Council took place during the examinations. During the fortnight it lasted, 12,464 examination seats were to have been occupied. Only 35 were vacant because of the strike. On the day, May 20, on which travel was most rigorously curtailed the corresponding numbers were 805 and 8 respectively. Some candidates had to walk long distances; some were under severe strain. Contrary to expectation, however, the general level of performance does not seem to have been lowered.

By ill luck, the printing of the question papers, delayed by the three-day week imposed because of the miners' strike, had not been completed when the Ulster Workers' Council strike was decreed. For a few papers, manually operated typewriters had to be used to cut stencils and these had to be run through manually operated duplicators. That I should mention such a prosaic affair is itself a revealing final comment.

I thank numerous colleagues for their generous help. Professor F. J. Smith, Director of the Queen's University of Belfast Computer Centre, provided information on the rise of computer power.

- ¹ *RUC Chief Constable's Report 1973* (edit. by Committee of Police Administration) (Police Authorities Printing Unit, Belfast, 1974).
- ² *Statistical Office of the United Nations, Demographic Yearbook 1970*, 708 (Publishing Services, United Nations, New York, 1971).
- ³ *Whitaker's Almanack*, 79, 454 (London, 1947).
- ⁴ *Bureau of the Census, US Department of Commerce American Almanac (1973): The US Book of Facts Statistics and Information*, 145 (Grosset and Dunlap, New York, 1974).
- ⁵ *Statistical Office of the United Nations, Demographic Yearbook 1971*, 740-2 (Publishing Services, United Nations, New York, 1972).
- ⁶ *UCCA Statistical Supplement to the 8th Report 1969-70*. (The Universities Central Council on Admissions, Cheltenham, Gloucestershire, 1971).
- ⁷ *UCCA 7th to 11th Reports 1968-9 to 1972-3* (The Universities Central Council on Admissions, Cheltenham, 1970 to 1974).
- ⁸ *Science Research Council, Reports of the Council for the Years 1968-69 to 1973-74*, Appendix III (Her Majesty's Stationery Office, London, 1969-74).
- ⁹ *Statistics and Economics Unit, Department of Finance, Northern Ireland Economic Report on 1973*, 22 (Her Majesty's Stationery Office, Belfast, 1974).

Contrasting styles of social responsibility

John Hall

Social responsibility in science manifests itself in completely different ways in Britain and the United States. In Britain the emphasis is on words, whereas the American approach is based on deeds.

A DIVERTING and not entirely misleading illustration of Old World and New World attitudes to social responsibility in science and technology is contained in the following two exchanges. The first features Art Tamplin, one of four resident scientists engaged by the Natural Resources Defense Council (NRDC) in the United States, who, when asked what his agency is doing as protector of the environment, reaches for a list summarising 100 lawsuits and related activities undertaken by 14 NRDC lawyers during the first four years of its existence.

The second exchange takes place at Fortress House, Savile Row, home of the 150-year-old British Association for the Advancement of Science (BA), where the avuncular Secretary and sometime television personality Magnus Pyke throws up his hands and exclaims that one must be truly ignorant if one does not know already about the association's activities in the region of social responsibility. He then lists categories of BA activity, which include a meeting of members in Scotland, meant in some sense to introduce science to the nation, the consideration by members of the effects of science on the body corporate, and the organisation of working parties in which experts consider specific problems.

But earnest consideration apart, one asks, what is the BA actually doing, for example, in the area of environmental protection? Two or three operations are going on, concedes Secretary Pyke, centred on universities, and employing the services of groups of schoolboys acting as 'information agents'. What does this involve? "Well, they can run out and collect sediment, and seaweed, and so on. Rather like winning battles with the Pioneer Corps."

Contrast

The comparison is not made in an uncharitable spirit, but simply to offer broad paradigms of British and United

States responses to similar problems. In Britain the tendency is first to define and discuss the problems, possibly (but not necessarily) hazarding recommendations, after which one might publish abroad the findings of one's working party in the hope that somewhere in the course of the democratic process attention will be paid to the deliverances of one's eminent panel. Actively engaging a problem, head on, is not a popular tactic. The favoured approach assumes that the good sense and standing of the working party's members is recognised outside their academic sphere of influence by, say, an Energy or Environment Minister, who on the one hand might have read politics at Ruskin and on the other might have flunked classics at Balliol.

In America a socially concerned science group is more likely to reach for its lawyers or take a government agency by the throat as a first line of defence against malfunctioning technology; education, study-group organisation and publishing are likely to constitute a continuing but secondary strategy. The Old World approach is to deal with conundrums raised by the possibilities of new science; the New World's is to regulate its confrontations.



Art Tamplin, Gus Spech, and Tom Cochrane (NRDC)

The self-help ethic and frontier spirit aside there are good business reasons for the different tactics: in the United States there are six or seven times as many lawyers as in the United Kingdom, and a contingency fee system which allows them to take something like 25 per cent of a client's damages if they win, and nothing if they lose. Since no similar sporting arrangement is allowed by the British legal establishment, there is little incentive for pressure groups and lawyers to join forces; over here the barristers can afford to win or lose, and their clients can't afford to do either.

This is not to say that the British system of organising committees to make sage deliverances is ineffective; but since the main agencies operating in this fashion deal with generalised and long term problems rather than with questions of specific and immediate concern, there is less chance of establishing a casual link (or absence of a link) between the group's activities and the eventual policy line directed by the government of the day. Thus, if Professor Bodmer's working party on Social Concern and Biological Advances says, through the agency of the BA, that the government should be prepared to act in order to prevent the abuse of research on *in vitro* fertilisation, it is difficult for them to claim credit when the government does just that. It is not a singularly original recommendation to make in the first place, but even if it were, the working party could scarcely claim a copyright on the idea. The NRDC and the Scientists' Institute for Public Information, on the other hand, can quite clearly claim that the Atomic Energy Commission's \$5 billion liquid metal fast breeder reactor programme was brought to a standstill because of their objections: the Court of Appeals register for the District of Columbia says so.

The BA deliberately avoids such hand-to-hand combat and the taking of partisan lines on issues of the day. As Dr Pyke points out: "We're against instant wisdom. When somebody has just read about something in the paper this week, and they're marching down the street with banners on the subject, usually they're wrong, and puerile. The BA's approach, and indeed its main usefulness, is to make plain the issues and the facts of the issues; we're in the communications business." And the reason why it's wisest to present facts rather than taking sides, says Dr Pyke, is that a group of scientists is rather like the criminal classes, in that it represents a broad cross section of the community, with every variation of opinion and foolishness that this implies. "I've seen so many scientists make asses of themselves on their own time; there's no reason why they should do so on behalf of the BA", says the Secretary.

Accordingly, although the BA may set people to work at issues of concern to society—like the uses made of scientific innovations by the police, or the ethical and legal problems of biomedical advances—the combined pressures of arriving at a consensus, and of not 'taking sides' can result in findings which are unexceptionable to the point of being platitudinous. After studying police methods for recording and computer-banking information about individuals, a working party will say that "serious thought needs to be given to people's attitudes to the recording of information by which they can be identified"; or after studying the complex problems surrounding AID births, that "the status of children conceived through artificial insemination by donor needs to be legally defined". My milkman might have reached the same conclusions while sorting his change.

Influential

The only British group of any standing which goes gunning for issues of immediate public concern is Parliament's own Select Committee on Science and Technology, an all-party committee which, although it can do no more than call witnesses and make recommendations, has proved im-

mensely influential, not to say embarrassing, in the formulation of official policy on energy. Unlike some parliamentary committees, the science and technology group is not confined to a limited area of study; it has an open brief to investigate and report on whatever topic it thinks fit, and so it can offer a forum for all shades of opinion on issues which are the subject of imminent governmental action if it chooses.

This is exactly what it did when it heard last year that Mr Heath's government was strongly in favour of American light water reactors for the next generation of nuclear power production in Britain. By summoning its own series of expert witnesses it provided, in effect, a second-chamber debate on the issue. For although the only exchanges were conducted between each witness and the committee, the publicity given to the hearings encouraged witnesses to refer to the evidence of others, so that the proceedings developed very much along the lines of a debate.

The chief advantage that the select committee has over other social responsibility groups is that it has the full powers of Parliament "to send for persons, papers and records". In other words, if the select committee calls, you've simply got to attend, and when you're there you're obliged to talk. The practical difficulty the committee members face, of course, is knowing who has something to say, so that he can be summoned and obliged to say it publicly. During the nuclear reactor hearings, for example, Sir Alan Cottrell didn't actually step up and volunteer the information that he had doubts about the pressure vessel integrity of the American hardware. But a whisper was heard to this effect and he was invited by the select



Dr Jeremy Stone

committee to make public his reservations. Possibly they would have been expressed through departmental channels in the normal course of events, but if the select committee had not had its ear to the ground so that the vital evidence could be made public at a fairly crucial point in the debate, the outcome might have been less certain.

A further strength in the select committee's dealings is that its evidence is privileged. So, if you're called, say, to give expert evidence as an employee of the electricity supply industry, you can say with impunity precisely what you think about the way supply industry chiefs are running and planning the industry. For some witnesses, coercion to give evidence of this kind is a positive pleasure.

During the seven years of its hearings the select committee has helped to force the government's hand on various issues (including the creation of British Nuclear Fuels, the establishment of the National Nuclear Corporation, and the creation of a department dealing with population problems), and it has established itself as an irksome pressure group which it would nevertheless be difficult for any government to dismiss. It has had to walk the fine line between pricking a government's conscience and actually challenging its authority. Its overall aim, according to its Chairman, Arthur Palmer, is "to do solid work but not to take so long that our conclusions are out of date before they're reached, while at the same time not following fashionable issues to the extent that we're not taken seriously."

The remaining British groups lack both the Parnassian elevation of the BA and the exceptional powers of the select committee. Broadly, they concern themselves on the one hand with the ethical possibilities of new technologies, and on the other with fostering grassroots involvement to defend the proletariat against developments seen as harmful, and with the radicalisation of scientists to assist this counter-offensive. The first category is represented by groups like the Council for Science and Society (CSS) and the Science Policy Foundation (SPF), which operate a traditional learned working party/publishing machinery; the secondary category (unless you include broadly based groups like Friends of the Earth) is represented by a single body, the British Society for Social Responsibility in Science (BSSRS), which relies on a combination of proselytising and missionary expeditions. It is difficult to say which category is the least effective, since the groups variously claim that they deal in unquantifiable forces (ethics don't measure), they are relatively new (nothing to measure yet), or that worker involvement may in itself be progress, although its specific campaigns have been abortive (radicalisation doesn't measure).

Practical men

The Council for Science and Society was formed last year with the object of "promoting the study of and research into the social effects of science and technology, and of disseminating the results thereof to the public". A registered charity funded with £80,000 over three years from the Leverhulme Trust, its 43 members include the clutch of Fellows of the Royal Society whose eminent names are virtually *de rigeur* on councils of this kind, along with assorted lawyers, industrialists, moral philosophers, theologians and 'others'. The essential distinction between the functioning of the CSS and the BA, according to the council's moving spirit Paul Sieghart, is that the council operates with "men concerned with practical affairs", whereas the BA (Bodmer's group excepted) tends to think that a body of scientists can supply the answers. The founding of the council followed the publication in *Nature* of a paper by an inter-disciplinary working party convened by ex-mathematician and lawyer Sieghart to consider the scientist's social responsibilities. He was interested in the interfaces between the disciplines of theoretical

ethics and science—the areas where controlled experiments were out of the question—where, he says, it seemed that a gap was developing into which mankind might fall and which he was concerned to bridge. His working party felt that diffuse debate on the subject could be brought into sharper focus, and, to quote its literature, it sought to analyse what were the special social obligations of the scientists, and to devise some practical means for discharging them.

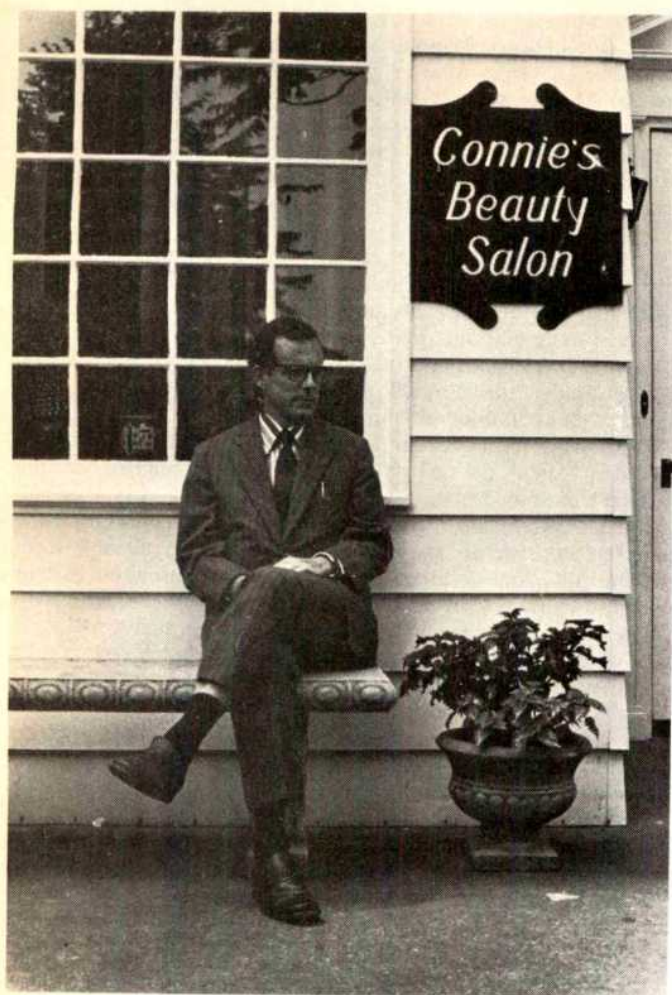
Its conclusion was that the primary need was for institutional machinery designed to identify problems in the field before they became acute, to stimulate well informed debate about them, and thus increase the time span for social decision making by the usual political process. Accordingly, the council is not a decision-making body, neither does it intervene in topics already well debated, or so far away as to border the realms of science fiction. Instead it "seeks to identify specific developments in science and technology whose social consequences lie just over the horizon, where no full scale debate has yet begun, but where intensive analysis of the actual and probable 'state of the art', and of the foreseeable social consequences can suggest a range of possible responses to those who will sooner or later have to take the necessary decisions".

Currently, working parties are discussing the standards to be applied and the procedures for applying them, in weighing 'acceptable risk' against anticipated benefits from new technologies; the use of surgical, pharmacological, psychological and other techniques for the control of individual behaviour deemed to be harmful or deviant; the problems involved in the development and use of 'harmless' weapons for the control of civil disorder; the problems posed to successful communication by the rapid growth in the volume of published technical information; the social and ethical implications of the nonclinical use of mood-control drugs; and the problem of monitoring technologies in those instances where technical competence is monopolised by a small number of institutions committed to the same interest.

The CSS will publish reports on each of these studies, outlining expected advances in the foreseeable future, the range of social consequences likely in the absence of controls, and the control mechanisms available if the consequences are to be modified. The overall aim is to arrive at an appropriate response "in the course of a responsible public debate conducted at leisure on the best information available, rather than by the hurried, ill informed and ill considered process which is apt to occur if the public does not become aware of the problem until too late in the day". Additionally, CSS makes itself available to government departments for advice, and apparently the appropriate departments have availed themselves of this facility already. On what issues? Confidential issues. Also, the council is available as confessor to scientists who have qualms about research projects they are working on. So far there has been one taker. On what issue? A confidential issue. The CSS probably has a rosier future than the BA in this area, because it engages younger, brighter people from mixed academic backgrounds, but for the moment there is nothing to measure but high intentions.

No criteria

The Science Policy Foundation (originally the Science of Science Foundation) has been operational since the middle 1960s, and has a similar difficulty in identifying its achievements, although in this case the claim is that there are no criteria for measuring the stock in trade: ideas, ethics, speculations. One thing abundantly in evidence at the SPF is a plethora of publications and eminent signatures in the guest book which also bears menus recalling the meals at which these eminents have taken their pleasures. The



Dr Jeremy Stone, outside the new headquarters of FAS

SPF looks like a one-man band, orchestrated by a former UNESCO science editor and *Reynolds News* writer, Maurice Goldsmith (although in fact he is answerable to a management committee). In 1964 Goldsmith was asked to edit a collection of articles to commemorate the 25th anniversary of J. D. Bernal's *Social Function of Science*. The collection was called *The Science of Science*, and after publication Sir Laurence Bragg, no less, suggested the formation of a Science of Science Foundation. No sooner was this said than done, with Mr Goldsmith at the helm, bearing a twin charter to look at science and technology internally in order to determine what were their growth mechanisms and to study the impact of science and technology on people.

To be fair, the SPF (the name changed because it became a bore to explain the meaning of the Science of Science at every turn) never actually undertook to do anything about social impact problems other than study them. In the event they have studied and published. Virtually everything the SPF touches involves a publication of some kind, and none takes a distinct line on a controversial issue. This is because the committee of management, which is another list of eminent names, covers such a wide spectrum of political views that no unanimous line emerges.

Two years ago the SPF set up a committee under Lord Avebury to take up contentious issues; the committee never reported. A similar committee was established to examine the Rothschild proposals for Government research and development; no agreed statement emerged, but the original report and the committee's deliberations over it were published, and sold. On energy the SPF takes the line that there is no need for an energy policy, but did not actually discuss reactor choices; on environmental pollution

it has made no statement, but, says Goldsmith, no member of the management committee would oppose the notion that pollution was a bad thing. Seminar fees, cash from publications, grants and donations roll in to the tune of £15,000 to £20,000 a year, and the prestigious dinner parties are entered in the book. "We do good by stealth," says Director Goldsmith, confiding that Cabinet Ministers receive "behind the scenes advice" at tables, where one may also find representatives of "key institutions", discussing things "pretty freely and off the record".

As a result of one *tête à tête* with Peter Walker (then Secretary of State for the Environment), Goldsmith and his colleagues at SPF were introduced to D. J. Lyons, Director General of Research Department of the Environment, and SPF was awarded the contract to publish the department's register of current research in the United Kingdom in the fields of town and country planning, environmental pollution, transport, building and construction. Not an insubstantial volume, but, says Mr Goldsmith, a non-profit making one.

Among the SPF's committees is one whose brief is to look at the foundation's terms of reference, with power to recommend that it be wound up if it is no longer a meaningful institution performing a useful function. The committee has never made such a drastic recommendation, although, says Goldsmith, if that's the way they feel, he hopes they will take the step. He adds that he doesn't expect this to happen while he is director.

The British Society for Social Responsibility in Science was formed at the Royal Society in 1969, with 40 Fellows among the founder members and a policy statement to which, it was said, the director of Porton could scarcely have objected. Most of the Fellows began to look worried when at the BA's 1970 meeting, the younger membership mounted a militant demonstration against British Army methods in Northern Ireland. A sort of Hippocratic Oath about scientists' moral responsibilities was taken by some members, and adopted as policy. Since then the society has agonised somewhat over its role and its political stance, and has emerged with a philosophy which says that moral oaths are pretty meaningless in a capitalist society, and that science is not simply a neutral body of knowledge, but a potent weapon for those who control it.

The abuses of advanced technology are not accidental, but spring from the nature of our society, says the BSSRS. Scientific and technical knowledge is "an important source of power, but only large institutions have the resources needed to exploit it. Advanced technology is used by our major corporations to create demand for new products, consumer or military. The government ensures, by economic management or direct intervention, that a market for these products will be found. . . . Thus science is used directly to increase the power of the already powerful and frustrate the expectations of the powerless". Naturally, the Royal Society Fellows are thin on the ground by now, but nobody at the BSSRS minds all that much, since, with the odd exception, Royal Society Fellows were found to be unwilling to do much more than lend their names to letterheads.

In terms of hard cash and administrative machinery the BSSRS looks distinctly shaky; its original Rowntree grant of £3,000 is almost spent, with no likelihood of renewal; membership stands at no more than 700 or 800; the society exists by courtesy of a largely part-time staff; and the £2,000 which it receives each year from endowments can be used only for educational purposes. Its office, also owned by Rowntrees, may not be available for much longer, and the work force is thinking of running the society as a collective. And yet these straightened circumstances, which might be the kiss of death to a traditional seminar/publishing-based body, organised on a centre-periphery administrative model, are less likely to kill a

loosely structured organisation like the BSSRS. It aims to develop an understanding of the social roles and functions of science and technology among people who do not normally expect to be consulted on such matters—the workers to whom its magazine *Science for People* is addressed—and its existence depends more on a network of grass-root operations than the survival of a central secretariat.

Already BSSRS scientists work in a consultative capacity with shop stewards' committees, mainly on issues like the toxicity or noise pollution of industrial processes—the strategy is to encourage people affected by technological nuisance to confront the problem themselves, with BSSRS supplying scientific expertise which the workers lack. They precipitated a strike by members of the National Union of Mineworkers over air pollution at a Coalite plant in Doncaster, action by a tenants' association attacking the plant responsible for the famous Battersea smell, and a strike by postgraduate workers at Edinburgh University which resulted in the raising of demonstration fees.

Urban collectives operate in Sheffield and Edinburgh, and autonomous BSSRS groups are active in half a dozen universities, beavering away at another favourite proposition of the society—that a non-elitist general education must emphasise the social implications of science and technology, and break down their isolation from other studies. All in all, the BSSRS might not be able to claim many successful campaigns against industry, but it can claim to have kindled community involvement, which is a success in itself, and it can only be a matter of time before its activities attract a grant from the Trade Union Congress.

Wide choice

In the United States there is an embarrassment of riches in the field of public interest science. An entirely arbitrary selection from the groups operating out of Washington yields the following bodies:

The Center for Science in the Public Interest (CSPI) operates a community involvement programme with aims similar to those of the BSSRS; the only difference is that it is more professional and more successful. The strategy is to introduce aggrieved citizen groups to experts capable of putting the finger on suspect planning decisions or industrial processes. Jointly with the public interest Economics Foundation, the CSPI has organised Professionals in the Public Interest, and come up with a meteorologist who helps groups to study air pollution from highways and power plants, an environmental engineer who is coordinating a survey of asbestos used in various consumer products, economists making evaluations of plans for interstate highways and cost/benefit analysis of public utility proposals, and a committee of scientists supplying community organisations with information on food additives.

The CSPI also has a healthy record running from asbestos to waste oil disposal, a list of 16 congressional and judicial testimonies last year, 48 administrative actions and requests, aimed at government agencies and individual senators, and two lawsuits, one requiring the Environmental Protection Agency (EPA) to reveal gasoline additives under the Federal Clean Air Act and the other attacking the EPA on asbestos, beryllium and mercury emission standards. Eleven major companies are opposing this action. The center's report on aerosols is thought to have cut market growth in that industry from 10% a year to 2½%.

This programme has been managed by a full-time staff of eleven, working for an average of \$360 a month, and a small army of volunteers. The CSPI started in 1971, with a staff of three working from a borrowed desk in the Oil, Chemical and Atomic Workers' office, and a budget of \$2,800 for the year. In 1972, the figure rose to \$19,600; in 1973 to \$39,600 and for the first nine months of this

year to \$72,600. Half of this money comes from the sale of publications and reports, the rest from foundations and donations.

Strategies, selected for the individual requirements of a problem, include the in-depth report, litigation, pamphleteering, citizen organising and what is known as the professional informational approach. Latest successes included an investigative report, *Interlocking Oil*, on the power of oil executives who also serve as bank directors and who manipulate instruments to drive out competitors. Using this study, several senators are writing legislation to eliminate these conflicts of interest. The CSPI also helped to organise a citizen's energy conference in Washington for 1,000 citizen activists from 47 states to develop strategies for increasing citizen power in the decisions on the uses and costs of energy resources.

The Federation of American Scientists (FAS) was founded in 1946 as the Federation of Atomic Scientists and is the country's only scientific lobbying body. It has a staff of three, eminent sponsors who include 29 Nobel Laureates, and a membership of 6,500 lawyers, doctors, engineers and scientists whose membership fees amount to \$90,000 of 'hard' money annually. 'Hard' money is tax exempt, but not deductible, and so it can be used for lobbying, whereas the 'soft' kind can only be used for education and publicity that does not attempt to influence legislation. The FAS tradition of lobbying started in 1946, when its briefings of congressmen helped to ensure civilian control of atomic energy. More recent successes have included an important role in holding up the building of an antiballistic missile system, and pioneering work on the promotion of scientific relations and exchanges between the United States and China (the People's Republic, that is).

Through its Director, Jeremy Stone, the FAS button-holes congressmen, gets them to listen to informed scientific opinion, and testifies at hearings. Its tax-deductible subsidiary, the FAS Fund, engages in research and educational functions, including the publication of a Public Interest Report which has taken strong lines on SALT 1, electronic eavesdropping, national science policy, research and development priorities, the Geneva Protocol on biological and chemical weapons, and the rights of Soviet scientists.

Stone (son of I. F.) has just toured the States trying to raise \$1 million from a few rich men in order to endow positions for three scientists, probably retired professors, to develop FAS staff expertise in the areas of environment, energy, medicine/public health, and development/population/food supply. According to the FAS the legislature hearings, although the heart of a publicly accessible debate over science and society issues, are often unattended by the press, and important laws get on to the books before alarms can be raised. The three new men will have the job of keeping an eye on such hearings, and making prompt reports, apart from providing briefings in their special fields at regular intervals.

The FAS, which calls itself the voice of science on Capitol Hill arranges two-way information exchanges between politicians and scientists, feeds congressmen's aides with intellectual grapes for scientific debates, and generally fixes things so that legislators do not make policies in areas whose workings they do not fully understand, without being fully aware of the alternative consequences. "We take positions on which reasonable men can hardly differ," says Stone. "We can't take eccentric lines; when you're going to Congress you've got to find the least common denominator".

The Natural Resources Defense Council, Inc., has a budget this year of £1,845,000. It employs four full-time scientists and 14 lawyers. It is four years old, and has a staff of 60 people working from headquarters in New

York, Palo Alto and Washington. It is the largest and most effective environmental law agency in America, and was founded to provide legal counsel to citizens and organisations seeking to protect the environment; to take legal action to protect the environment on its own initiative; to monitor the performance of government regulatory agencies whose activities affect the environment; and to encourage the legal profession to play a greater role in defending the environment.

In deciding whether or not to help citizen groups to find legal redress beyond their means in order to protect the environment, the NRDC uses two criteria: first, that the potential for establishing a legal precedent exists, and second, that without the NRDC's help, some valuable or unique environmental resource will be destroyed. Through

its recent actions it has saved for the time being much of the country's existing system of railroad track through a test case against the Interstate Commerce Commission, blocked the Atomic Energy Commission's liquid metal fast breeder reactor programme, forced the EPA to issue its standards on atmospheric lead standards, won a decision which challenged the clear-cutting of trees, sued to change the utility rate structures favouring big industrial users (granted by public utility commissions), opposed strip mining, offshore oil drilling and noise pollution in cities, and successfully petitioned the EPA to stop the use of four lethal poisons used by the Fish and Wildlife Service for predator control. To understand the full weight of the NRDC's effort you have to read the book listing its legal actions.

Murder involving discovery and first application of fluorescence of tyre prints

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Details are given from a murder investigation in which the fluorescence of tyre prints was discovered. The prints enabled the tyres and the vehicle in which the body was carried to be identified and fluorescence spectroscopy demonstrated a correlation between the fluorescence of one of the tyres and the corresponding print. Circumstances favouring the formation of fluorescent prints and the importance of the technique for forensic science are discussed.

PART of the evidence for the prosecution in a recent case of murder arose from the discovery by the investigating police officers of a set of fluorescent tyre prints. In our view the discovery will initiate developments of widespread significance to forensic science. We wish to describe, therefore, the circumstances of the case so far as they bear on this striking and hitherto unrecognised phenomenon.

The pertinent facts established in the enquiry, and subsequently accepted by the defence were that on the evening of Saturday, July 7, 1973, a Mini Estate vehicle, apparently deliberately set ablaze, was discovered in a field off Glasshouse Lane on the outskirts of Kenilworth. Immediate enquiries revealed that the owner was a local person, whose elder son, last seen that morning, proved to be missing. The son's bedroom and other parts of the house were heavily bloodstained. The investigation was therefore pursued as a case of murder, to be justified 5 days later in the discovery of the youth's body on a rubbish tip.

The parents of the youth had left home early that Saturday morning. At 0730h his younger brother was collected by a John Lees, who employed youths at weekends to assist in his work as a driver and labourer for a building company. Lees took the younger son to a building site, gave instructions on the work, and returned to the house, where he battered the still sleeping elder son to death. The body was rolled in the bed sheets, removed downstairs to the garage and into the family's Mini Estate vehicle, which was driven to a building site at Albion Street. Lees parked the car in number five of a newly completed and cleaned row of garages which he had worked on. That evening he drove to the tip, dumped the body on the tip face,

and covered the body with rubbish. He finally left the car ablaze in the field off Glasshouse Lane, and departed from the area. On his return 10 days later he was arrested, and subsequently, on January 21, 1974, at Birmingham Crown Court, pleaded guilty to the murder. He was sentenced to life imprisonment.

Our report concerns the Albion Street garage. Three days before the discovery of the body, when the movements of the car and any connections with Lees were crucially important matters, a bloodstained axe and a mutilated wallet belonging to the deceased were found close to the garages. There followed an inch-by-inch search that revealed in garage number five a small oil spot at the point expected had a front-engined car been reversely parked there. The oil proved to be largely Duckham's 20W/50, matching by thin layer chromatography and by fluorescence and infrared spectroscopy, oil from the Mini Estate. Otherwise, nothing whatsoever remained on the apparently featureless concrete garage floor to identify or to indicate even vaguely the type of vehicle that had evidently stood there.

It was decided that the floor should be examined in ultraviolet light. This was done with an Allen's portable lamp, emitting mainly at 366 nm, operated from a 12 V battery. Four brightly fluorescing tyre marks were revealed. (Figs 1 a and b). The precise definition of the marks enabled accurate measurements to be made that indicated, for the car concerned, a front track width of 122 cm, a rear track width of 117 cm, and a wheel base of 213 cm. Only Mini Estate vehicles correspond in these dimensions, for which the manufacturers quote 121, 116, and 214 cm respectively. The tread patterns reproduced in the marks are unquestionably of Goodyear G800 tyres at the front, and of Kelly Springfield KRI tyres at the rear. The burnt-out Mini Estate carried these makes of tyres in the same positions. These results, together with the matching oil samples established a vital link in the chain of evidence.

Part of the front near-side mark and samples of nonfluorescent concrete were chipped from the floor and removed to the laboratory. Before the examination of this material, however, experiments were made to determine the origin of the effect, which, it was found, can be readily demonstrated. When a tyre is contacted with a strip of polyester-backed thin layer chromatography adsorbent (silica gel or alumina) for periods

of as little as 3 min, fluorescence appears at all contact points. The fluorescence is blue-green in colour (with excitation at 366 nm), varies slightly in shade between different tyres and adsorbents, increases with time and pressure of contact, and reproduces the tyre pattern in detail. Analysis of the fluorescent material (by chromatography on the adsorbent *in situ* by spectrofluorimetry *in situ*, and by the latter technique and by spectrophosphorimetry after solvent extraction from the adsorbent) indicates that the fluorescence is caused by the transfer of extender and process oils, antioxidants and polynuclear aromatic hydrocarbons from the tyre rubber to the adsorbent. All these factors vary with the make and the use of the tyres (J. B. F. L., unpublished) but the fluorescence originating from most tyres in current use is caused mainly by extender oils.

Figure 2 compares spectra from chloroform extracts of prints from a selection of tyres (Fig. 2a). The spectra, partly quenched by this solvent to increase the detail resolved, were synchronously excited at an interval of 30 nm (ref. 1). The sensitivity of our equipment to ultraviolet fluorescence was low and therefore the emissions recorded are those occurring mainly in the visible region. The spectra clearly demonstrated, however, the extent to which fluorescence can vary with the originating tyre.

The nature of the receiving surface determines the intensity of the fluorescence obtained. Level, fresh adsorbent surfaces, concrete or brickwork, for example, have exhibited distinguishable prints within a contact time of 8 min. On soiled or aged surfaces, or where tyres have been in repeated contact, no distinguishable fluorescent prints are formed. Repeated contact either produces a diffuse area of fluorescence, or transfers so much ultraviolet absorbing material that all fluorescence is

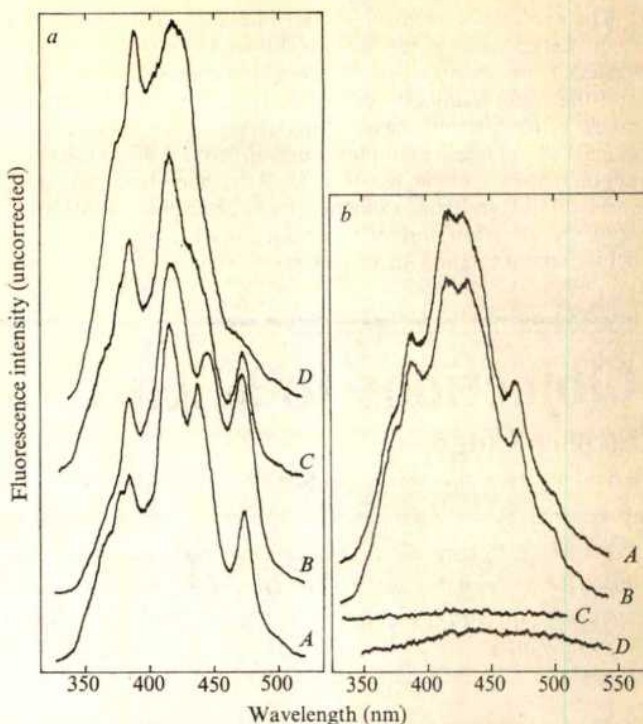


Fig. 2 *a*, Synchronously excited (30 nm interval) fluorescence emission spectra of chloroform extracts from prints, on silica gel, of various tyre samples: *A*, Michelin X; *B*, Goodyear G8 remould; *C*, Goodyear G8; *D*, Pleak remould. *b*, Synchronously excited (30 nm interval) fluorescence emission spectra of chloroform extracts of: *A*, nonfluorescent concrete from scene after contact overnight with tyre from Mini Traveller; *B*, concrete from fluorescent tyre print found at scene; *C*, concrete-underlying that used in *B*; *D*, nonfluorescent concrete from Albion Street garage.

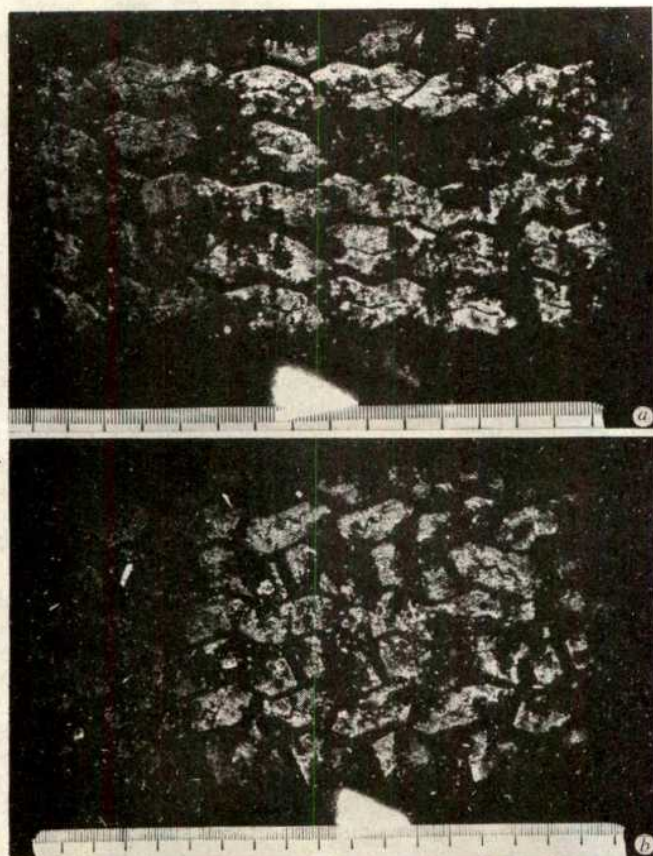


Fig. 1 *a*, Tyre print fluorescence, rear offside position at the Albion Street garage; *b*, tyre print fluorescence, front offside position at the Albion Street garage. Illumination in both cases was with an Allen's 12 V battery operated ultraviolet lamp. Photographed with a 35 mm single lens reflex camera fitted with a Wratten 8 filter on FP4 film exposed 600 s at *f*/5.6 and developed in Acutol.

quenched. Even so, many surfaces lie between the extremes. Thus, in our experience, fluorescent prints are often apparent in concrete-surfaced public car parks. No prints occur on bituminous surfaces, but many others, even of glass, collect material that, although not visibly fluorescent, yields fluorescent solvent extracts. For instance, a tyre dirt mark on paper is not fluorescent, but yields fluorescent solutions suitable for spectrofluorimetric analysis on solvent extraction. The effect is due to the minute particles of rubber of which such marks are composed (J. B. F. L., unpublished). That rubbers are intrinsically fluorescent has long been known, but this fluorescence, which is entirely suppressed by carbon black in tyre rubbers, is not extracted by organic solvents².

The circumstances at the garage were clearly ideal to the observation of tyre print fluorescence. Experiments made with a vehicle parked in an adjacent garage indicated that within 30 min four marks became apparent, after 1 h the fluorescence was considerably intensified, especially where the more heavily laden tyres contacted. This intensification continued over 8 h when all the marks had become equal in intensity. No visible changes occurred after this time, although the continued transfer of fluorescent material over extended periods of time can be demonstrated by spectrofluorimetry of solvent extracts.

Figure 2 also shows spectra from the material collected at the garage and from the corresponding tyre (Fig. 2b). The top surfaces of the chippings from the mark were scraped to yield 10 mg of powdered concrete, an extract of which in chloroform (0.5 ml) gave the spectrum shown. Further fragments of concrete, from the new surface exposed, contained no fluorescent material, in confirmation of the expected superficial distribution of fluorescence. Fluorescent material from the tyre was collected on nonfluorescent concrete taped to the tyre surface overnight. The spectrum of the extract obtained is also shown (Fig. 2b).

The excellent agreement shown in Fig. 2b between the two fluorescences added further weight to the highly significant evidence already obtained. We feel, however, that these results and the observations we have described will be important in many other circumstances. Apart from the characterisation of parked vehicles, examples, some already applied (J. B. F. L., unpublished), include the transfer of rubber from car accessories in hit and run accidents, from shoe soles and heels in scuff marks, from erasers to erasure marks, from the rubber debris contaminating surfaces heavily exposed to tyre contact

to people and articles themselves in contact, and, indeed, from any item liable to shed traces of rubber or fluorescent rubber additives at a scene of crime.

We wish to thank Detective Inspector J. F. Bradley of the Warwickshire Constabulary for the photographs reproduced and for experiments made at the scene.

¹ Lloyd, J. B. F., *Nature phys. Sci.*, **231**, 64-65 (1971); *J. forens. Sci. Soc.*, **11**, 83-94 (1971).

² Morris, V. N., *Ind. Engng Chem.*, **26**, 107-111 (1934).

Objections to science

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Professor Cotgrove discusses the view that science is not as objective as its adherents claim. He considers the misuse of science, especially the use of pseudo-science to justify social practices.

RECENT years have seen a spate of critical writings on science. Much of this is concerned with the uses and abuses of science. But, some of the objections go deeper, questioning the objectivity and neutrality of science. Indeed, the Brook report to OECD went so far as to express concern at "the world-wide culture of educated youth which is deeply concerned with ecological perspectives and . . . could conceivably even adopt anti-rational views and could become more influential in the next decade than our extrapolations suggest"¹⁻³.

Science and culture

A dominant theme in the attack on science is its threat to humanistic values. The penetration of the world views of natural science into contemporary culture, it is argued, has encouraged ways of thought which will have disastrous consequences for the future of humanity. Specifically, it is the mechanisation and mathematisation of the world view of nature which is attacked: "Our hypnotic enslavement to the numerical aspects of reality has dulled our perception of non-quantitative moral values; the resultant end-justifies-the-means ethics may be a major factor in our undoing"⁴. Such objections in their more extreme forms go so far as a rejection of the primacy of reason and rationality and the celebration of emotion, feeling and unstructured spontaneity in thought and action^{5,6}. It is with such objections of course that the prevailing youth culture is so sympathetic and which may have particularly important implications for the future of science.

Those who see great dangers in the implications of science for human values have therefore sought to bring to light the meta-physical and ideological assumptions implicit in modern science and thus to challenge its claims to special objectivity. Second, they have exposed the ways in which science has been used as a justification and legitimation for social and political policies (scientism), thus negating its claim to neutrality. It is to these issues that I now turn.

Mechanistic approach to nature

The nub of the debate is the argument that the influence of natural science on culture is to reduce man to mechanism. Post-Newtonian physics has all too successfully exorcised the ghost in the machine. In place of the 'primary' human experiences of taste, touch, sight, sound and smell, nature is now explicable to modern science only in terms of matter in motion, or more recently, forces and fields. So, the Baconian dream of mastery over nature for the "relief of man's estate" has been

turned into a nightmare in which science becomes the basis for the dehumanising not only of nature but also of man. In the last analysis, mind and human personality, purposes and values, freedom and dignity—all are reducible to matter in motion. Man too, as a part of nature, thus becomes the object of manipulation and control. The mastery of nature becomes the basis for the oppression and repression of all that is human.

The reaction, understandably, has been to challenge the hegemony of the mechanistic view in a variety of ways. A particularly powerful attack has emerged in recent years on the more simplistic positivistic views of science as being objective knowledge independent of human judgement. Some of this critique stems from historians of science who have investigated the actual process by which new theories come to be accepted in science. Distinguished scientists such as Medawar and Polanyi have similarly stressed the crucial role of personal judgement—the distinctive vision of the scientist who discovers a new way of looking at nature—often in spite of the facts. It is this crucial role of theory as a screen between the senses and 'reality' which makes science an essentially human and fallible activity, akin to artistic creativity in its higher reaches⁷⁻¹⁰.

Such a perspective opens the way to an exploration of the external factors which influence the vision of the scientist as he seeks to interpret the shadows dancing on the wall. Thus, for example, Dijksterhuis concludes, "The strong influence which Newton's religious ideas exercised on his scientific thought is revealed, among other things, in his belief in the existence of absolute space and absolute time . . . Compared with the mechanistic idea of impact as the only cause of a change in the state of the motion, there is a spiritual or animistic flavour about the idea of an incomprehensible force operating at a distance which does not seem out of keeping with an anti-materialistic philosophy of nature." Needless to say, Newton's use of the concept force was strongly opposed by many of the leading natural philosophers of his day precisely on the grounds that it was an occult quality and unmechanistic (ref. 8, page 487 and ref. 11, chapter 7).

As an extension of this line of argument, the objectivity of the contemporary mathematical view of nature is questioned by asking what it is in fact that we now know about? Modern physics is a far cry from the attempts to understand nature in terms of the primary qualities ascertainable by the five senses. "Modern physics is not really concerned with 'things', but with the mathematical relations between certain abstractions which are the residues of vanished things . . . All we do in fact know is that we read our instruments—the number of clicks in the Geiger counter, or the position of a pointer on a dial—and interpret the signs according to the rules of the game . . ." (ref. 4, page 544). The 'facts' of nature are whatever meaning we can give to the readings on the dials. Or, as Bertrand Russell has succinctly observed, the only thing we know about the physical world is its mathematical properties.

Such observations then are used to support two main conclusions. Firstly, it is claimed, simplistic notions of the objectivity of science cannot be sustained. Galileo's view that "... the conclusions of natural science are true and necessary, and the judgement of man has nothing to do with them", is decisively rejected. Secondly, the limits and limitations of scientific knowledge are stressed. 'Certainty' is bought at the price of losing a massive amount of information and 'knowledge' of nature in the search for mathematical models.

Perhaps the weakest aspect of the challenge to the overriding dominance of the mechanistic view is its practical success, and the absence of any satisfactory notion of an alternative science. Here, the opponents of modern science turn to the recent history of science for examples of the way in which alternative views may exist side by side, each consistent with the known data. For example, Hesse shows that not only were theories of continuous action and action at a distance both equivalent in form and identical in experimental content, but both contributed to advances in electromagnetic theory (ref. 11, pages 216-222). It is argued too that alternative 'organic' views of nature have had some success in science. In the eighteenth century the divide between science and the humanities had not yet occurred, and speculation in natural philosophy embraced not only the experimenters but also poets and philosophers in its discourse including men like Shelley and Coleridge who turned to 'science' to combat the materialist mechanistic view. By contrast, they emphasised those aspects of science which stressed the unity of nature, the primacy of force over matter, and the unity of all forces. This Romantic viewpoint had an important influence on many scientists and in fact played a major part in the emergence of concepts of energy, and in many specific discoveries including the connection between electricity and magnetism, and Davy's discovery of potassium. In other words, an alternative paradigm free of the humanistic shortcomings of the mechanistic view has proved of value in the past and may do so again¹².

Reductionism and holism

Although modern physics is essentially reductionist, it is in biology where the feud between reductionism and holism has been fought with all the ferocity of a theological dispute. Monod, for instance, refers tersely to "a very stupid and misguided quarrel, which merely testifies to the 'holists' total lack of understanding of scientific method..."

Although some of the protagonists dismiss the attack on reductionism as a return to vitalism, in essence, the debate is about whether we can explain the properties of complex phenomena solely in terms of the characteristics of the parts of which they are made; whether that is to say, there are emergent properties of systems that are not discoverable simply from an analysis of their components. The debate can be examined at two levels. Firstly reductionism can be tested like any other theory (or perhaps meta-theory) for its explanatory power. Secondly, and what is perhaps more interesting, the ideological undercurrents and implications can be identified.

The case against reductionism is explored intensively and extensively in *Beyond Reductionism*, the report of the Alpach Symposium at which a group of distinguished scientists explored the growing criticisms of much biological orthodoxy, and the 'robotomorphic' mechanistic view of man implied in behaviourist psychology. Weiss, for example, drew attention to a number of difficulties in the reductionist position. Analysis, he argues, by focusing on smaller and smaller parts, inevitably results in the loss of information, notably information about interaction between the parts. It is in this neutral sense, he maintains, that the whole is greater than the sum of the parts—that is, what is discovered and recorded about the parts by the process of analysis. What is lost is information about the orderly relations among the parts. We need, he argues, to take account of the regulatory and controlling mechanisms which are to be found in the larger systems of which the subsystem is a part¹³.

By contrast, Monod's reductionist emphasis looks at first sight to be dramatically opposed. This is in part a reflection of the fact that Monod's emphasis and examples focus strongly on the base of the hierarchy—on the regulatory mechanisms of DNA and the genetic code. But on closer examination, Monod's descriptions look less unlike those of Weiss despite the very different positions each have taken in relation to reductionism. Monod too speaks of microscopic systems, and of "the cybernetic system of the simplest cell", and "that all activities that contribute to the growth and multiplication of that cell are interconnected and intercontrolled..."¹⁴. Monod too recognises that much remains to be demonstrated. But he decides as it were, to back a different horse.

It is highly probable that the issue will be resolved by further advances in microbiology. It is in any case an internal debate which will have to be left to the biologists to resolve. But of particular interest are the wider issues raised by the debate. Why, for example, are the protagonists so ready to accuse each other of such heresies as being unscientific, and so quick to identify the dogma in their opponents' positions? Both would seem to admit when pushed that neither position is dogmatically tenable and that the case is essentially unproven. For example, Commoner claims that the term 'central dogma' is often used in the literature on DNA. He quotes the opening comments of the chairman at the annual meeting of the Federation of American Societies of Biology (1965) as saying: "Let me review quickly the essential doctrines which comprise the dogma of modern genetics", and a participant as saying: "The reason we call this 'dogma' is that it depends on personal bias, not logic"¹⁵. Why then, if the tentative nature of theory is recognised, the impassioned partisanship?

Neutrality and commitment

In one sense, some measure of partisanship among scientists is what we would expect from what is now known about the process of discovery in science and especially the role of theory. But there are dimensions of this particular debate that are of special interest. For example, Monod has a section "dangers of genetic degradation in modern societies". This is the familiar argument which has raged since Galton and Spencer, that natural selection has been suspended. Monod's reasoning is worth quoting in full: "... statistics, as everybody knows, show a negative correlation between intelligence quotients (or cultural level) and the average number of children per couple. The same statistics also demonstrate that there is a high positive correlation of intelligence quotients between marital partners. This is a dangerous situation, which could gradually drain the highest genetic potential into an elite, shrinking in relative numbers"¹⁴.

Now there are two observations. First, this is a breathtakingly partisan statement and vast oversimplification of the nature-nurture debate about the nation's alleged declining intelligence. But more central to this discussion the reductionist position favours the 'nature' side of the equation—emphasising genetic factors rather than environmental influences.

What is being argued here is that such beliefs are embedded in and exemplify a more general ideology or *weltanschauung* which can be typified very crudely as antiliberal, and tough minded. The existence of such clusters of beliefs and attitudes is now well documented¹⁶. I am not here referring to the kind of ideological issues which have been traditionally associated with the left-right dimension in politics, but rather with a cluster of beliefs and attitudes which cut across such a conception of a conservative/radical dichotomy: in simple terms, an individual's attitude towards 'the bomb', and a range of 'Home Office' humane and moral issues. Thus support for the extension of nationalisation is no indication of a person's attitude towards capital punishment or the sterilisation of sexual offenders, or restriction on immigration. So it can be argued that support for the reductionist position is the expression of a more deeply rooted tough-minded philosophy of life, which not only values tough, logical rigorous and possibly rigidly linear thinking, but

is prepared to follow such logic to its perhaps disturbing conclusions, and in this sense allows means to determine ends and logic to dominate values. It is the expression of a basically pessimistic (though possibly realistic) view of human nature, aware of the need for tough and disciplined measures to control man's wayward tendencies and to nurture man's tenuous hold on civilisation. So, it would be claimed, we have to face the facts of the basic inequality of human genetic endowment and devise educational systems based on a recognition of such facts.

By contrast, it is those who incline to the opposite position, have a basically optimistic view of man's nature, and who locate the frustrations to the full flowering of human potential in impoverished environments and repressive social institutions, who are likely to find the implications of reductionism disturbing. Indeed, it is precisely from such radical critics of society and from the basis of the liberal-humanist tradition, that the most vigorous attack has been mounted—including Roszak, Dubos, and Habermas. From such perspectives, reductionism is seen as an expression of the mechanistic Baconian approach to science which sees science as a source of domination and control over nature (and man), as distinct from the more organic perspective of understanding and relating to nature¹².

Science as ideology

It is when the findings of 'science' are used as the basis for rationalising and justifying particular economic and political doctrines (scientism)¹⁷ that scientists are most open to attack for their partiality. There is a long tradition of justifying inequality, conflict, hierarchy, domination and competition through crude applications of biological notions to social dynamics (ref. 3, pages 259–263; ref. 18). Indeed, it can be argued that while crucial uncertainties are part of the human condition, there is need for folk sciences functioning as ideologies to provide comfort and reassurance. Seen in this light, the new natural philosophy of the seventeenth century "with its disenchanted and dehumanised world of nature and its appreciation of closely controlled experience, itself functioned as a folk science. . . ." And the extent to which 'science' persists as the dominant folk science can also be seen in the resistance to psi-phenomena as possible evidence of the powers of mind independent of matter, which if accepted would constitute a threat to the contemporary world view¹⁹.

Such scientism has entered a new lease of life with the current intellectual revival of ethology and its popularisation through such best-sellers as Desmond Morris's *Naked Ape*²⁰, and the earlier work of Lorenz. Despite the claim of the blurb that this study is "objectively scientific", Morris's book is in fact both partisan and prescriptive. Man is essentially a primate, the argument goes, and "still follows fundamental patterns of behaviour set down by his hunting-ape ancestors . . . and there is no hope of quickly shrugging off the accumulated genetic legacy of his whole evolutionary past". For example, the fact that *Homo sapiens* has evolved as the sexiest of all the primates is used to explain the fact that "whatever obscure, backward tribal units are doing today, the mainstream of our species expresses its pair-bonding character in its most extreme form, namely long term monogamous matings". So monogamy is safe: it's all in the genes; "the space ape still carries a picture of his wife and children with him in his wallet as he speeds towards the moon".

Now whatever the case for or against monogamy or the biological basis of religion, or aggression, the gap between genetic codes and such complex behaviour is so wide as to require a remarkable leap of faith to discover the bases of social relations and religious beliefs in the macromolecules of the DNA code. The most serious objection of the critics, however, is not so much that such claims put the objectivity of science into question, but that appeals to nature or to human nature are basically conservative. As Reich²¹ points out, they are used

to shift the problem from the social to the biological sphere where nothing can be done about it. Those who are anxious to change rather than to conserve, to free man from outworn constraints, to pursue new heights of discovery and self awareness, are understandably suspicious of theories which underpin a kind of fatalism; and an acceptance of what has been seen as natural, inevitable and in this sense, right²¹.

It could be argued that the attack on science is largely misdirected. It is the extravagant claims made for science which have generated a backlash. However elegant (and powerful) the mathematical equation, it is as partial to claim totality for such an approach to understanding nature as is the total rejection of science by those terrified at the prospect of a world without poetry. But science can hardly be blamed if some of its enthusiasts attempt a takeover bid for human culture. And if some scientists have used science as a pawn to promote the kind of world they want, one beyond 'freedom and dignity', or in which biological imperatives replace the messy and untidy human search for meaning and purpose, then they must not be surprised if such 'unscientific' behaviour stimulates some non-scientists to mount powerful antiscience arguments.

But to single out science as the cultural base for oppression, repression and various inhuman and antihuman policies is to overlook the repertoire of elaborate legitimations and justifications, both religious and secular that human ingenuity has invented for historical excursions into domination. It is one thing to point to the fact (with some justification) that science is and can be an instrument of human repression and quite another to argue that it is a major dynamic force marshalling the human race like the Gadarene Swine to rush headlong towards the destruction of all that is civilised.

Whatever legitimate criticisms can be mounted against the behaviour of some scientists and against some of the more simplistic versions of the nature of science, it can claim to be a special kind of knowledge in the very limited sense that it has been spectacularly successful in building a consensus of certified knowledge, which goes beyond the subjective judgment of any one individual. Whatever may be the human failings of individual scientists, and whatever the enthusiasms of the individual for his own theory, the success of science may lie in the way in which it has collectively regulated and harnessed the passions of such individuals to the shared enterprise of advancing public knowledge without deadening the enthusiasm necessary to sustain the often intense commitment to a theory. What keeps the individual creative scientist from being swept away by his own enthusiasm is the institutionalised code of conduct, which ensures that his work is subject to the critical scrutiny of his peers. It is at this level that for all its faults and blemishes, science can claim to have achieved a remarkably high level of objectivity. It is this quality of scientific knowledge which commands respect. The critics of science who question the objectivity of a science increasingly involved in military and industrial applications may well have a point. Such developments could conceivably damage the mechanisms of quality control²² and lose for scientific knowledge any special claim which it may have. And the antics of individual scientists who parade their ideological preferences under the guise of scientific objectivity outside the controlling mechanisms of the community of science, similarly threaten the high standards of their calling.

Beyond its more routine and humdrum reaches, science is an imaginative and creative act: the attempt to construct a meaningful picture of nature. And this is a picture which is in a constant state of flux and periodically undergoes revolutionary changes whenever a creative genius sees a new pattern in the ambiguous dance of the flames in the fire—a picture which excites the imagination of his contemporaries and generates a burst of activity to test its explanatory power. But in the last analysis the result is the subjective conviction of fallible men. What is accepted as a scientific truth is what survives the negotiations and interchanges within the scientific community. Whether and in what sense we can ever know 'objective reality' is a philosophical question beyond the scope of this paper.

- ¹ Cotgrove, S., *New Scientist*, **59**, 82 (1973).
- ² Toulmin, S., in *Civilization and Science: in Conflict or Collaboration* (CIBA Foundation Symposium) (Associated Scientific Publishers, Amsterdam, 1972).
- ³ Easlea, B., *Liberation and the Aims of Science* (Sussex University Press; Chatto and Windus, London, 1973).
- ⁴ Koestler, A., *The Sleepwalkers*, 552 (Penguin, Harmondsworth, Middlesex, 1959).
- ⁵ Roszak, T., *The Making of a Counter Culture* (Faber, London, 1969).
- ⁶ Roszak, T., *Where the Wasteland Ends: Politics & Transcendence in Post-Industrial Society* (Faber, London, 1973).
- ⁷ Kuhn, T. S., *The Structure of Scientific Revolutions* (University of Chicago Press, Chicago and London, 1970).
- ⁸ Dijksterhuis, E. J., *The Mechanization of the World Picture* (Oxford University Press, 1961).
- ⁹ Medawar, P., *The Art of the Soluble* (Methuen, London, 1967).
- ¹⁰ Polanyi, M., *Personal Knowledge* (Routledge and Kegan Paul, London, 1958).
- ¹¹ Hesse, M. B., *Forces and Fields* (Nelson, London, 1961).
- ¹² Whitehead, A. N., *Science and the Modern World* (Free Press, Illinois, 1967).
- ¹³ Weiss, P. A., in *Beyond Reductionism: New Perspectives on the Life Sciences* (edit. by Koestler, A., and Smythies, J. R.), 3–55 (Hutchinson, London, 1969).
- ¹⁴ Monod, J., *Chance and Necessity* (Collins, Glasgow, 1972).
- ¹⁵ Commoner, B., *Science and Survival*, 41–42 (Gollancz, London, 1966).
- ¹⁶ Brown, R., *Social Psychology*, chapter 10 (Collier-Macmillan, London, 1965).
- ¹⁷ Jaki, S. L., *The Relevance of Physics*, chapter 11 (University of Chicago Press, London, 1966).
- ¹⁸ MacRae, D., *Ideology and Society*, chapters 11 and 12 (Heinemann, London, 1961).
- ¹⁹ Ravetz, J., *Scientific Knowledge and its Social Problems*, 386–397 (Clarendon Press, Oxford, 1971).
- ²⁰ Morris, D., *The Naked Ape* (Cape, London, 1967).
- ²¹ Reich, C., *The Greening of America* (Allen Lane, Penguin Press, Harmondsworth, 1970).
- ²² Young, R. M., in *The Limits of Human Nature* 235–274 (edit. by Benthall, J.), Allen Lane, Harmondsworth, 1973).

Science and works of art

J. Plesters Brommelle and N. S. Brommelle

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Over the past few years, considerable advances have been made in the application of scientific method to the conservation of works of art. Slow changes in paintings with exposure to light are still the subject of research but recent developments in the protection of outdoor sculptures are encouraging.

DESPITE much publicity about the intervention of science in the realm of art, comparatively few of the world's museums and galleries housing the fine and applied arts have their own scientific departments and the number of scientists engaged in this field is small compared with that of research workers on any subject of comparable breadth and complexity in universities or industry. Consequently, a particular technique or instrument may be available only in one museum laboratory in the world, not necessarily in Britain. The international scene in our special field of study is not densely populated and economic considerations are probably the limiting factor in the application of science to works of art.

There are three main areas of application: (1) examination to determine the physical and chemical composition of the object, the nature of past, present, and possible future deterioration and, on occasion, to aid dating or authentication; (2) conservation in the sense of providing the optimum environment to prolong the life of the object; (3) conservation in the sense of restoration, that is treatment of the object to repair existing damage or deterioration. Obviously all three are interdependent. For example, examination is a prerequisite of treatment and to return a treated object to an environment which does not minimise the risk of future damage is to nullify in part the benefits of restoration.

Paintings

European easel paintings, the kind seen in the National Gallery, London, and usually dating from about 1300 onwards and painted on wood panel or canvas, are a rather special category. Every component of the multi-layer structure which comprises a painting—wood, canvas or metal of support, ground or priming layers, pigment

and medium of paint layers, varnish or surface coating—can be subjected to scientific examination, and the layer structure itself merits close study. Although such examinations often aid conservation and sometimes research in art history, it should be stressed that the study of artists' materials and techniques is a proper subject in its own right.

Starting with the lowest layer of the picture, the support, identification by microscopical methods of the wood species used in panels gives an indication of the provenance of the picture, but little hint of its date. Carbon-14 dating, indispensable in archaeology, is not usually applicable to painting because of limitations of sample size and the small time-span involved, but an allied dating technique—dendrochronology, the measurement of annual growth rings of trees—has proved useful. First applied in Hamburg to panel paintings of Rembrandt and Wouwermans, the technique has been developed at the Research Laboratory for Archaeology and the History of Art at Oxford where a new reference curve for slow-grown oak has been compiled and measurements made on oak panels including paintings by Holbein and Rubens in the National Gallery and National Portrait Gallery¹. The quarter-sawn planks often had 200–300 rings. For pictures independently dated, the tree-ring date usually worked out to be the date of painting plus about five years for seasoning of the wood. Unfortunately the technique is unlikely to be extended to Italian panel paintings since these are mostly painted on poplar wood.

One of the most important developments has been in the identification of paint media which up to about 10 years ago could only be roughly classified. Gas chromatographic analysis, pioneered by the National Gallery laboratory² is now used in several other museum laboratories in various parts of the world to detect the fatty acids present in dried oil and egg films, the ratio of which indicates whether oil or egg was originally present and the type of drying oil. It was found, for example, that Piero della Francesca's "Baptism" was painted in egg tempera, but his "St Michael" in drying oil, specifically walnut oil. Occasionally different areas or even different layers of the same picture have different types of medium. For this reason it is as well to use some microscopical technique,



Virgin and Child by Pyrgotelis, Church of St Maria dei Miracoli, Venice. *a*, Detail before treatment, *b*, after cleaning and consolidation (carried out by K. Hempel, Victoria and Albert Museum, London, and G. Musumeci, Venice).

such as biological staining, on a paint cross section before analysis. Although ^{14}C dating is not generally used, detection in the United States of recent forgeries, even of late 19th century pictures, has proved possible by measuring the increase of ^{14}C in artists' materials—in this instance linseed oil—resulting from the atmospheric testing of nuclear weapons³.

Identification of pigments is still often done by well established methods of chemical microscopy, a less arduous exercise than it would seem in that the majority of artists' pigments in the past were simple compounds of metals such as oxides, carbonates or sulphides, or minerals or other crystalline materials having well defined particle characteristics and optical properties. Emission spectrography and X-ray diffraction have also long been used, the latter indicating the compound present rather than the elements only. The electron microbeam probe would be ideal for layer-by-layer analysis of the mounted paint cross section but its cost and complexity prevent its acquisition. Over the past decade we have been grateful to have occasional samples examined on instruments belonging to other institutions but a long run of coordinated samples is needed for significant results when buying or borrowing time on an instrument. That it can be done successfully is proved by a paper published by the Louvre laboratory on the scanning of sections of early Italian paintings⁴. Lacking an electron microbeam probe on the premises, the National Gallery has just acquired a laser microspectral analyser. The use of laser microspectrography in analysis of works of art was introduced in the laboratory of the Boston Museum of Fine Arts some years ago. In the apparatus now in London the laser is used to evaporate a minute amount of the sample, thereby forming a crater the dimensions of which may be varied between 10 and 100 μm . The firing of the laser is arranged to coincide with the sparking of carbon electrodes and the rest is emission spectrography.

All the above methods require removal of a sample, however small, so a non-destructive method of scanning the surface of the painting for elements is desirable. X-ray fluorescence analysis is an obvious choice, with hopes pinned on the energy-dispersive form. Even here the layer structure cannot be ignored for the primary X-ray beam may penetrate the uppermost paint layer with confusing results. Also the range of elements is greater than in, say bronzes or silver coins. Sensational in their possibilities are a whole range of analytical techniques based on the application of nuclear energy, again a facility not to be found in the museum itself. In the mid 1960s work was carried

out in both Delft and Munich on neutron activation analysis of trace elements of lead white. Results indicated variations not only with date but also with geographical location. In the United States autoradiography of paintings has been used to identify pigments, as has isotope mass spectrometry, and one method for dating lead white depends on measuring its natural radioactivity. It is an achievement in itself to have evolved or applied these techniques. One hopes that these will not be isolated experiments but the start of some serious data collecting. The Doerner Institute at Munich recently put out a paper in which the author had compiled a table of periods of use and terminal dates of artists' pigments from his extensive experience of analysing pigments from a vast number of pictures of different schools and dates, using fairly traditional methods. Simultaneously with analytical methods examination of paintings by X radiography, infrared and ultraviolet photography still goes on. A recent advance is infrared reflectography, developed in the Netherlands particularly for revealing carbon black underdrawing on white grounds. It involves the use of infrared light at a wavelength of about 2 mm and an infrared-sensitive television camera instead of infrared-sensitive plates or film⁵. The National Gallery in London now has such an apparatus. The need to control the humidity and temperature of the atmosphere in which paintings are hung, particularly wood panel paintings, was recognised as long ago as the 1930s, and the first fully air-conditioned room with humidity control, cooling, warming, dust filtration and removal of SO_2 was opened in the National Gallery in 1950 to house some of the most important panel paintings. Eventually all the west wing was air conditioned. Control of illumination took longer to gain acceptance. Now, in addition to having ultraviolet filters on all light sources, whether daylight or artificial, the visible light has been reduced to 150 lx. Obvious damage by light is fading of pigments and dye-stuffs but photochemical degradation of any organic component, whether medium, varnish or canvas, is also likely to occur. It is often assumed that after a hundred years or so paintings must have reached some sort of end point in the changes of colour they may undergo. There is no objective evidence for this assumption. In rare cases where part of a picture has been protected from light the contrast with the exposed part is alarming. Research has been going on in the National Gallery to find methods of recording very slow changes in colour of paintings. The recent acquisition of a reflectance spectrophotometer, designed for the purpose by W. D. Wright, is a significant step

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forward. The new extension to the National Gallery which is to open next year, will have temperature, relative humidity, sulphur dioxide and light levels automatically controlled and recorded by a data logger for future use, but there can be no cause for complacency while the east wing of the present building still lacks air conditioning.

Restoration

In the treatment of paintings science plays rather less part than is the case for other types of works of art, for example objects of metal and stone. Although it might make his task less hazardous and certainly more interesting, it is doubtful whether a knowledge of the mechanism of polymerisation of drying oils or the optics of paint films would enable a picture restorer to produce a visually satisfactory final effect in cleaning, retouching or varnishing a picture. There seems to be no substitute in this case for skill and experience. In the postwar years it had seemed likely that modern synthetic materials of a high degree of stability would replace the traditional natural materials used in picture restoration. This expectation was only partly fulfilled. In this country at any rate restorers often show reluctance to use as varnishes or retouching media materials which differ markedly in handling properties from those they are accustomed to, and this includes some polymers with otherwise desirable properties, for example polyvinyl acetate and some acrylic copolymers. For final varnishing many prefer the lower molecular weight polycyclohexanone resins despite the fact they have only a modest advantage over the natural resins in rate of discolouration; their handling qualities are, however, similar. On the backs of pictures modern synthetic materials are more frequently used, for example the non shrinking epoxy resin adhesives instead of animal glue.

Recently the standard treatment of canvas supports, that of sticking a new canvas on the back of an old and weakened one to strengthen it, has come under scrutiny. Lining of canvases dates at least from the late 17th century, the earliest adhesives being glue and flour paste, in this century frequently replaced by beeswax mixtures. Now in its turn the soundness of wax lining has been questioned and in April this year a three-day international conference met at the National Maritime Museum, Greenwich, to discuss lining techniques. Alternative types of adhesive were proposed, such as emulsions of vinyl polymers, and lining canvases of polypropylene or glass fibre were demonstrated. Also on show were improved types of vacuum hot tables which enable lining to be carried out without the heavy ironing previously needed. One of these had been designed at the Courtauld Institute of Art, University of London. Although no clear-cut resolution seems to have been taken in favour of any one method the conference was an opportunity for discussion of a rather neglected but necessary aspect of restoration.

For art objects, technical examinations has been directed towards problems of dating and authentication, supplementing the art historian's style criticism, and towards investigating details of structure, composition and condition. Those methods which supplement visual examination—optical microscopy, X radiography, ultraviolet fluorescence and infrared photography have of course been available for many years. Wet chemical analysis has been in use since the 19th century for metal objects. The methods of physical analysis, developed mainly since 1900 and particularly since the Second World War, are applicable as much to art objects as to those usually regarded as falling within the field of archaeology. They have been used more sparingly in museum specialising in post-classical material where there is still a certain reluctance to turn to scientific aids, particularly when sampling is involved.

Of the methods of absolute dating and the consequent possibility of authentication where stylistic evidence and details of provenance are insufficiently positive, three of the methods of the archaeologist are available: ^{14}C dating, dendrochronology and thermoluminescence. It is seldom feasible to use ^{14}C dating for art objects of comparatively recent date, while dendrochronology though applicable for example to the study of furniture and wooden sculpture does not seem so far to have been used for the former, and not extensively for the latter. Neither of these methods is in any case linked directly with the date of production of the art object. Much interest has been shown, however, in the technique of thermoluminescence (TL) for absolute dating and the detection of fakes in ceramic objects, this has been developed at, among other centres, the Research Laboratory for Archaeology and the History of Art at Oxford. The techniques were until recently of value mainly for dating pottery more than 500 years old. Advances since 1969 have extended the range to more recent times⁸. A study of authenticity of Italian Renaissance terracottas, in comparison with documented forgeries of high quality by a nineteenth century sculptor, has demonstrated the possibilities of solving problems of recent art history, though unfortunately not to the extent that the work of master and pupil can be distinguished. These methods have been used during the past few years for an extensive study of Chinese Tang dynasty ceramics (618–906 AD) with good dating results for genuine works and clear distinction between these and imitations.

Most of the physical methods of analysis of value in archaeological research find a use in attempting to solve particular problems in the decorative arts, though extensive studies of groups of objects of particular periods, districts or craftsmen, sufficient for making judgements for inclusion or rejection of doubtful pieces on analytical grounds, are rare. Moreover, comparison between analytical results from different institutions is often of dubious value because of differences in sampling techniques and absence of information on precision and accuracy. Of the methods available (which there is no space to list in detail here) X-ray fluorescence analysis, being non-destructive, is naturally attractive to the curator of valuable art objects, though the fact that it is less accurate than atomic absorption spectroscopy and that analysis is only of the surface layers of an object, which are usually of different composition from the body of the object, make its use less valuable, except for specific studies of superficial layers. Recent attention both in the United Kingdom and elsewhere has centred on the development of energy dispersive X-ray fluorescence spectrometers. Their increased sensitivity compared with crystal dispersion types makes it possible to use radio isotope sources or miniature X-ray tubes, thus imparting a degree of portability, and the multi-channel analyser greatly increases the speed of analysis. One form of this apparatus, the Isoprobe, referred to above in connection with paintings, and developed at the Oxford laboratory⁷ has recently begun operation as part of the York Glaziers' Trust Research Programme for studying the chemical composition of 12th century stained glass from York Minster. Most analytical methods require a sample such as a fine drilling from an unobtrusive part of an object, but the X-ray diffraction techniques described by Bimson⁸ for examining the crystalline constituents of soft and hard paste porcelain for authenticity or attribution requires a sample so small that the method could be almost described as non-destructive.

Environment

In a modern museum of the decorative arts, the tendency to display in one gallery art objects of every kind of a

particular period provides problems of environmental conservation over and above those of a gallery of paintings alone. The control of light levels, of vital importance for textiles, where both dyestuffs and their fabric substrates are damaged, is made difficult by the complexity of the 'visual problem' of local lighting for particular objects and reflections from show cases, so that disability glare factors impel the curator to call for light levels higher than the 50 lx generally accepted as desirable⁹. The problem of damage by light was first studied scientifically at the Victoria and Albert Museum (then the South Kensington Museum) in 1888. Studies relevant to paintings but also of general value have been made continuously at the National Gallery since the 1950s and experiments on the fading of textiles, begun at the Victoria and Albert Museum in the 1960s are continuing.

Requirements for temperature and relative humidity and the elimination of air pollution are broadly similar for most art objects even though the properties of particular objects may require localised conditions. For the general ambience the tendency, where it is possible, to install complete air conditioning is to recommend more complete elimination of pollutant gases, notably sulphur dioxide, than previously specified, and, particularly where textiles are involved, the elimination of a high percentage of grime in the submicron range (60% plus on test dust No. 1).

Turning to the many problems of the restoration of art objects, apart from those which require fine craftsmanship in repair work, specialists in the United Kingdom are taking part in notable developments in stone¹⁰ and textile conservation while close attention is being paid to problems of deterioration and protection of painted and stained glass¹¹. British workers are in the forefront of developments in these fields. One of these, the problem of arresting the decay of calcareous stone and marble sculpture (and more generally the stone of buildings), in modern industrial atmospheres was, as recently as 1963, declared to be insoluble¹². Consolidation treatment when the sculpture could be brought indoors was confined to wax impregnation. Deterioration out of doors is a consequence, still not fully understood, of the complex interaction of a more or less porous aggregate containing calcium carbonate, with water, carbon dioxide and sulphur dioxide together with soluble salts, including, in some situations, sodium chloride. If the sculpture can be brought indoors consolidation treatment is usually needed, in conjunction with cleaning. If the sculpture must remain *in situ* total loss of the object as a work of art must be expected eventually unless treatment is given. Recently, notable advances in treatment have been made and the prospects are now

brighter than in 1963. Consolidation inevitably involves impregnation with a material which enters the pores of the superficial deteriorated layers, preferably penetrating into or bonding with the sound stone beyond. Dutch and American investigators have been using selected epoxy resins, vinyl fluoride polymers and silicon esters and successful work has also been done with a solution of barium hydroxide and urea leading to the deposition of barium carbonate. A different approach has been adopted for external sculpture in Venice by English conservators¹⁰ who apply a monomer of trimethoxymethyl silane which penetrates easily and deeply into marble and polymerises with water to a hard cross-linked solid *in situ*.

In 1950 the late F. I. G. Rawlins, then Scientific Adviser to the National Gallery, announced in *Nature*¹³ the formation of a new international institute for the coordination and advancement of research in conservation. This was the International Institute for Conservation of Historic and Artistic Works of which he was Secretary-General for some years until one of us (N.S.B.) took over that post. The institute, which celebrates its 25th anniversary next year with its sixth international congress, in Stockholm, has its headquarters in Trafalgar Square, London. Its quarterly technical journal, *Studies in Conservation*, first published in 1952, contains original work and reviews on advances in conservation and restoration. This with its *Art and Archeology Technical Abstracts* (twice yearly) and the publications arising from its congresses enables its world-wide membership, which includes most of the world's leading conservators, to keep abreast of developments.

¹ Fletcher, J., *The Burlington Magazine*, **116**, 250 (1974).

² Mills, J. S., in *Proceedings of the Lisbon Congress, 1972, Conservation of Paintings and the Graphic Arts*, 701, International Institute for Conservation of Historic and Artistic Works.

³ Keisch, B., and Miller, H. H., *Nature*, **240**, 491 (1972).

⁴ Delbourgo, S., *Proc. Lisbon Congress, 1972*, 107.

⁵ van Asperen de Boer, J. R. J., *Studies in Conservation*, **14**, 96 (1969).

⁶ Fleming, S. J., *Archaeometry*, **15**, 13 and 29 (1973); **16**, 91 (1974).

⁷ Hall, E. T., Schweizer, F., and Toller, P. A., *Archaeometry*, **15**, 53 (1973).

⁸ Bimson, M., *Studies in Conservation*, **14**, 83 (1969).

⁹ Thomson, G., *Studies in Conservation*, **6**, 49 (1961).

¹⁰ Hempel, K., and Moncrieff, A., in *The Treatment of Stone* (Centro per la Conservazione delle Sculture all'Aperto, Bologna, 1972).

¹¹ Newton, R. G., *Art and Archaeology Technical Abstracts*, suppl., **10**, 132 (1973).

¹² *Building Research Station Digest*, First Series, No. 128 (1963).

¹³ *Nature*, **165**, 903 (1950).

Rutherford's Cavendish

Edward Bullard

Madingley Rise, Madingley Road, Cambridge, UK

Sir Edward Bullard recalls his early days at the Cavendish Laboratory in Cambridge where, under the watchful eye of Lord Rutherford, he worked, and struggled, as a research student in the company of people like Blackett, Kapitza, and Cockcroft.

I FIRST saw the Cavendish nearly 50 years ago, in December 1925, when I made an unsuccessful attempt to get a scholarship. The physics practical exam was held in the large room to the right of the arch as you come in. I was

a little surprised to find that my galvanometer was permanently jammed and that the assistants and demonstrators did not think it at all odd. Indeed, it was not odd: most of the equipment in the lab was like that.

The following year I went up to Clare and embarked on the Natural Science Tripos. I found the Part I Physics lectures deeply disappointing. Alex Wood, on mechanics, roughly, covered the first book of the *Principia* and the Cavendish experiment, together with a little about gyroscopes. I found that the lack of generality and the attempts to avoid all but the simplest calculus were tedious. I went off



Mr. F. Lincoln, the head of the Cavendish workshop

Cavendish Laboratory

to listen, a little uncomprehendingly, to Pars on analytical mechanics, and also, very briefly, to Larmor. By part II the lectures had become better and they gave a sense of contact with contemporary physics.

It was, I think, early in my third year that, coming out of a lecture, I met Patrick Blackett who asked me what I was doing. I replied that I had been listening to Aston talking about mass spectroscopes. "Why?", he said, "everything he says is in his book". It came to me as a revelation, and I went to no more lectures, except to Rutherford, whom I could not resist. He would boom on, talking about all kinds of interesting things, occasionally producing gems like "integral $y \cdot dx$; dx is small, we will neglect this". He talked so easily and informally, he knew it all in an instinctive, relaxed way and the answer always somehow came out right. As someone said: "The α particles were his friends, he knew what they would do" (there was a story, which I have not verified, that in one of his early papers the mass of an α particle came out as 3.3 on which he commented: "we take four as the nearest integer").

Although the practical classes interested me I learnt almost nothing useful in them. A practical class can serve three purposes: it can teach the techniques of measurement; it can allow the student to go over the path by which the principles of physics were discovered; or it can help to give contact with contemporary physics. The practicals in the Cavendish were largely historical, tempered with some feeling that, if the equipment was available (which it wasn't), there ought to be some experiments on modern physics. As it was, we swung pendulums which had their bobs in buckets of water and tried to find the frequency of a tuning fork with a phonic motor once used by Lord Rayleigh. (The usefulness of that potentially interesting experiment was reduced by the absence of a clock that could match the stability of the tuning fork.) The trouble was, I suppose, that there was very little money for equipment and that most of the very able and distinguished men running the classes had more important and exciting things to do. Indeed, when, in my third year as an undergraduate I tried to devise some new experiments for the Part II class, I found every encouragement and immediate acceptance of innovation.

I think the physics course in the 1920s was, in an important way, preferable to that of today. It was not very difficult. A reasonably intelligent man, who was interested in the subject, who took pains to understand it, and who could write in a way that would show that he did understand it, could get a first in the tripos without wearing himself out or concentrating too exclusively on science. He could take up politics or hiking, or learn a foreign language, without feeling that he was not giving enough time to physics.

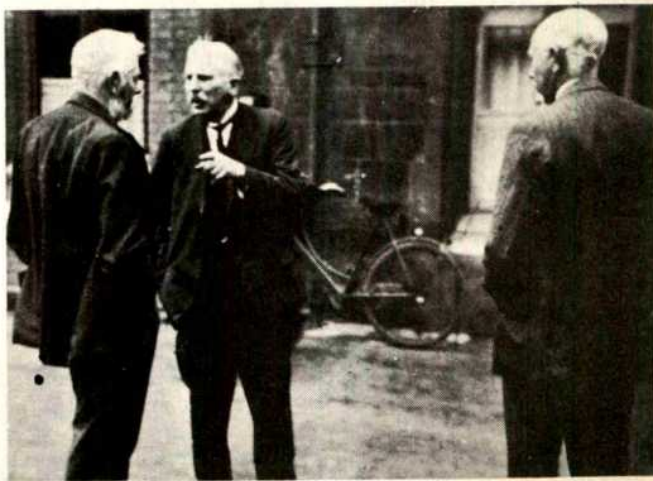
In the Cavendish one absorbed an attitude to knowledge,

and an intellectual style. A real physicist ought to be able to estimate any quantity within a factor of 10 without looking in a book, using only a pencil and the back of an envelope. The central idea was that when you really understood anything it would seem simple. When I was an undergraduate, this spirit filtered down through research students a few years older than I was, particularly through Jimmy Braddick, who was in a constant state of indignation about things that Rutherford had said to him, Paul White, a much more serious character, a mathematician and low-church Christian, and P. I. Dee, for a while my supervisor, who carried apparent omniscience with an engaging charm.

In 1929 Rutherford accepted me as a Ph D student. In those days it was usual for those starting as research students to spend the long vacation working in the 'kindergarten', an institution run by Chadwick. This was supposed to do what the undergraduate practical courses so clearly failed to do, that is, teach the techniques of modern physics. As I was going to study the scattering of slow electrons in gases I was set to learn vacuum techniques by measuring the vapour pressure of tap grease.

It proved a most instructive project and, more important, it enabled me to get to know Chadwick a little. Tap grease was a perennial problem in the Cavendish. It was normally made by a chemist in Southampton named Everett, the son of 'old Everett' who was J. J. Thompson's assistant. Old Everett had been mentioned in an agreement between J. J. and Rutherford, which specified that J. J.'s share of the lab funds would be £150 per annum "out of which he would pay his assistant". This tap grease was made by heating rubber bands with vaseline and adding paraffin wax. As Everett's product was sometimes overcooked and smelt of burnt rubber, the more fussy amongst us made our own. At about this time C. R. Burch, who worked for Metropolitan Vickers, introduced a much superior product prepared by distillation; it was, however, enormously expensive (I think £1 per pot) and in order to get it all of Oliphant's powers of persuasion were needed.

In 1929 I started work in a large room already occupied by Mark Oliphant, Phillip Moon and J. K. Roberts, all of whom were doing experiments involving vacuum techniques. After a few months I was joined by Harrie Massey. The room was just down the corridor from Rutherford's office. He would come round about once a week with Chadwick, and at odd times when he felt like blowing off steam. Once, he came in in the most cheerful good humour and said to Roberts "You know, Roberts, the time has come to consider whether your experiments are worth the liquid air they consume". On another occasion he told me that he never wanted to see me in the lab again. The next morning I got in early and managed to meet him on the stairs; he was particularly friendly. I never really got



Cavendish Laboratory

G. F. C. Searle, Rutherford and Aston



Lady Rutherford directing a gang of research students reconstructing her rock garden. The author, in shirt sleeves, looks on. Mr. Lincoln assembled these gangs on days when Rutherford was in London.

Cavendish Laboratory

to know him well but, as time went on, and particularly after I had left the Cavendish, I found that he would always listen and help me if I needed anything. I remember asking him for help in getting funds to enable one of Townsend's students to leave Oxford and come to work with me. Rutherford's reply was: "Well, I am never averse to any scheme for doing down Prof. Townsend". There was a half humorous resentment between them, dating, I suppose, from the days when they worked together in J. J. Thomson's Cavendish. On another occasion, when Townsend had made a rather intemperate attack on Massey and myself in the Royal Society's Proceedings, Rutherford met me in the corridor and, in great glee, said: "Have you seen what Townsend has said about you? What are you going to do about it". "Nothing," I replied: "Quite right," said Rutherford, "if you answer him he will wait 20 years, but he will get you in the end". It was, I suppose, a totally unjustified view of Townsend but it was enormously encouraging for me; Rutherford had a gift for that sort of thing, he could make you feel a colleague in a great enterprise. His boys were the best boys, surrounded by foolish and wicked men who did not understand what physics was really about. I think he felt himself to be an honest New Zealand farmer's boy fallen into a corrupt society; sometimes that made him rather rude to his more pompous colleagues and rather terrifying to his students. I recently asked Kapitza if he was scared of Rutherford. "Yes", he said; "terrified".

My period in the Cavendish was just before the *annus mirabilis* in which artificial disintegration and the neutron were discovered. Much has been written of this, and it was indeed the source of modern nuclear physics and engineering. My most dramatic memory is of going to the Kapitza Club (a weekly meeting of physicists, usually in Cockcroft's rooms in St Johns) and hearing Chadwick describe the Curie-Joliot results on 'penetrating γ rays', and then going the next week and hearing Chadwick again, explaining that they were really new particles—neutrons—if he had suspected the truth on the first occasion, he gave no clue).

The fame of these discoveries has obscured the feelings that preceded them. Many young men coming into the subject in the late 1920s felt that it was no time to start to work on radioactivity. The existing techniques had been pushed near their limits and many, including myself, did not realise what could be done with the emerging tech-

nology of electronics. It was Wynn-Williams and Cockcroft who, in different ways, broke the barrier. This involved not only inventing things, but also overturning deep seated traditions of do-it-yourself and not spending money. Cockcroft's elegant solution to the problem of producing 300kV of d.c. power was preceded by some very odd schemes which included using an induction coil given to the University by a collector of scientific equipment, and a Tesla coil (which was noisy enough to produce a complaint from the Master of Corpus, on which Rutherford is said to have commented: "If they don't like the noise why did they build the college there"). An early breakthrough in spending money was the purchase of the doughnut magnet, designed by Cockcroft, which was used to focus α particles. Rutherford, when showing it to a visitor, said: "That cost as much as a research student for a year—but it does twice as much work".

The feeling for economy went all through the organisation; I remember the store man trying to maintain that the existing stock of valves, World War I bright-emitter triodes, must be used up before modern types could be bought. The day-to-day detail was looked after by Mr Lincoln, the fierce head mechanic, whose bark was much worse than his bite. In my third year as an undergraduate I complained about the absence of bandages in the first-aid box, and he said: "What's the use of my keeping bandages, people only come and use them". On another occasion I asked for six inches of one inch steel tube and was surprised when he said: "Certainly I have got just the thing". He went into the room behind the workshop and produced a bicycle left, I suppose by some undergraduate, and told me to saw six inches off the cross bar.

It is easy to tell funny or slightly discreditable stories about the Cavendish in those days, but the fact remains that it was uniquely successful both in discovery and in training many of the most able and effective men of their generation—Blackett, Cockcroft, Kapitza, Oliphant and many others. The defects of parsimony and, to some extent of arrogance, were easily cured in the years that followed. In many ways the experience of the war ideally complemented and compensated for the defects of the training in the Cavendish 10 years before.

For me, Rutherford and Blackett represent most of what is best in physics, and I am deeply conscious of the exceptional advantages that I had from being a student in the right place at just the right time.

(Continued from p. 742)

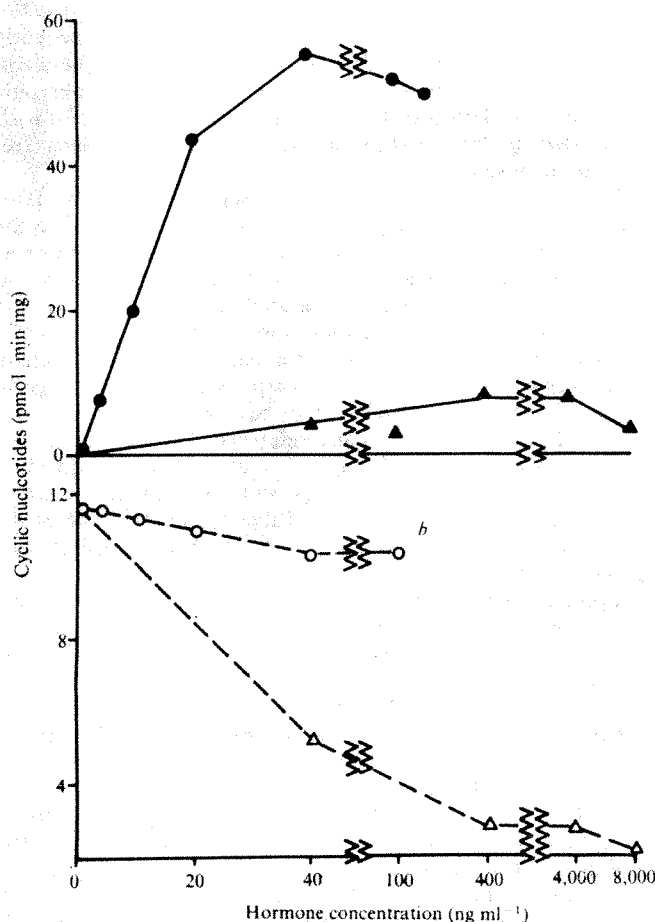


Fig. 3 Changes in membrane-bound cyclase activities. BALB/c 3T3 cells were grown to confluency in 10% serum as in Fig. 1a. After washing and detaching them as in Table 1, and washing further in 0.01 M potassium phosphate (pH 7.4), 0.15 M NaCl, the cells were resuspended in Buffer A (Table 1) and lysed under nitrogen cavitation at 6000 pound inch⁻² in an Artisan Pressure Homogeniser (diameter of orifice 0.068), 5 min being allowed for equilibration. The homogenate was made 0.001 M in EDTA_{Na}, and centrifuged at 2,000 g for 5 min. Plasma membrane fractions were isolated (method to be published). Approximately 2.3 mg of protein was recovered with specific activity for 5'-AMPase of 108 nmol hydrolysed per h per mg protein at 37° C compared with 3 nmol per h per mg in the unfractionated lysate. Reaction mixtures (100 µl) contained for a, guanylyl cyclase (—●—, —▲—): 0.04 M HEPES (pH 7.6), 0.006 M MnCl₂, 0.002 M GTP, 0.0005 M EDTA_{Na}, 0.0005 M DTT, 0.3 M sucrose, 0.01 M theophylline, 0.02 M caffeine, 12 µg of plasma membrane protein, 20 µg BSA or for b, adenyl cyclase (—○—, —△—) 0.006 M MgCl₂ replaced MnCl₂ and 0.002 M ATP, 0.015 M creatine phosphate, 4 µg of creatine phosphokinase replaced GTP. Reactions were incubated for 10 min at 37° C and terminated as described in Table 1. Cyclic nucleotides synthesised were estimated with the radioimmuno assay (Fig. 1). Either varying concentrations of FGF (—●—, —○—) or insulin (—▲—, —△—) were added to incubation mixtures and results are expressed as pmol of cyclic nucleotides synthesised per min per mg of plasma membrane protein, after deduction of the value for a 10 min incubation with no hormone addition for the guanylyl cyclase mixtures (10.5 pmol) or after deduction of the zero time point for the adenyl cyclase mixtures (2.9 pmol). For both reactions synthesis was proportional to time of incubation up to 20 min. Control reactions with added cyclic nucleotides showed no appreciable hydrolysis (less than 2 pmol min⁻¹ mg⁻¹). Boiling FGF for 5 or insulin for 1 h in neutral buffers destroyed their capacity to increase cyclic GMP levels and DNA synthesis in cultured cells and their effects on cyclase enzymes *in vitro*. Addition of 1 µg ml⁻¹ BSA or purified gelatin had no effect on cyclase enzymes *in vitro*.

concentrations of FGF in the presence of hydrocortisone^{9,10} or high, non-physiological concentrations of cyclic GMP⁵ can initiate DNA synthesis in mouse fibroblasts in the absence of

exogenously-added serum. All these observations strongly implicate cyclic GMP as a positive signal which starts the chain of events leading to the initiation of DNA synthesis and cell division. The apparent transience of the increase in intracellular cyclic GMP does not necessarily negate this, since similar variations are observed in intracellular cyclic AMP concentrations after exposure of cells to adrenergic or peptide hormones whose actions are mediated by cyclic AMP²².

The observed increases in intracellular cyclic GMP concentration and DNA synthesis upon addition of high, non-physiological concentrations of insulin is probably an indirect consequence of additional decreases in intracellular cyclic AMP. Hydrocortisone, as it does not immediately change intracellular cyclic nucleotide levels or activities of the membrane-bound cyclase systems almost certainly exerts its permissive effect through the internal cellular machinery¹³.

The concept of a dualistic theory of cellular control involving cyclic AMP and cyclic GMP both in their antagonistic physiological effects²³ and in control of fibroblast cell growth⁵ may thus be extended to the receptor systems for adenyl and guanylyl cyclase of the plasma membrane and their respective hormones. These systems may possibly initiate cell growth through cyclic GMP or expression of specialised and differentiated functions through cyclic AMP. Though such a general model holds promise not only for physiological events but even more for developmental processes, there are important differences from this relationship. Analysis of cyclic AMP and cyclic GMP concentrations throughout the cell cycle in synchronised fibroblasts show that the specific, transient increase in cyclic GMP occurs early in the G1 phase, whereas cyclic AMP exhibits rhythmic changes during the cell cycle⁵. Results with insulin also stress the possible importance of a negative interference between the two cyclic nucleotide systems. It would not be surprising if growth induction in different organs or tissues in the intact animal were to be primarily controlled by a new class of polypeptide hormones, from the pituitary and submaxillary glands⁹⁻¹¹, which bind to the cell surface and activate the guanylyl cyclase system, the specificity of the inductive process being determined by differences between surface receptors on the various target cells. This could then liberate the adenyl cyclase system for use by those hormones or factors concerned with the synthesis and release of specialised products.

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¹ Hershko, A., Mamont, P., Shields, R., and Tomkins, G. M., *Nature new Biol.*, **232**, 206-211 (1971).

² Rudland, P. S., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 750-754 (1974).

³ Dulbecco, R., *Nature*, **227**, 802-806 (1970).

⁴ Holley, R. W., and Kierman, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 300-304 (1968).

⁵ Seifert, W., and Rudland, P. S., *Nature*, **248**, 138-140 (1974).

⁶ Otten, J., Johnson, G. S., and Pastan, I., *J. biol. Chem.*, **247**, 7082-7087 (1972).

- ⁷ Sheppard, J. R., *Nature new Biol.*, **236**, 14–16 (1972).
- ⁸ Hadden J. W., Hadden, E. M., Haddox, M. K., and Goldberg, N. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3024–3027 (1972).
- ⁹ Gospodarowicz, D., *Nature*, **249**, 123–127 (1974).
- ¹⁰ Rudland, P. S., Seifert, W. E., and Gospodarowicz, D., *Proc. natn. Acad. Sci. U.S.A.*, (in the press).
- ¹¹ Armelin, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2702–2706 (1973).
- ¹² Robinson, G. A., Butcher, R. W., and Sutherland, E. W., *Cyclic AMP* Academic Press, NY 1971).
- ¹³ Ballard, P. L., and Tomkins, G. M., *J. Cell Biol.*, **47**, 222–234 (1970).
- ¹⁴ Temin, H. M., *J. cell. comp. Physiol.*, **69**, 377–384 (1967).
- ¹⁵ Hardman, J. G., and Sutherland, E. W., *J. biol. Chem.*, **244**, 6363–6370 (1969).
- ¹⁶ White, A. A., and Aurbach, G. D., *Biochim. biophys. Acta.*, **191**, 686–697 (1969).
- ¹⁷ Hardman, J. G., Beavo, J. A., Gray, J. P., Chrisman, T. D., Patterson, W. D., and Sutherland, E. W., *Annals N.Y., Acad. Sci.*, **185**, 27–35 (1971).
- ¹⁸ Steiner, A. L., Parker, C. W., and Kipnis, D. M., *J. biol. Chem.*, **247**, 1106–1113 (1972).
- ¹⁹ Jiménez de Asua, L., Surian, E. S., Flawia, M. M., and Torres, H. N., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1388–1392 (1973).
- ²⁰ Illiano, G., and Cuatrecasas, P., *Science*, **175**, 906–908 (1972).
- ²¹ Beavo, J. A., Hardman, J. G., and Sutherland, E. W., *J. biol. Chem.*, **246**, 3841–3846 (1971).
- ²² Illiano, G., Tell, G. P. E., Siegel, M. I., and Cuatrecasas, P., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2443–2447 (1973).
- ²³ Goldberg, N. D., O'Dea, R. F., and Haddox, M. K., in *Advances in Cyclic Nucleotide Research* (edit. by Greengard, P., and Robison, A. G.), vol. 3, (Raven Press, New York, in the press).
- ²⁴ Pierson, R., and Temin, H. M., *J. cell. comp. Physiol.*, **79**, 319–330 (1972).
- ²⁵ Kimura, H., and Murad, F., *J. biol. Chem.*, **249**, 329–331 (1974).

Evidence for a unique kind of α -type globin chain in early mammalian embryos

MAMMALIAN embryonic haemoglobins are synthesised in nucleated erythroid cells derived from the yolk sac¹. They are structurally different from foetal and adult haemoglobins. It has been generally assumed that the difference of embryonic to foetal or adult haemoglobin is solely due to an embryonic globin chain structurally related to the β chain. It was believed that embryos either synthesise no α -type chain at all or an α chain structurally identical to the adult α chain^{2,3}.

In mice and in rabbits we show the existence of a unique embryonic globin chain (x chain). The different x chains of the two species are closely related in respect to their primary structure and are very similar to the ζ chain of the human haemoglobin Portland I^{4,5}. The ζ chain can therefore be considered to be a human x-type chain. It was suggested that a haemoglobin found in early human embryos is identical with Portland I (refs 6–8).

The presented structural data and the functional relationship of x and α chains prove that the x chains are in fact α -type chains. Furthermore, we find more structural similarities between the x chains of different species than between the x and α chains of the same species. This indicates an early evolutionary divergence of x- and α -chain genes and demonstrates another case of gene duplication of globin genes during evolution⁹.

The embryonic haemoglobins of BALB/c mice and of New Zealand white rabbits were isolated. Embryonic haemoglobins can be separated from adult haemoglobins by column chromatography or acrylamide gel electrophoresis. Using CMC-urea columns, three embryonic haemoglobins are observed in mice: HbE_I, HbE_{II}, HbE_{III}. The tetramer of HbE_I consists of a pair of non- α and a pair of non- β chains: $\alpha_2\gamma_2$; HbE_{II} and HbE_{III} consist of a pair of α and a pair of non- β chains $\alpha_2\gamma_2$ and $\alpha_2\zeta_2$ respectively^{10,11}. γ and ζ chains are similar to the β chain and can be regarded as ϵ chains (refs 11–13 and our own observa-

tions, to be published). Using a pH gradient and CMC columns, we found that embryonic haemoglobins of the rabbit consist of at least six haemoglobin fractions: a group of three early eluting embryonic haemoglobins HbE_I–HbE_{III} with the chain composition $\alpha_2\epsilon_2$ plus a group of late eluting embryonic haemoglobins HbL_I–HbL_{III} with the chain composition $\alpha_2\epsilon_2$. There are at least two structurally different ϵ -chains (ref 11 and our results).

Mice and rabbit x chains were separated by chromatography^{14–16}. In mice the y chain frequently eluted close to the x chain; in rabbits separation between x and ϵ chains is usually incomplete. The x chains were digested by trypsin and the tryptic peptides were separated by the fingerprinting method. The amino acid composition of the tryptic peptides is shown in Table 1. They were compared with α and β chain sequences of mouse, rabbit and carp. According to the principle of least dissimilarity, the best correspondence was obtained using the α chains as a reference. Two peptides of the x chains are identical with typical α -chain peptides (residues 93–99 and 140–141). Several other peptides of the two x chains are similar to α -chain sequences (Table 1, Fig. 1). None of the peptides shows an amino acid composition with closer correspondence to β than to α chain peptides.

Comparing the difference of x and α chains of the same species we found that in the mouse x chain 29 amino acid substitutions are necessary in order to match a sequence of 87 amino acid residues of the α chain. The minimum difference of this stretch is 33%. The difference, if the complete chains are compared, is probably higher, since several x chain peptides are so different from α -chain peptides that they could not be matched at all (Table 1).

In the rabbit x chain we found 24 amino acid substitutions in comparison to a corresponding sequence stretch of 67 amino acid residues of the rabbit α -chain which would reflect a structural difference of 36%. The difference of the human x to the human α chain is in the same order of magnitude with 11 substitutions of a corresponding α chain sequence 38 amino acids long.

Human, mouse and rabbit x chains show much more sequence homology than the x and α chains of the same species. The difference between corresponding peptides with 49 amino acid residues of mouse and rabbit x chains is nine amino acid substitutions, whereas the difference between mouse x and mouse α chain is 19 substitutions. The difference between mouse and human x chains of a sequence of 39 amino acid residues is six substitutions, whereas the difference between mouse x- and mouse α -chain is 13 substitutions. Finally, the difference between rabbit x- and human x-chains is six sub-

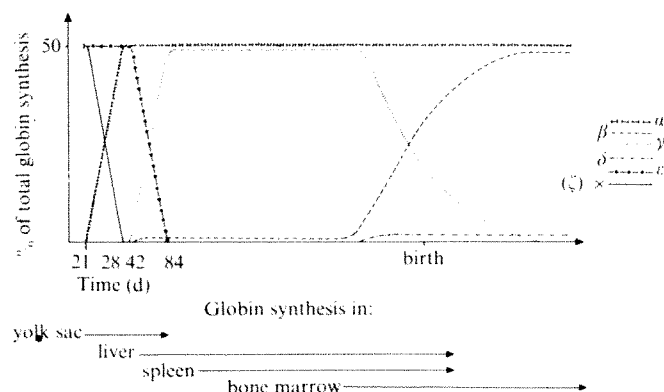


Fig. 1 The amino acid compositions of the tryptic peptides of the human ζ chain and of mouse and rabbit x chains (Tables 1 and 2) were sufficiently similar to some α -chain peptides. The composition data of the human ζ chain were taken from Capp et al.⁵. The sequence homologies are shown.

Amino acid pos. no.	57	61	62	63	63a	70	71	82	83	89	90	92	93	99				
x human	Ala	His	Gly	Ser	Lys			*	Leu	Ser	Glx	Leu	His	Ala	Tyr	Ile	Leu	Arg
x mouse	Ala	His	Gly	Phe	Lys	Val	Met	Val	Asx	Ala	Ile	Thr	Gly	Ala	Lys			
x rabbit	Ala	His	Gly	Thr	Lys	Val	Ala	Val	Asx	Ala	Leu	Ala	Gly	Ala	Lys	Glx	Glx	Ala
α human	Gly	His	Gly	Lys	Lys	Val	Ala	—	Asp	Ala	Leu	Thr	Asn	Ala	Val	Ala	His	Val
α mouse	Gly	His	Gly	Lys	Lys	Val	Ala	—	Asp	Ala	Leu	Ala	Thr	Ser	Ala	Gly	Ala	His
α rabbit	Ala	His	Gly	Lys	Lys	Val	Ser	—	Gln	Ala	Leu	Thr	Lys	Ala	Val	Gly	His	Leu
α carp	Lys	His	Gly	Lys	Lys	Val	Ile	Met	Gly	Ala	Val	Gly	Asp	Ala	Val	Ser	Lys	Ile
Amino acid pos. no.	32					40				128					139	141		
x human	Leu	Phe	Leu	Ser	His	Pro	Glx	Thr	Lys	Phe	Leu	Ala	Ser	Val	Ser	Thr	Glx	Leu
x mouse	Leu	Phe	Cys	Ser	Tyr	Pro	Glx	Thr	Lys	Phe	Leu	Ala	Ser	Ile	Ser	Thr	Asx	Leu
x rabbit	Leu	Phe	Cys?	Ser	His	Pro	Glx	Thr	Lys	Phe	Leu	Leu	Ser	Val	Ser	Thr	Asx	Leu
α human	Met	Phe	Leu	Ser	Phe	Pro	Thr	Thr	Lys	Phe	Leu	Ala	Ser	Val	Ser	Thr	Val	Leu
α mouse	Met	Phe	Ala	Ser	Phe	Pro	Thr	Thr	Lys	Phe	Leu	Ala	Ser	Val	Ser	Thr	Val	Leu
α rabbit	Met	Phe	Leu	Gly	Phe	Pro	Thr	Thr	Lys	Phe	Leu	Ala	Asn	Val	Ser	Thr	Val	Leu
α carp	Met	Leu	Thr	Val	Tyr	Pro	Gln	Thr	Lys	Phe	Phe	Gln	Asn	Leu	Ala	Leu	Ser	Gln

* Lys or Arg

* Lys or Arg

Fig. 2 Scheme of the switches of human haemoglobin chains during ontogenesis. The type of haemoglobin which is synthesised is not organ specific with the possible exception of embryonic haemoglobins. For the time course of erythropoiesis in yolk sac, liver, spleen and bone marrow we used the data cited by Metcalf and Moore¹.

stitutions of a stretch of 39 amino acid residues, the difference between rabbit x and rabbit α chain is 13 amino acids. It is only possible to place a rabbit x-chain peptide into position 71-82 if the insertion of one amino acid in position 63a is accepted. This additional amino acid is also present in the α chain of the carp (Fig. 1).

We also tried to obtain data on the time of synthesis of the embryonic globin chains in the course of embryogenesis. Mouse yolk sac erythropoiesis starts at day 7 of gestation and is succeeded by liver erythropoiesis at day 12-14 (ref 10). It was previously shown that the relative rates of synthesis of the three

embryonic haemoglobins change during the course of embryogenesis. Synthesis of HbE_I decreases markedly whereas the synthesis of HbE_{II} increases during embryonic development. The synthesis of HbE_{III} seems to decrease as well¹⁷.

The rate of synthesis of embryonic globin chains was determined by labelling embryonic mouse yolk sac cells at day 9.5 of gestation with ³H-leucine at 37° C for 6 h in leucine-free tissue culture medium, and at day 11.0 with ¹⁴C algae hydrolysate in amino acid free medium¹⁵. Our data show that during the time from day 9.5 to day 11.0 of gestation the rate of synthesis of the ε-type globin chains (y and z) stays constant;

Table 1 Amino acid composition data of the tryptic peptides of mouse and rabbit embryonic x chains

Amino acid	A. Mouse: Residue no. which homologised to known α chain sequences																Peptides that could not be homologised							
	1-7 a* b†	17-31 a b	32-40 a b	41-56 a b	57-61 a b	62-70 a b	71-82 a b	83-89 a b	90-92 a b	93-99 a b	128-139 a b	140-141 a b	a b	a b	a b	a b	a b	a b	a b	a b	a b	a b	a b	a b
Lysine			1.4	1		0.9	1	1.0	1				0.8	1										
Histidine					3.0	3																		
Arginine		1.0	1		0.8	1																		
Aspartic Acid					1.0	1																		
Threonine		1.9	2	1.0	1	0.7	1																	
Serine				1.0	1	1.0	1																	
Glutamic Acid		4.0	4	1.1	1	2.2	2																	
Proline		1.2	1	1.0	1	1.0	1																	
Half Cystine			0.5	1																				
Glycine		1.0	1		0.8	1	0.9	1	1.0	1														
Alanine		3.2	3				1.2	1	2.2	2														
Valine							1.8	2																
Methionine		0.8	1				0.8	1																
Isoleucine		0.9	1				0.9	1																
Leucine		0.9	1																					
Tyrosine			0.9	1	2.2	2																		
Phenylalanine			1.0	1	2.3	2	0.9	1																
No. of residues yield [10 ⁻⁹ M]		15	9	16	5	10	7	3	7	1	12	2	8	10	2	4	30							
B. Rabbit:																								
Lysine	0.9	1		1.0	1		1.0	1	0.8	1	0.8	1												
Histidine				1.4	1		0.9	1																
Arginine																								
Aspartic Acid	1.0	1																						
Theonine			0.9	1		0.6	1																	
Serine			0.9	1																				
Glutamic Acid			1.1	1		1.9	2	1.0	1															
Proline			1.0	1		0.4	1																	
Half Cystine			§	1																				
Glycine	1.0	1				1.1	1	1.0	1	1.0	1													
Alanine	2.3	2				1.1	1	4.5	4	2.2	2	1.1	1											
Valine	1.0	1				1.5	2	1.1	1															
Methionine																								
Isoleucine																								
Leucine	0.9	1		0.8	1																			
Tyrosine																								
Phenylalanine			0.9	1																				
No. of residues yield [10 ⁻⁹ M]	7	10	8	10	5	10	9	14	10	3	12	6	15	23	9	10	12							

*a: residues.

†b: nearest whole number of residues.

‡ Phenylalanine is present.

§ Half cystine is usually destroyed during hydrolysis.

|| Proline is present.

The embryonic globin chains of mouse and rabbit were separated as described (11,16). The x chains were digested with trypsin and after separation of the tryptic peptides by fingerprinting the amino acid composition analysis was carried out as described (15). Line 1 indicates the residue numbers that were homologised to known α-chain sequences. The composition data of peptides that could not be homologised to α-chain peptides are also given in the last columns of the Table.

on the other hand the synthesis of the χ chain decreases from 18% to 9% while there is an increase of α -chain synthesis from 82% to 91%. The decrease of χ -chain synthesis is thus compensated by an increase of α -chain synthesis.

Similar data have been obtained for the embryonic haemoglobins of the rabbit. Those haemoglobins which contain χ and ϵ chains (HbE_I-HbE_{III}) show a decrease of the rate of synthesis, whereas those containing α and ϵ chains (HbL_I-HbL_{III}) increase correspondingly during yolk sac differentiation¹¹. These data show that χ - and α -chain synthesis in both species is inversely correlated.

This and the above cited structural evidence proves that the χ chain takes the place of the α chain during early embryogenesis. The χ chain therefore could be expected to compensate for the α chain in situations where no α -chain synthesis is possible. Haemoglobin Portland I tetramer containing $\chi_2\gamma_2$ is observed in infants with homozygous α -thalassaemia^{7,8}.

These data taken together with suggestive evidence of others on the human χ chain⁶⁻⁸ lead us to propose the following scheme of development of globin chain synthesis in man (Fig. 2). As in mice and rabbits we expect, during early yolk sac erythropoiesis, predominantly haemoglobins of the constitution $\chi_2\epsilon_2$. Later, but during yolk sac erythroid differentiation, the α chain replaces the χ chain to give haemoglobins of the constitution $\alpha_2\epsilon_2$. The ϵ chain is replaced by the β chain after the switch of erythropoiesis from the yolk sac to the foetal liver.

In view of this scheme, the existence of ϵ_4 in human foetuses remains unexplained^{2,3}. No ϵ_4 -type haemoglobins have been observed by us during the embryonic phase of erythroid differentiation in mice and rabbits. It might well be that the globin χ chains of the embryonic haemoglobins $\chi_2\epsilon_2$ are degraded at a different rate thus leading to the formation of the ϵ_4 tetramer which is found in human foetuses.

A structural comparison of χ and α chains of mice, rabbits and of man⁵ shows the existence of a group of embryonic χ -type chains which have many features in common. Our data show that the χ -chain genes of all three species have evolved from a common ancestor gene which in turn is derived from an ancestral α -globin gene by gene duplication. We do not know when this duplication occurred, but the many similarities within the three investigated mammalian χ chains and the large common difference as compared to the α -chains suggest that the divergence of α and χ chains is much older than mammalian evolution.

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¹ Metcalf, D., and Moore, M. A. S., *Haemopoietic Cells*, 172-195, (North-Holland, Amsterdam and London, 1971).

² Huehns, E. R., Dance, N., Beaven, G. H., Keil, J. V., Hecht, F., and Motulsky, A. G., *Nature*, **201**, 1095-1097 (1964).

³ Hecht, F., Motulsky, A. G., Lemire, R. J., and Shepard, T. E., *Science*, **152**, 91-92 (1965).

⁴ Capp, G. L., Rigas, D. A., and Jones, R. T., *Science*, **157**, 69-66 (1967).

⁵ Capp, G. L., Rigas, D. A., and Jones, R. T., *Nature*, **228**, 278-280 (1970).

⁶ Kaltsoya, A., Fessas, P., and Stavropoulos, A., *Science*, **153**, 1417-1418 (1966).

⁷ Todd, D., Lai, M. C. S., Beaven, G. H., and Huehns, E. R., *Br. J. Haematol.*, **19**, 27-31 (1970).

⁸ Weatherall, D. J., Clegg, J. B., and Boon, W. H., *Br. J. Haematol.*, **18**, 357-367 (1970).

⁹ Dayhoff, M. O., Hunt, L. T., McLaughlin, P. J., and Jones, D. D., in *Atlas of Protein Sequence and Structure* (edit. by Dayhoff, M. O.), (17-30) (Washington, DC, 1972).

¹⁰ Fantoni, A., Bank, A., and Marks, P. A., *Science*, **157**, 1327-1329 (1967).

¹¹ Steinheider, G., Melderis, H., and Ostertag, W., in *Int. Symp. Synthesis, Structure and Function of Hemoglobin*, 1971 (edit. by Martin, H., and Novicki, L.), 225-235 (Lehmanns, München, 1972).

¹² Gilman, J. G., Thesis (University of Wisconsin, 1973).

¹³ Gilman, J. G., and Smithies, O., *Science*, **160**, 885-886 (1968).

¹⁴ Huisman, T. H. J., Martis, E. A., and Dozy, M. A., *J. lab. clin. Med.*, **52**, 312-327 (1958).

¹⁵ Ostertag, W., Melderis, H., Steinheider, G., Kluge, N., and Dube, S., *Nature new Biol.*, **239**, 231-234 (1972).

¹⁶ Dintzis, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **44**, 247-261 (1961).

¹⁷ Fantoni, A., De La Chapelle, A., and Marks, P. A., *J. biol. Chem.*, **244**, 675-681 (1968).

Hormonal control of oestrogen receptor in uterus and receptivity for ovoidimplantation in the rat

THREE distinct states of the rat uterus with respect to ovoidimplantation have been described. The prereceptive stage lasting from day 1 to day 4 is followed by receptivity during day 5; finally, during a postreceptive period from day 6 onwards, transferred mature blastocysts do not implant if implantation on day 5 has been prevented¹. Analogous situations can be created in the castrated animal by treatment with a precise sequence of hormones: a minimum of 2 d of progesterone preparation is necessary to obtain, 18 h after the subsequent administration of a small dose of oestrogen the state of receptivity for ovoidimplantation which will last for approximately 12 h. Again, a period during which the fertilised egg cannot implant will follow the time of receptivity, as shown by egg transfer experiments¹.

Thus in both intact and castrated animals oestrogen seems to be effective only after progesterone treatment. The possible involvement of the specific high affinity intracellular oestrogen receptor (see papers of the groups of Jensen, Gorski, King, Erdos, Bresciani, Jungblut and this laboratory in ref. 2) in the control of tissue responsiveness for oestrogen has therefore been examined. We have studied the influence of progesterone and oestradiol on the level of oestrogen receptor in both the endometrium and myometrium of the castrated rat uterus. We have compared the results with the variations observed during early pregnancy. There is strong evidence that the presence of such receptors in the cytoplasm is necessary to mediate the action of oestrogen hormones on the target tissues².

We separated uterine endometrium from myometrium by gentle scraping with a scalpel. A Scatchard³ plot was made to show the binding of homogenised endometrium and myometrium supernatant fraction to ³H-oestradiol (as described previously⁴ (Fig. 1). A consistent difference between the apparent equilibrium dissociation constants of the receptor-hormone complexes of the endometrium $K_d = 3 \times 10^{-10} - 5 \times 10^{-10}$ M) and myometrium ($K_d = 6 \times 10^{-10} - 9 \times 10^{-10}$ M) may reflect different degree of interference of low-affinity sites on the binding equilibrium, since straight line Scatchard plots indicating a single class of high-affinity binding sites are obtained when the whole uterus homogenate is used as the source of the supernatant receptor^{2,4}.

The variations in the receptor concentration during pregnancy up to day 8 are shown in Fig. 2. Whether related to total soluble protein or to DNA content of the tissue, the endometrium receptor concentration first decreased

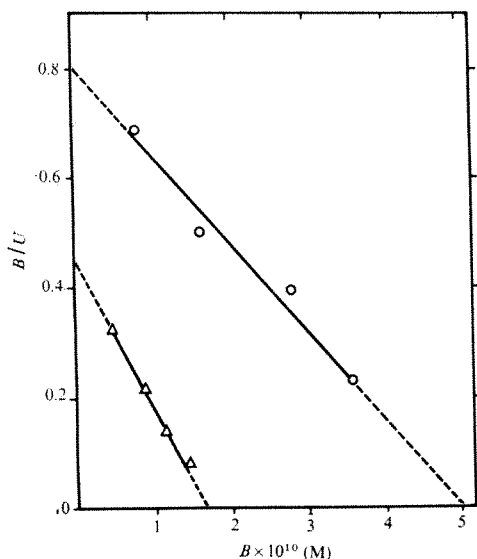


Fig. 1 Determination of the receptor concentration in the endometrium (Δ — Δ) and pooled or individual myometrium (\circ — \circ) homogenates. The uterus was removed from a 2 day pregnant rat; the endometrium and myometrium were homogenised separately in respectively 1 ml or 2 ml of the sucrose (0.25 M)— MgCl_2 (3 mM) medium. After low speed centrifugation ($800g \times 10$ min), the supernatant fractions were incubated with radioactive oestradiol for 30 min at 30°C . The receptor bound to unbound (B/U) hormone ratio and the concentration of hormone bound (B) to low-affinity receptor sites in equilibrium solutions (0.2 ml) containing 0.2, 0.5, 1.0 or 2.0 nM ^3H -oestradiol were determined by adsorption with charcoal (0.25% Norit A)—dextran (0.0025%, molecular weight 90,000) suspension (0.5 ml) for 10 min at 30°C (ref. 4.) The concentration of the oestrogen receptor binding sites is indicated by the intercept of the experimental straight lines on the abscissa; the inverse slopes of the respective straight lines are equal to the equilibrium dissociation constants ($3.5 \times 10^{-10}\text{M}$ for the endometrium, $6.25 \times 10^{-10}\text{M}$ for the myometrium preparation). Although by this technique only directly available specific binding sites are measured, it has been demonstrated¹⁰ that the concentration of endogenous oestrogen in cytosol is negligible under physiological conditions (oestrus cycle and early pregnancy) when compared to the amount of receptor. The measurement of the receptor concentration in low-speed and in high-speed ($105,000g \times 60$ min) supernatant preparations of rat uterine tissue has been shown to yield identical results¹⁰.

slightly from day 1 to day 2 to rise strongly thereafter. The receptor concentration decreased between days 5 and 8 as seen clearly when expressed on a DNA basis. The values for the myometrium receptor are considerably lower and the variations less marked.

It is known that the secretion of both oestradiol and progesterone in the pregnant rat increases from the evening of day 2 and remains substantially elevated from days 3 to 10 (refs 5,6). Therefore we performed an ovariectomy on the morning of day 2 of pregnancy. We measured the influence of oestrogen and progesterone on the level of oestrogen receptor in the uterus of rats 3 weeks after the ovariectomy. The results are shown in Fig. 2. Both hormones, alone and in combination, caused an increase in the endometrial receptor level. The increase was due partly to cell proliferation but also to a rise in the cellular concentration of the receptor. There was no additivity of oestrogen and progesterone (simultaneously administered) under these experimental conditions. Progesterone showed little effect on the myometrium, whereas oestradiol caused a large increase in the content of myometrium receptor, compared with soluble protein and total DNA. The oestradiol effect on myometrium was significantly counteracted by a simultaneous progesterone administration.

Next we examined the hormone requirements for the maintenance of the elevated endometrium receptor concentration during pregnancy. Rats were castrated on the morning of day 4, and subsequently treated either with progesterone alone or with a combination of progesterone and oestradiol. The receptor concentrations were measured on the following day and compared with these in untreated castrated rats and in intact pregnant controls. We found that neither castration nor the subsequent hormone replacement lead to marked differences in the endometrium receptor level within 24 h. This observation is in agreement with the slow turnover rate of uterine oestrogen receptor (half-life about 5 d) reported by other authors⁷.

In summary, although the presence of oestrogen receptors in the rat endometrium cells may be necessary (maximum levels coinciding with the period of oestrogen sensitivity and implantation), it does not seem to be sufficient to explain the oestrogen effects, since a high level of receptor is found also at stages of pregnancy when oestradiol fails to induce endometrium receptivity for ovoimplantation¹. Progesterone has only been clearly shown to influence the level of

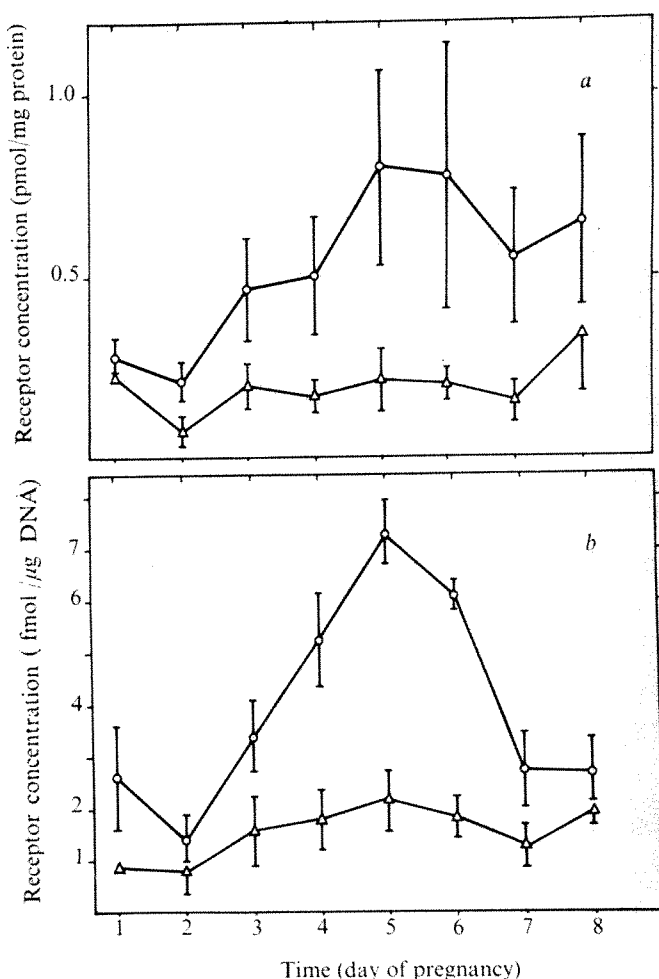


Fig. 2 Endometrium and myometrium oestrogen receptor concentrations in the rat during early pregnancy. The oestrogen receptor concentration in the low speed supernatant preparations was determined as shown in Fig. 1 in endometrium (\circ — \circ) or myometrium (Δ — Δ) from individual rates throughout the first 8 d of gestation or in a pool of four myometria (day 1). The results are shown as receptor concentration related to total soluble protein (a) or DNA content (b) of the tissues. Vertical bars show the standard deviations of the mean of at least four determinations.

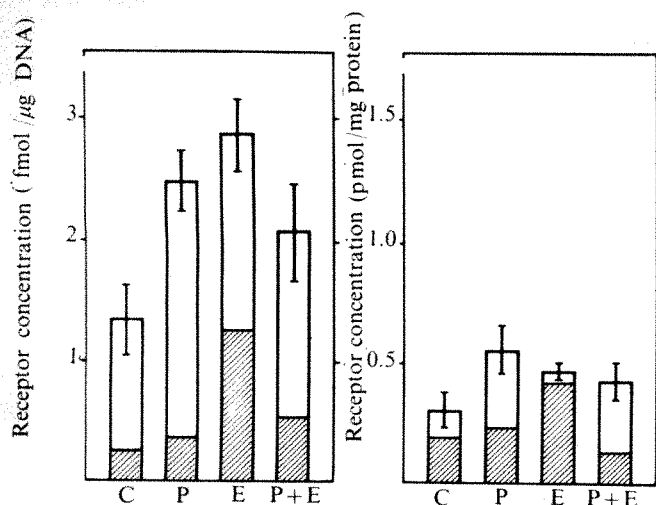


Fig. 3 Effect of progesterone and oestradiol treatment on the endometrium and myometrium oestrogen receptor concentrations in castrated rats. The concentration of oestrogen binding sites in the low speed supernatant preparation was determined as shown in Fig. 1 in endometrium of individual rats (whole bars) or in pooled myometrium (shaded part of the bars, four rats per determination). The rats received subcutaneously: the vehicle (propylene glycol) alone (control, C), progesterone (P, 5 mg per injection), oestradiol (E, 0.25 μ g per injection) or both hormones (P+E). The treatment was repeated twice with an interval of 24 h and the animals were killed 24 h after the second injection. The vertical lines represent standard deviation of the mean (four determinations).

oestradiol receptors in the endometrium, the changes in the myometrium being disputable. This, together with the lack of systematic study, may account for the fact that the progesterone effect on the uterine oestrogen receptor has escaped earlier detection. Whether the progesterone effect on oestrogen receptor is realised through the stimulation of receptor synthesis or activation in all or only certain cells, or through the preferential stimulation of mitotic activity of oestrogen-responsive cells, remains to be explored.

Perhaps of even greater interest is the antagonistic effect of progesterone in the oestradiol stimulation of the oestrogen receptor level in the myometrium. The observed variations in the receptor concentration in the endometrium and myometrium between days 2 and 5 of gestation can best be interpreted by simultaneous action of the two hormones. There is therefore a possibility that by blocking the increase of oestrogen receptor level in the myometrium, progesterone inhibits the excitatory action of oestradiol⁸ and thus favours implantation. This negative aspect of progesterone action could be as important as the increase of the endometrium oestrogen receptor which it provokes. A study of changes in the progesterone receptor has recently been published⁹. This work provides another model system where the antagonistic and synergistic effects of oestradiol and progesterone may be studied at a molecular level.

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- ¹ Psychoyos, A., in *Vitamins and Hormones* (edit. by Harris, R. S., Diczfalussy, E., Munson, P. L., and Glover, J.), **31**, 201-256 (Academic Press, New York and London, 1973).
- ² *Advances in Biosciences* (edit. by Raspé, G.), **7** (Pergamon Press, Oxford, 1971).
- ³ Scatchard, G., *Ann. N.Y. Acad. Sci.*, **51**, 660-672 (1949).
- ⁴ Mester, I., Robertson, D. M., Feherty, P., and Kellie, A. E., *Biochem. J.*, **120**, 831-836 (1970).
- ⁵ Nimrod-Zimrod, A., Ladany, S., and Lindner, H. R., *J. Endocr.*, **53**, 249-260 (1972).
- ⁶ Hashimoto, I., Henricks, D. M., Anderson, L. L., and Melampy, R. M., *Endocrinology*, **82**, 333-341 (1968).
- ⁷ Sarff, M., and Gorski, J., *Biochemistry*, **10**, 2527-2563 (1971).
- ⁸ Takeda, H., in *Hormonal Steroids* (edit. by Martin, L., and Pecile, A.), 203-211 (Academic Press, New York and London, 1965).
- ⁹ Milgrom, E., Luu Thi, M., Atger, M., and Baulieu, E. E., *J. biol. Chem.*, **248**, 6366-6374 (1973).
- ¹⁰ Feherty, P., Robertson, C. M., Waynforth, H. B., and Kellie, A. E., *Biochem. J.*, **120**, 837-844 (1970).

Methylmercury is a potent inhibitor of membrane adenyl cyclase

THE toxicity of methylmercury has taken on added importance since it was discovered that inorganic mercury can be converted to methylmercury by bacteria in bottom sediments of lakes and homogenates of rotting fish^{1,2}. The mechanism for methylation of inorganic mercury by bacteria has been described in some detail^{3,4}. Although there have been several documented incidents involving the toxicity of alkylmercury derivatives⁵⁻⁷, the basis for methylmercury toxicity has not been defined unambiguously. One possible target site for methylmercury—the plasma membrane—is described in this communication.

Many macromolecules in mammalian cells could be modified chemically by methylmercury. In principle, any enzyme with an essential sulphhydryl group is a potential target for methylmercury. However, components of the plasma membrane must be given primary consideration since methylmercury is lipid-soluble⁸ and the plasma membrane is the cellular organelle most vulnerable to external agents. An examination of central nerve tissue from victims of the Minamata disaster in Japan revealed considerable morphological damage to plasma membranes⁹ and methylmercury has been reported to catalyse the hydration and hydrolysis of plasmalogens, when the reaction was examined in methylene chloride¹⁰. Alternatively, it is important to determine whether or not functional proteins in plasma membranes are sensitive to methylmercury. Crucial membrane activities known to be inhibited by sulphhydryl reagents include adenosine triphosphatases, acetylcholine esterase, adenyl cyclase, transport systems, depolarisation phenomena in nerve and muscle tissue and hormone binding¹¹. We have now found that methylmercury is a potent inhibitor of adenyl cyclase in rat liver plasma membranes.

Table 1 Inhibition of membrane adenyl cyclase by sulphhydryl reagents

Sulphydryl reagents	*Concentration for 50% inhibition (M)
Iodoacetic acid	3×10^{-3}
Iodoacetamide	3×10^{-3}
N-ethylmaleimide	$\sim 1 \times 10^{-3\dagger}$
p-CMB	$5 \times 10^{-3\dagger}$
p-Chloromercuriphenyl sulphonate	$4 \times 10^{-6\dagger}$
5-Bromo-5-phenyl-barbiturate	6×10^{-6}
Mercuric chloride	$\sim 1 \times 10^{-6}$
Methylmercury chloride	1×10^{-7}

* Rat liver membranes incubated for 15 min at 30°C with the sulphhydryl reagent.

† Rat liver membranes incubated at 22°C for 15 min¹⁵.

‡ Heart muscle adenyl cyclase at 37°C (ref. 16).

Methylmercury chloride was synthesised by the method of Imura *et al.*¹². Purity was verified by thin-layer silica gel chromatography developed with diethyl ether: petroleum ether (3:7). The concentration of aqueous solutions of methylmercury chloride was quantitated using a Coleman mercury analyser. Rat liver plasma membranes were prepared by the Neville procedure as modified by Rodbell¹³ and adenylyl cyclase was assayed by the Krishna technique¹⁴. The final assay media contained 25 mM Tris, pH 7.6, 1 mM EDTA, 5 mM MgCl₂, 5 mM theophylline, 15 mM NaF, 1 mM ³H-ATP (2.5 μ Ci), 2 mM cyclic AMP, 20 mM creatine phosphate, 1 mg ml⁻¹ creatine kinase and 0.1% bovine serum albumin. Membranes were treated with methylmercury chloride by incubating a sample (80 μ g protein) with 200 μ l of 0.01 M phosphate buffer, pH 7.00, containing various amounts of methylmercury chloride. After 15 min of incubation, 5 ml of phosphate buffer was added and membranes were centrifuged at 20,000g for 10 min at 4° C. The pellet was dispersed carefully in buffer using a 20 gauge hypodermic needle and assayed in quadruplet for NaF stimulated adenylyl cyclase activity. Enzyme activity is expressed relative to the control which consisted of membranes treated as described above with buffer containing no methylmercury chloride. The control membranes catalysed the formation of 1.00 nmol cyclic AMP per mg protein per 10 min in the presence of NaF.

Adenylyl cyclase activity in rat liver plasma membranes proved to be extremely sensitive to methylmercury chloride (Fig. 1). Concentrations of methylmercury chloride as low as 10⁻⁸ to 10⁻⁹ M partially inhibited enzyme activity. Fifty per cent inhibition occurred at 1 \times 10⁻⁷ M when incubation was carried out at 30° C for 15 min. This inhibition was not due to inhibition of the ATP regenerating system in the assay since the membranes treated with methylmercury were washed thoroughly before assaying for adenylyl

cyclase activity, and creatine kinase would have been in excess of methylmercury even without washing. It is interesting that when membranes were incubated with methylmercury at 4° C adenylyl cyclase was not inhibited. It is possible that inhibition of membrane adenylyl cyclase activity requires diffusion of the inhibitor through the membrane phase, which could be reduced drastically below the thermal transition point of the lipids. Inhibition by methylmercury at 30° C was reversed by mercaptoethanol but only 80% of the original activity could be restored.

The effectiveness of methylmercury as an inhibitor of adenylyl cyclase is compared with several other sulphhydryl reagents in Table 1. Methylmercury is the most potent inhibitor of adenylyl cyclase reported so far. Two properties of methylmercury probably account for this: first, organomercurials generally react quite rapidly with protein sulphhydryl groups; secondly, methylmercury is quite lipid-soluble and would be expected to concentrate in the membrane phase when distributed between an aqueous phase and membranes.

The molecular basis for methylmercury toxicity is not known and there may of course be multiple effects. But, any consideration of alkylmercury toxicity must take into account the sensitivity of adenylyl cyclase to methylmercury and the pivotal role this enzyme plays in the metabolism of mammalian cells.

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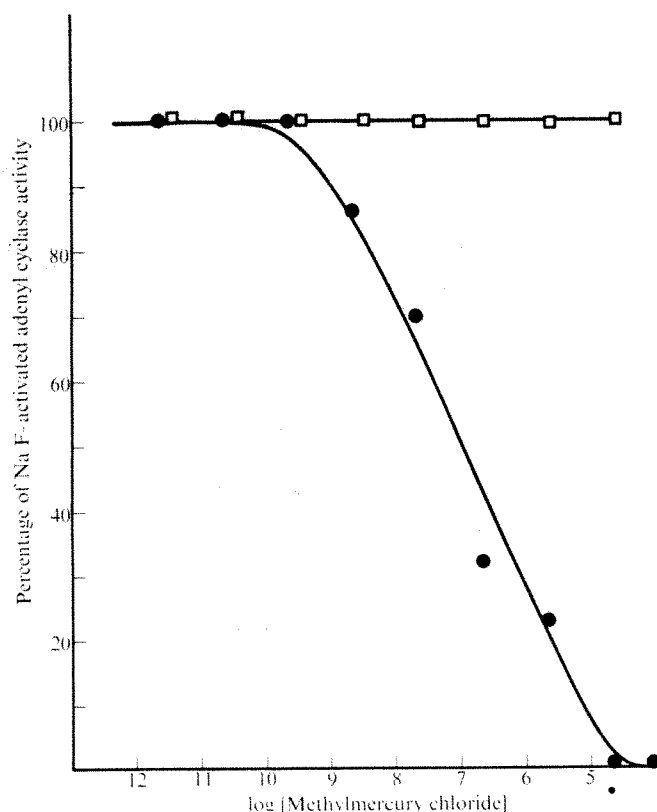


Fig. 1 Concentration dependence for methylmercury inhibition of adenylyl cyclase. Rat liver plasma membranes were incubated with various concentrations of methylmercury chloride for 15 min at 30° C (●) or 4° C (□). After incubation, the membranes were assayed for NaF-stimulated adenylyl cyclase activity as described in the text.

- ¹ Jensen, S., and Jernelov, A., *Nordforsk Biocidinformation*, **10**, 4 (1967).
- ² Jensen, S., and Jernelov, A., *Nature*, **223**, 753 (1969).
- ³ Wood, J. M., Kennedy, F. S., and Rosen, C. G., *Nature*, **220**, 173 (1968).
- ⁴ DeSimone, R. E., Penley, M. W., Charboneau, L., Smith, S. G., Wood, J. M., Hill, H. A. O., Pratts, J. M., Ridsdale, S., and Williams, R. J. P., *Biochim. biophys. Acta*, **304**, 851 (1973).
- ⁵ Fujiki, M., *Kimamoto, Igk. Z.*, **37**, 10 (1963).
- ⁶ Westoo, G., Sjostrand, B., and Westermarck, T., *Var Fodo*, **5** (1965).
- ⁷ Westoo, G., *Acta chem. scand.*, **21**, 1790 (1967).
- ⁸ Berlin, M., Ullberg, S., *Arch. Environ. Hlth*, **6**, 610 (1963).
- ⁹ Takevchi, T., in *Minamata disease* (edit. by Kutsuna, M.), 141 (Kumamoto University, Japan, 1968).
- ¹⁰ Segall, H. J., and Wood, J. M., *Nature*, **248**, 456 (1974).
- ¹¹ Rothstein, A., in *Current topics in membranes and Transport*, **1**, 135 (1970).
- ¹² Imura, N., Sukeyawa, E., Pan, S. K., Nagao, K., Kim, J. Y., Kwan, T., and V Kita, T., *Science*, **172**, 1248 (1971).
- ¹³ Rodbell, M., in *Methods in cyclic nucleotide research* (edit. by Chasin, M.), Ch. 4 (Dekker, New York, 1972).
- ¹⁴ Krishna, G., Weiss, B., and Brodie, B. B., *J. pharmac. exp. Ther.*, **163**, 379 (1968).
- ¹⁵ Pohl, S. L., Birnbaumer, L., and Rodbell, M., *J. biol. Chem.*, **246**, 1849 (1971).
- ¹⁶ Weinryb, I., Michel, I. M., Alicino, J. F., and Hess, S. M., *Archs. Biochem. Biophys.*, **146**, 591 (1971).

Effects of thyroid state on adrenoceptor properties

RECENT studies with frog hearts have shown that the adrenoceptors that mediate inotropic responses are altered qualitatively by ambient temperature¹⁻³. The characteristics of these receptors changed from β to α when the temperature of the isolated hearts was reduced through a critical range of 17°-22° C, which suggested that α and β adreno-

Table 1 Effect of altered thyroid state on the sensitivity of rat left atria to adrenergic agonists

	Control	<i>P</i>	Thyroidectomised	<i>P</i>	Thyroidectomised +T ₃ or T ₄
Isoproterenol	8.83 ± 0.16* (10)	<0.005	7.81 ± 0.19 (13)	<0.005	9.45 ± 0.21 (6)
Noradrenaline	7.12 ± 0.09 (30)	<0.005	6.20 ± 0.04 (40)	<0.005	7.27 ± 0.14 (13)
Phenylephrine	5.45 ± 0.09 (20)	<0.05	5.74 ± 0.10 (32)	<0.05	5.38 ± 0.11 (8)

* Mean + s.e., error of pD_2 (negative logarithm of molar concentration required to produce half-maximal response). Numbers of experiments are given in parentheses. *P* values indicate significance of differences between adjacent groups.

ceptors represent allosteric conformations of the same structure³. Although a change in adrenoceptor characteristics qualitatively similar to that found in the frog heart seems to occur in the mammalian myocardium^{1,4,5}, the physiological significance of a temperature-induced change is obviously limited in homeothermic species. But, several observations indicate that conditions which promote α -adrenoceptor properties, including cold¹⁻³, iodoacetate or fluoroacetate⁶ or dinitrophenol¹, inactivity of skeletal muscle⁷ and increased vagal influence on the myocardium⁸, are associated with a decrease in metabolic activity. Therefore, it seemed possible that hypothyroidism would produce similar changes in receptor properties. We report here that the characteristics of adrenoceptors mediating inotropic responses in hypothyroid rats are similar to those in normal hearts at low temperatures.

Hypothyroidism was induced in male Sprague-Dawley rats (180–220 g) by surgical thyroidectomy. Experiments were performed on isolated left atria 6–10 weeks after the operation. By this time growth was retarded and the fur was dry. Serum thyroxin levels determined by the competitive protein binding assay⁹ were significantly lower (25 ± 4 ng ml⁻¹) than those of control animals (57 ± 6 ng ml⁻¹). Control preparations were from either sham-operated age-matched, or weight-matched animals. The results from the two control groups were almost identical and were pooled. A third group consisted of thyroidectomised rats injected daily with thyroxine (T₄, 1.0 mg kg⁻¹) or triiodothyronine (T₃, 0.25 mg kg⁻¹) for 1 week before use. The animals were anaesthetised with ether and the hearts were removed immediately. The left atria were suspended in Krebs-Henseleit solution at 31°C bubbled with 5% CO₂ in O₂, and were stimulated through platinum electrodes with square-wave pulses at a voltage slightly above threshold, a duration of 3 ms and a rate of 1 Hz. Isometric contractions were recorded by a force-displacement transducer and Grass polygraph. Cumulative concentration-response curves for various agonists were determined before and after a 40 min incubation with propranolol (0.04–4.0 μ M) or ³H-phenoxybenzamine (³H-POB, specific activity 33 mCi mmol⁻¹, 7.3 μ M). Responses are expressed as percentage of the maximal control response. Atria exposed to ³H-POB were washed for 2 h and then dismantled and freeze dried. The dry tissue was weighed and dissolved in NCS tissue solubiliser; radioactivity was measured in a liquid scintillation counter. The retained radioactivity is expressed as d.p.m. per mg dry weight.

Thyroidectomy did not significantly alter either the basal contractile force of left atria or the maximal increase in force produced by the sympathomimetics tested. But, selective changes in sensitivity to the various amines were re-

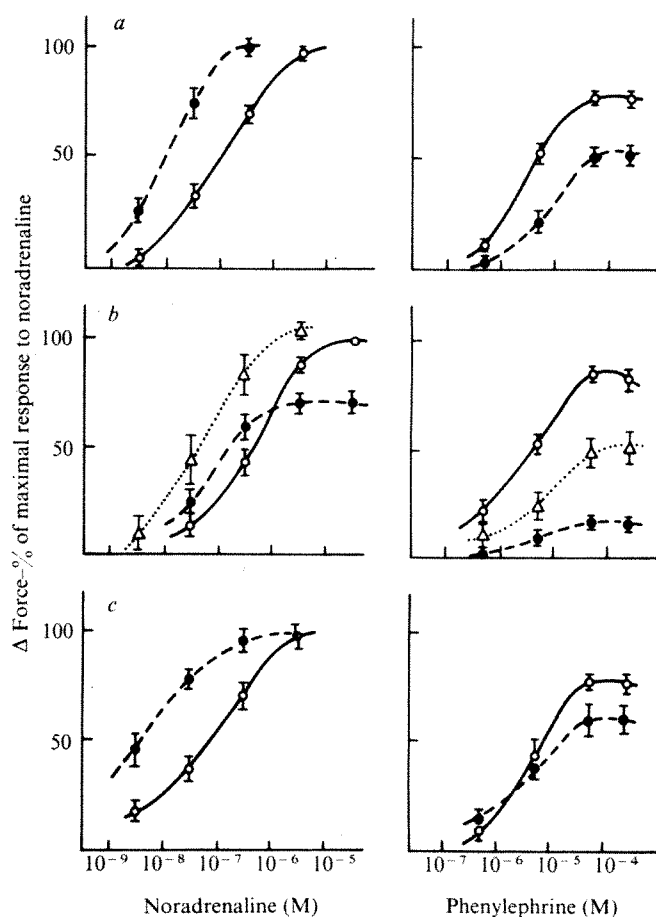


Fig. 1 Effects of phenoxybenzamine on inotropic responses of rat left atria to noradrenaline and phenylephrine. Rats were normal (a), thyroidectomised (b); and thyroidectomised +T₃ or T₄ (c). O, Control responses; ●, after exposure to phenoxybenzamine (7.3 μ M); Δ , after exposure to phenoxybenzamine (7.3 μ M) in the presence of phentolamine (26.5 μ M). Means \pm s.e. are shown.

Table 2 Effect of altered thyroid state on the inhibitory potency of propranolol

Propranolol (μ M)	Control	<i>P</i>	Thyroidectomised	<i>P</i>	Thyroidectomised +T ₃ or T ₄
0.04	0.70 ± 0.09* (11)	<0.05	0.37 ± 0.07 (7)	<0.05	0.75 ± 0.10 (5)
0.4	1.84 ± 0.15 (11)	<0.005	0.70 ± 0.10 (8)	<0.005	1.70 ± 0.21 (5)
4.0	3.01 ± 0.37 (5)	<0.005	1.39 ± 0.07 (8)	<0.005	2.54 ± 0.38 (5)

* Mean + s.e. of log dose-ratio (logarithm of the ratio of NA ED₅₀s after and before propranolol). Numbers of experiments are given in parentheses. *P* values indicate significance of differences between adjacent groups.

flected in significant changes in the concentrations producing half-maximal responses. Table 1 shows that the sensitivity of atria to isoproterenol and noradrenaline, agonists with predominantly β -adrenoceptor activity in the heart, was significantly lower, whereas sensitivity to the α -adrenoceptor agonist phenylephrine was significantly higher in the thyroidectomised than in the control rats. Sensitivities to all three agonists were returned to or beyond the control values in the thyroidectomised animals treated with thyroid hormone. There were also reciprocal changes in the effectiveness of α and β -adrenoceptor antagonists. The inhibitory potency of propranolol was at least ten times less in the thyroidectomised than in either the control or the thyroidectomised, hormone-treated groups (Table 2). Conversely, POB inhibited inotropic responses more effectively in hypothyroid than in the other groups (Fig. 1). In control atria POB potentiated inotropic responses to noradrenaline and moderately inhibited those to phenylephrine. In preparations from thyroidectomised rats a similar exposure to POB partially inhibited responses to noradrenaline and blocked those to phenylephrine significantly more than in the controls. Maximal responses of these preparations to CaCl_2 were unaltered by POB. Thyroid hormone treatment reduced the effectiveness of POB on responses to both noradrenaline and phenylephrine to about the control level. In association with the increased blockade, significantly more ^3H -POB was bound to the hypothyroid myocardium ($25,122 \pm 1,365$ d.p.m. ml^{-1} dry weight) than to preparations from either control ($19,310 \pm 1,176$) or thyroidectomised, hormone-treated rats ($16,384 \pm 857$). When atria from thyroidectomised rats were exposed to ^3H -POB in the presence of $26.5 \mu\text{M}$ phentolamine, protection of α adrenoceptors was shown by a significant reduction in both the blocking effectiveness (Fig. 1, dotted lines) and the binding of ^3H -POB ($18,212 \pm 1,190$). These data indicate the presence in hearts from hypothyroid rats of α adrenoceptors that are absent or are unreactive with POB in euthyroid animals. They also suggest that, as in frog hearts at low temperatures³, α adrenoceptors represent a considerably higher percentage of the total binding sites for POB in hypothyroid myocardium than in normal vascular smooth muscle¹⁰.

The observed shift in the balance of α and β adrenoceptors involved in inotropic responses can be interpreted either as due to reciprocal changes in the sensitivity or availability of two independent pools of receptors, or to a single type of adrenoceptor that is qualitatively altered by changes in thyroid state. The experiments reported here do not distinguish between these possibilities. But, analogies between this model and temperature-induced receptor transformation, where the second interpretation was supported by the observation that β adrenoceptors did not appear at high temperatures after α adrenoceptors had been alkylated irreversibly at a low temperature³, suggest that the changes in responsiveness produced by altered thyroid hormone levels reflect an interconversion of myocardial α and β adrenoceptors. In view of the reported sensitivity of allosteric systems to thyroid hormones^{11,12}, this interconversion can be envisaged as an allosteric change in a single basic type of adrenoceptor, as proposed earlier³.

Such a change in adrenoceptor characteristics due to altered thyroid hormone levels could explain several published observations, including a decreased blocking potency of propranolol in the hearts of hypothyroid rats¹³, a selective increase in adrenergic stimulation of the aorta of hypothyroid rabbits¹⁴, a diminished lipolytic response to noradrenaline in adipose tissue from hypothyroid human subjects¹⁵, a decreased adrenergic vasoconstriction, reversed by pronethalol, in the hind limbs of hyperthyroid dogs¹⁶, and an increased sensitivity to phenylephrine and decreased sensitivity to isoproterenol of atria from propylthiouracil-pretreated rats^{17,18}.

Although both α and β adrenoceptors can mediate posi-

tive inotropic responses in the mammalian myocardium¹⁹, the underlying mechanisms may differ. It has been reported that stimulation of α adrenoceptors is not associated with an 'oxygen-wasting' effect²⁰, and is not accompanied by increased accumulation of cyclic AMP²¹. It is therefore tempting to speculate that changes in adrenoceptor characteristics play a role in the adaptation of mechanical responses of the myocardium to its metabolic state.

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- ¹ Kunos, G., and Szentiványi, M., *Nature*, **217**, 1077 (1968).
- ² Buckley, G. A., and Jordan, C. C., *Br. J. Pharmac.*, **38**, 394 (1970).
- ³ Kunos, G., Yong, M. S., and Nickerson, M., *Nature new Biol.*, **241**, 119 (1973).
- ⁴ Benfey, B. G., Kunos, G., and Nickerson, M., *Br. J. Pharmac.* (in the press).
- ⁵ Kunos, G., Vermes-Kunos, I., Boyd, G. N., and Nickerson, M., *Fedn Proc.*, **32**, 691 (1973).
- ⁶ Nickerson, M., and Nomaguchi, G. M., *Am. J. Physiol.*, **163**, 484 (1950).
- ⁷ Szentiványi, M., Kunos, G., and Juhász-Nagy, A., *Am. J. Physiol.*, **218**, 869 (1970).
- ⁸ Szentiványi, M., Kunos, G., and Juhász-Nagy, A., *Acta physiol. hung.*, **37**, 427 (1970).
- ⁹ Murphy, B. E. P., and Pattee, C. J., *J. clin. Endocr. Metab.*, **24**, 187 (1964).
- ¹⁰ Yong, M. S., and Nickerson, M., *J. Pharmac. exp. Ther.*, **186**, 100 (1973).
- ¹¹ Monod, J., Changeux, J.-P., and Jacob, F., *J. molec. Biol.*, **6**, 306 (1963).
- ¹² Wolff, J., *J. biol. Chem.*, **237**, 230 (1962).
- ¹³ Hirvonen, L., and Paavilainen, T. O. A., *Acta physiol. scand.*, **69**, 284 (1967).
- ¹⁴ Rosenquist, U., and Boréus, L. O., *Life Sci.*, **11**, 595 (1972).
- ¹⁵ Rosenquist, U., *Acta med. scand.*, **192**, 353 (1972).
- ¹⁶ Zsotér, T., Tom, H., and Chappel, C., *J. Lab. clin. Med.*, **64**, 433 (1964).
- ¹⁷ Nakashima, M., Maeda, K., Sekiya, A., and Hagino, Y., *Jap. J. Pharmac.*, **21**, 819 (1971).
- ¹⁸ Nakashima, M., and Hagino, Y., *Jap. J. Pharmac.*, **22**, 227 (1972).
- ¹⁹ Govier, W. C., *J. Pharmac. exp. Ther.*, **159**, 82 (1968).
- ²⁰ Privitera, P. J., and Rosenblum, I., *J. Pharmac. exp. Ther.*, **156**, 325 (1967).
- ²¹ Benfey, B. G., *Br. J. Pharmac.*, **43**, 757 (1971).

Stereopsis in dynamic visual noise

A NOISE signal has the useful property that convolution of the output spectrum with the reciprocal of the input spectrum gives a measure of the characteristics of the transmitting system. In visual perception it is not possible to obtain the output spectrum directly, but an observer can report on features of the 'perceptual output' of the visual system¹. One striking characteristic which may be observed is the generation of stereoscopic depth merely by an interocular delay in transmitting binocular dynamic visual noise.

Ross² has recently described the effects of an interocular delay in perception of random dynamic noise (an electronic snowstorm) generated by a sophisticated computer technique. He interpreted his results as showing that a temporal rather than disparity between the two eyes may act as a signal for stereoscopic depth. The concept of stereopsis from temporal disparity is a radical one and needs to be critically examined before being fully accepted. I shall describe some observations and a theoretical viewpoint which seems to provide an explanation for dynamic noise stereopsis within the framework of stereopsis from spatial disparity.

The display used for the observations consisted of

random visual noise generated by a detuned television receiver. An interocular delay of up to 100 ms may be produced by the classic technique of a neutral density filter in front of one eye³. Observation of the dynamic noise with a one log unit filter (creating a delay of about 30 ms at 10 trolands when fully adapted³) gave rise to a number of perceptual experiences which have been spontaneously confirmed by six observers. The noise exhibits a considerable depth, perhaps 10% of the viewing distance, and also a streaming motion which is leftwards in front of the point of fixation and rightwards behind fixation with the filter over the left eye. Direction of movement reverses with the filter over the right eye. The motion had the characteristics of motion in a landscape viewed from a moving train, such that points near fixation rotated slowly whereas points well in front of or behind fixation moved more quickly. Movement is leftwards in front of fixation with the filter over the left eye. Direction of movement reverses if the filter is switched to the right eye.

The range of interocular delays for depth probably depends on the density and spatial distribution of the dynamic noise. In my display depth could be perceived with as little as 5 ms delay and as much as 70 ms, the maximum obtainable. One interesting observation was that depth and in particular movement were enhanced by tracking the movement of one depth plane across the screen. Tracking also seems to enhance the unity and salience of the plane which is being tracked. In contrast tracking the conventional Pulfrich pendulum against a plain background has the effect of abolishing depth.

As a control for any peculiarities of the television noise which may have influenced the results, observers tilted their heads from vertical to horizontal. The scan rate in the transverse plane through the two eyes changes from 64 μ s to 40 ms between vertical and horizontal. The plane of movement rotates to remain parallel to the two eyes,

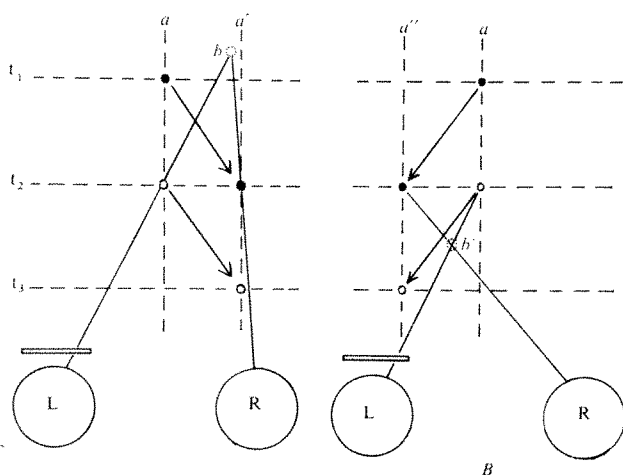


Fig. 1 A, Sequence of possible events at three points in time. Left and right eyes are depicted as viewing events only at t_2 . A spot at point a is transmitted by the right eye (●) at time t_1 . As a result of the interocular delay the same spot is transmitted to the left eye at time t_2 (○). If a second spot happens to appear at a' to the right of a such as to be transmitted at time t_2 , a spatial disparity is produced and a spot will be perceived in a different depth plane at b . The monocular sequences of a spot at $a(t_1)$ followed by a spot at $a(t_2)$, and a spot at $a'(t_2)$ followed by one at $a'(t_3)$ are both preconditions for monocular apparent movement to the right, which may therefore be associated with the spot at depth b . B, reversal of both depth and movement when the second spot appears to the left of a at a'' rather than the right. In a random display both sequences A and B will occur, producing both rightward movement behind the point of fixation and leftward movement in front. The distances from a to a' and a to a'' will vary with a Poisson distribution depending on the density of the visual noise.

and otherwise no change in the percepts described was reported.

These observations confirm that stereopsis may be obtained by interocular delay in viewing random noise. In this situation there is no correlation between the signals from the two eyes at any instant in time. It is therefore difficult to understand what can give rise to a perception of a range of disparities in the stimulus. The model I propose is based on the assumption that the two percepts of depth and movement arise from the same operation on the dynamic noise stimulus. Thus Ross's hypothesis of depth produced by temporal disparity also implies that movement can be produced by temporal differences alone, whereas logically movement involves both temporal and spatial displacement. To resolve this difficulty, I considered the microstructure of the dynamic noise, rather than regarding it as random and therefore unpatterned. Figure 1 shows how depth and movement both arise from chance associations of points at different times in the random display. The single postulate of an association between depth and movement arising from the same pair of points is all that is required to produce the percept of a rightward-moving spot behind the plane of fixation. Such an association between the monocular movement and binocular depth is not unlikely since stationary monocular stimuli tend to be drawn to a stereoscopically defined depth⁴.

It is difficult to reconcile Ross's description of a single depth plane with the dense range of depths reported by my observers.

Preliminary observations of my dynamic noise stimulus with a dark central square confirm that depth is now perceived predominantly to the rear of the plane of the card. This may be due both to gestalt figural organisation favouring an underlying as against overlying interrupted surface, and to the difficulty of making tracking eye movements with the square present.

My observations support the hypothesis that an interocular delay produces a random distribution of spatial disparities in a dynamic noise stimulus. Each disparity is associated with a certain rate of apparent movement, giving rise to the perception of moving depth planes. The observations may thus be accommodated within the conventional framework of stereoscopic theory.

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¹ MacKay, D. M., *J. gen. Psychol.*, **72**, 181 (1965).

² Ross, J., *Nature*, **248**, 363 (1974).

³ Rogers, B. J., and Anstis, S. M., *Vision Res.*, **12**, 909 (1972).

⁴ Gogel, W. C., *Psycho. Bull.*, **64**, 153 (1965).

Contractility in *Spirostomum* provides for nonelectrogenic calcium regulation through energy-dissipative metabolic processes in the absence of membrane excitability

ATTEMPTS to carry out *in vivo* pharmacological analyses of the events leading to contraction in fast acting muscle systems have been hampered by problems due to, for example, intracellular diffusion, cell-cell interactions and extracellular control mechanisms, which arise in part because of the multicellular organisation of many vertebrate muscle systems. Their effect is to impede accurate measurement of rapid changes in the physiological and ultrastructural states throughout the system in response to

specific stimuli. The most troublesome aspects of these problems can be bypassed through the use of single-celled models which have the same properties of excitability as do muscle systems.

An essential feature of contraction in the ciliated protozoan *Spirostomum*, as in muscle, is the requirement for calcium to couple stimulation and contraction. There is a temporal relationship between calcium release into the cytoplasm and the onset of contraction in cells micro-injected with the calcium-sensitive, bioluminescent protein aequorin¹. In spite of the similarity in calcium dependency, systems such as *Spirostomum* and muscle differ in the ultra-structural organisation and energy requirements of their contractile apparatus²⁻⁴. In addition, *Spirostomum* responds to many chemical and physical stimuli in the absence of recordable changes in the electrical properties of the cell membrane¹. The response is a very specific set of contractions and provides a means of examining the biochemical pathways which may regulate calcium release during pharmacomechanical stimulation.

Dikstein⁵ suggested that several redox systems in cells are in such tight equilibrium with redox substrates and

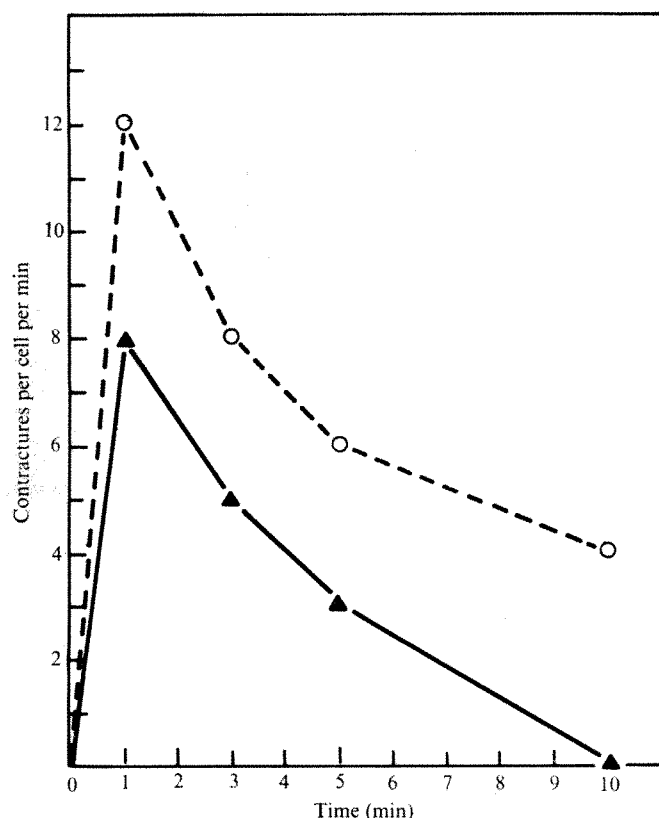


Fig. 1 Time of course and magnitude of the contractile responses of *Spirostomum* to treatment with 10^{-4} M (O) and 10^{-5} (Δ) PMS.

products as to affect ATPase activity, energy coupling and excitation. Such regulation of metabolic activity can be studied by varying the redox ratio NADPH: NADP⁺ (and NADH: NAD⁺ ratio through transhydrogenation). Some NADPH is generated during the biosynthesis of pentose monophosphates, and it functions as a reducing equivalent in the biosynthesis of fatty acids, steroids and certain purines. Some of the reducing equivalents from NADP are oxidised through the microsomal respiratory chain mediated by cytochrome P-450 and atmospheric oxygen as part of the hydration of toxic substances⁶. The availability of NADPH can be controlled by an intermediate hydrogen carrier, phenazine methosulphate (PMS),

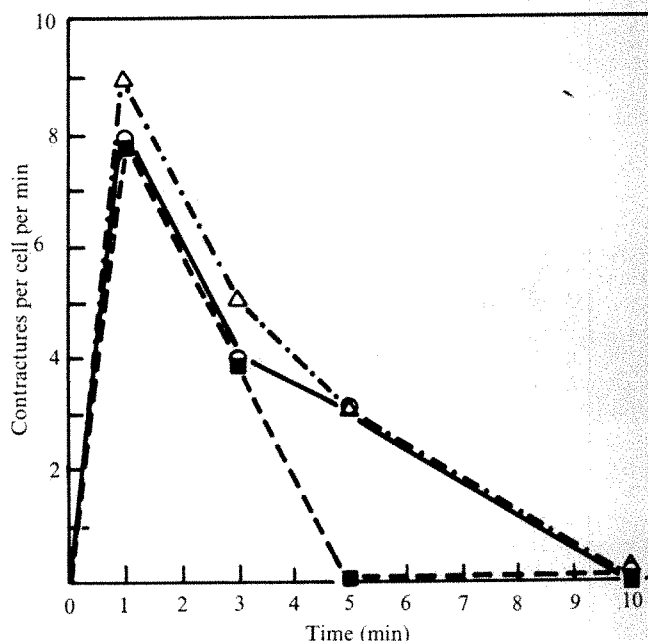


Fig. 2 Influence of PMS on cells depolarised in high KCl medium. The most significant effect occurs at 40 mM KCl when there is a 33% decrease in the maximum number of contractures exhibited by the cell under conditions of maximal stimulation. Concentrations of KCl: O, 10 mM; Δ, 20 mM; ■, 40 mM.

which accepts hydrogen from NADPH (and NADH) and transmits it to a suitable oxidising agent such as neotetrazolium or oxygen. This generates an energy sink and deprives mitochondria of reduced substrates.

When we treated *Spirostomum* with 10^{-4} M and 10^{-5} M PMS in a medium consisting of 2 mM NaCl; 0.5 mM KCl; 0.05 mM CaCl₂; 0.1 mM KH₂PO₄, and 0.1 mM KOH (pH 6.3) contractures were induced in each cell (Fig. 1). Data were tabulated according to the method of Sleigh⁷ who counted contractures per minute for several cells at regular intervals as a direct assay of their behavioural responses to pharmacomechanical stimuli. At the higher concentration of PMS each cell made a maximum

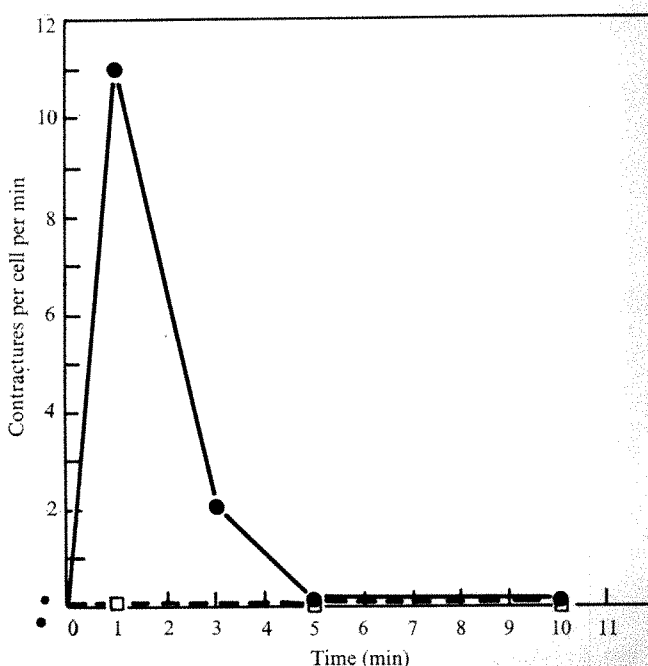


Fig. 3 Net effect of the calcium ionophore A-23187 at 10^{-3} M (●). Total inhibition of PMS induced contractures occurs in calcium-free Chalkey's medium (□).

of twelve contractures per minute during the first minute of stimulation, followed by a slower exponential decay at a rate of two contractures per minute until contraction stopped within 30 min. Further treatment with PMS did not elicit further contractures. To insure that PMS was not initiating contractures by depolarising the cell membrane, the concentration of KCl in the medium was adjusted at 10 mM, 20 mM and 40 mM to depolarise the membrane before treatment. Figure 2 shows that a maximum of 33% depression of 10^{-4} M PMS-stimulated contractures occurred when cells were preincubated in 40 mM KCl.

At the cessation of PMS-induced contractures, the terminal vacuole of the cell enlarged, occupying a third to a half of the cell volume. This indicated that the osmotic activity of the vacuolar space increased, possibly due to the sequestration of Ca^{2+} during relaxation after PMS-induced contractures. Vacuolar calcium concentration has been reported⁸ to increase after repeated mechanical and electrical stimulation of *Spirostomum*, suggesting that PMS action is metabolically linked to cyclical calcium movements in the cellular compartments. To test this hypothesis, the calcium ionophore A-23187 (Eli-Lilly) was added to calcium-free and complete medium in a concentration 10^{-5} M for 10 min before stimulation with PMS. Preincubation did not affect the ciliary locomotory activity. As Fig. 3 shows, in the presence of 0.05 mM Ca^{2+} the cell still made the maximum number of contractures, but these decayed at two to three times the initial rate going to baseline in 5 min. In calcium-free medium, preincubation in A-23187 followed by PMS stimulation produced a single contraction from which the organisms failed to recover. These results suggest that the cell accumulates calcium from the medium, as Jones⁹ suggested, which would then be available for activation. Pautard¹⁰ indicated that some membrane-limited calcium is bound tightly in the form of hydroxyapatite in *Spirostomum*. Our results might also indicate the slow chelation of stored calcium by the ionophore and subsequent free diffusion of the ion away from sites of calcium storage and release. The terminal vacuole failed to enlarge

during treatment with the ionophore and PMS, indicating that calcium was being removed through chelation with the ionophore, which then prevented calcium sequestration.

Figure 4 shows the effect of chlorpromazine and dicyclohexyl carbodiimide (DCCD), which Maran *et al.*¹¹ reported to inhibit PMS activation of *Vorticella*. Both substances inhibit enzymatic reactions which involve transfers of inorganic phosphate. Our results indicate that DCCD eliminated PMS induced contractures and elicited no further responses. Chlorpromazine depressed the PMS response. This drug, however, showed some delayed activity of its own, independently inducing contractures between 3 and 5 min after application.

Our results support the concept that calcium movements, and hence contractility, can be influenced directly by changes in the metabolic state of a contractile cell apparently independently of any alteration in the external concentration of ions governing the electrical properties of the membrane or of contractility. Storage of Ca^{2+} within cellular compartments against its electrochemical gradient represents a source of chemical potential energy. Presumably, the release or efflux of calcium can be coupled to the synthesis of energy-rich intermediates which may be used for resequestration and for the active processes involved in contraction¹². Ultimately in *Spirostomum*, such a system would run down because the energy available to re-establish a calcium chemical potential is continually depleted in the presence of PMS by other active processes within the cell. We suggest that motile or contractile processes such as dividing cells, amoeboid movement, cytoplasmic streaming and other processes with an implied calcium dependency, may well depend on local changes in metabolism which in turn regulate the amount of free calcium within various cytoplasmic compartments.

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- ¹ Ettienne, E. M., *J. gen. Physiol.*, **56**, 168 (1970).
- ² Ettienne, E. M., *J. Cell Sci.* (in the press).
- ³ Lehman, W. J., and Rebhun, L. J., *Protoplasma*, **72**, 153 (1971).
- ⁴ Huang, B., and Pitelka, D. R., *J. Cell Biol.*, **58**, 704 (1973).
- ⁵ Dikstein, S., *Naturwissenschaften*, **58**, 439 (1971).
- ⁶ Chayen, J., Altman, F. P., and Butcher, R. O., in *Fundamentals of cell pharmacology* (edit. by Dikstein, S.), 196 (Thomas, Springfield, Illinois, 1973).
- ⁷ Sleight, M. A., *Prog. Protozool.*, **Third Int. Cong. Protozool.**, 165 (Nauka, Leningrad, 1969).
- ⁸ Osborn, D., Huang, J. C., and Eisenstein, E. M., *Behav. Biol.*, **8**, 665 (1973).
- ⁹ Jones, A. J., *Protozoology*, **14**, 220 (1967).
- ¹⁰ Pautard, F., *Biochim. Biophys. Acta.*, **35**, 229 (1959).
- ¹¹ Maran, M., Himmelstein, R., and Dikstein, S., *Comp. gen. Pharm.*, **3**, 363 (1972).
- ¹² Tonomura, Y., and Yamada, S., in *Organisation of energy transducing membrane* (edit. by Nakao, M., and Packer, L.), 107-112 (University Park Press, Baltimore, 1973).

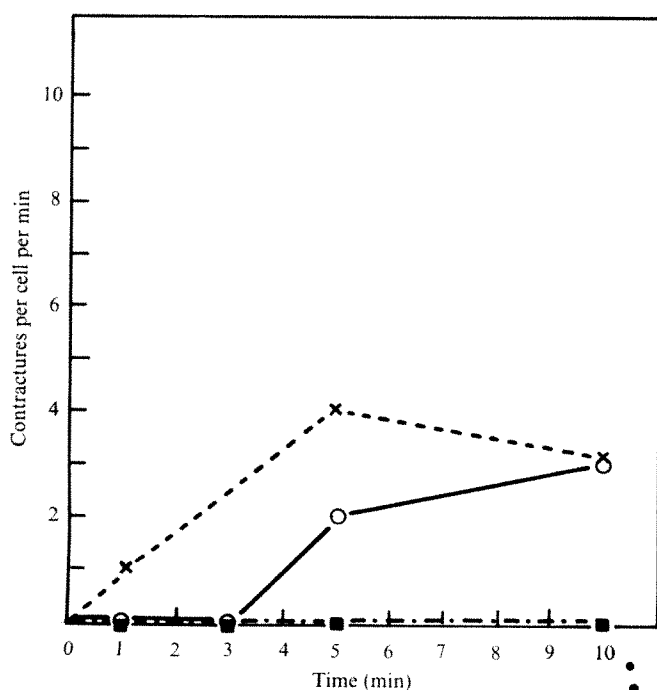


Fig. 4 Chlorpromazine depresses the response to PMS and acts as a delayed independent stimulus. Dicyclohexyl carbodiimide (DCCD) inhibits PMS-induced contractures by blocking PO_4 transfer reactions. x, 10^{-5} M chlorpromazine as inhibitor of PMS; O, 10^{-5} M chlorpromazine as stimulus; □, 10^{-5} M DCCD, before and after PMS.

Inhibition of interferon action by plant lectins

IN spite of various theories, the mechanism of the antiviral action of interferon¹⁻³ remains obscure. Previous work indicated that it does not have to penetrate into target cells to exert its effect. Mouse interferon preparations, when covalently

Table 1 Effect of lectins on antiviral activity of Sepharose-bound interferon

Addition to cell monolayer	Lectin pretreatment of beads cells		Viral yield after pretreatment with			
			PHA	Con A	Lotus lectin	Orange lectin
Control Sepharose	—	—	4,096	4,096	4,096	2,048
Control Sepharose	+	—	4,096	4,096	(8,192)	Not done
Control Sepharose	—	+	4,096	2,048	(4,096)	Not done
Control Sepharose	+	+	4,096	4,096	(4,096)	2,048
IF - Sepharose	—	—	32	64	(8,192)	64
IF - Sepharose	+	—	128	4,096	(64)	Not done
IF - Sepharose	—	+	2,048-4,096	1,024	(2,048)	Not done
IF - Sepharose	+	+	4,096	4,096	(512)	32
					(8,192)	

Mouse L cells were cultivated in 35 mm plastic Petri dishes (5×10^5 cells per dish) in Eagle's minimal essential medium (MEM) and Hanks salt solution, containing 10% calf serum. After removal of medium the cells were washed once with 1 ml PBS and incubated for 1 h at 20° C in the presence of respective lectin solutions in PBS (0.5 ml). Thereafter the lectin solutions were removed and the cells washed once with 1 ml of PBS. IF-Sepharose, prepared as described previously⁴, was treated with lectin by incubating 5×10^4 beads with 0.5 ml lectin solution in PBS for 16 h at 20° C. Thereafter the beads were washed once with 4 ml PBS and suspended in 1 ml MEM without serum. Control Sepharose 4-B beads (Pharmacia Fine Chemicals) were treated identically. IF-Sepharose or control Sepharose beads were then added to the cells (one bead per ten cells) and incubated for 5 h at 37° C. After removal of the beads the cells were challenged with EMC at a multiplicity of infection of 0.1. (In the experiment with con A the cells were washed once with 1 ml of 0.5 M α -methyl mannoside PBS before infection with virus to minimise toxic effects of con A.) Viral yield was determined after 16 h of incubation at 37° C by haemagglutination of human red blood cells of type O in serial two-fold dilutions of virus suspensions⁵. The numbers represent the reciprocal of the highest dilution that showed haemagglutination. The lectin preparations were used at the following protein concentrations⁷: PHA (Phytohaemagglutinin, M form, Grand Island Biological Co.), 4.1 mg⁻¹ ml; con A (A grade, Calbiochem), 1.6 mg⁻¹ ml; lotus lectin (Fucose-Binding Protein, Miles), 0.22 mg⁻¹ ml or 0.67 mg⁻¹ ml (in parentheses). Orange lectin (prepared from Osage orange seeds according to Ahmed *et al.*⁸, using the material precipitating between 0-85% ammonium sulphate saturation after dialysis against PBS), 12 mg⁻¹ ml. The same negative results as shown for Orange lectin were obtained with partially purified lectins from *Lens culinaris* and *Ulex europaeus*. Lens lectin was prepared as described by Sage and Green⁹; crude extracts were fractionated with ammonium sulphate and the fraction precipitating between 33 and 66% saturation was dialysed against water. The resulting precipitate was dissolved in PBS to a protein concentration of 22 mg⁻¹ ml. Extracts of *Ulex europaeus* seeds were fractionated with ammonium sulphate as described by Osawa and Matsumoto¹⁰. Crude haemagglutinin 1 and 2 separating at 0-40 and 40-70% ammonium sulphate saturation were dialysed against PBS and used at protein concentrations of 4.5 and 7.5 mg⁻¹ ml, respectively. When assayed in serial twofold dilutions, all lectin solutions used agglutinated human red blood cells, type O, at maximal dilutions of 1/64-1/128, except the orange lectin solution, which gave haemagglutination at a maximal dilution at 1/512.

bound to Sepharose beads, retained full antiviral activity, even after multiple cell-to-cell transfers⁴. Physical restriction of Sepharose-bound interferon to a well defined area on the cell monolayer protected only cells in immediate contact with the insoluble interferon preparation, but not those unable to make such contacts. This excluded the possibility that soluble interferon diffused from the Sepharose beads to which it was attached⁶. If indeed interferon induces its antiviral effect by interaction with the cell surface, interferon-sensitive cells should possess specific receptor sites. We report here the inhibitory action of certain plant lectins on the action of interferon.

We used Sepharose-bound interferon (IF-Sepharose) to assay for possible inhibitory effects of plant lectins on the induction of the antiviral state. This approach facilitates

removal of unabsorbed lectin by simple washing, which is not possible with soluble interferon. Either mouse L cells, IF-Sepharose or both were preincubated with a solution of lectin in phosphate-buffered saline (PBS). In control experiments Sepharose 4-B beads were treated identically. IF-Sepharose or equal quantities of control beads were then incubated with mouse L cells for 5 h at 37° C in serum-free medium. After removal of the beads, the cells were challenged with encephalomyocarditis virus (EMC) and viral yield was determined by haemagglutination (Table 1).

Lectins derived from *Canavalia ensiformis* (concanavalin A, con A), *Phaseolus vulgaris* (phytohaemagglutinin, PHA) and *Lotus tetragonolobus* (lotus lectin) abolished the antiviral effect of IF-Sepharose, when both IF-Sepharose and the cell monolayer were preincubated. No effects were seen with lectins

Table 2 Reversal of interferon inhibitory action of lectins by lectin-specific substances

Lectin bound to IF-Sepharose	cells	Treatment with	Viral yield
—	—	None	32-64
—	PHA	None	2,048-4,096
—	PHA	D-galactose	2,048-4,096
—	PHA	Fetuin	256
Con A	—	None	2,048-4,096
Con A	—	D-galactose	4,096
Con A	—	α -Methyl-mannoside	256
—	Con A	None	2,048
—	Con A	D-galactose	2,048
—	Con A	α -Methyl-mannoside	64

L cell monolayers or IF-Sepharose beads were incubated with lectin solutions as described under Table 1. Cells were washed once with 1 ml PBS, followed by incubation with 0.5 M D-galactose, 0.5 M α -methyl mannoside, or 10 mg⁻¹ ml Fetuin (Sigma) in PBS. After 15 min at 20° C the saccharide or glycoprotein solutions were removed, cells were washed once with 1 ml PBS and incubated with fresh IF-Sepharose beads as described under Table 1. Con A-treated IF-Sepharose beads were washed with 4 ml PBS, then incubated for 15 min at 20° C with 1 ml D-galactose (0.5 M) or α -methyl mannoside (0.5 M), followed by washing with 4 ml PBS. The washed IF-Sepharose beads were then layered on to L cell monolayers and their antiviral activity was determined as described in Table 1.

derived from Osage orange seeds, *Lens culinaris* and *Ulex europeus* when used under comparable conditions. When only the cell monolayer was preincubated with lectin, PHA still inhibited interferon action completely, whereas con A and lotus lectin were less inhibitory. In contrast, preincubation of IF-Sepharose alone had little effect in the case of PHA, yet pronounced effects were seen with con A and lotus lectin. The haemagglutinating titres of the lectin solutions used, when assayed with human red cells of group O, were quite comparable. They remained unchanged when the lectin solutions were titrated after preincubation with cells or beads. Similarly, no significant decrease in protein concentration was found after preincubation with either cells or beads. Therefore the amounts of lectins that remained bound to either cells or beads seem to have been rather small, undetectable in our assay conditions. Preincubation of cells, beads or both with these lectins had no detectable effect on viral yield when determined by haemagglutination (Table 1).

As Table 2 shows, the interferon inhibitory action of PHA was almost completely reversed by fetuin, a glycoprotein with high affinity for this lectin. No reversal was observed after treatment with galactose. Likewise, treatment of con A-preincubated cells or IF-Sepharose beads with α -methylmannoside almost completely restored the antiviral effect of IF-Sepharose. Identical treatment with galactose had no effect. We were unable to reverse the interferon inhibitory action of lotus lectin using solutions of L-fucose, a sugar of high affinity for this lectin. This might indicate that interferon inhibitory action by this lectin either is unrelated to its carbohydrate-binding property, or is due to very tight binding to oligosaccharide components that cannot be replaced by this monosaccharide.

Recent data indicate that mouse interferon is a glycoprotein with strong affinity for con A (ref. 11). Likewise, rabbit interferon has been shown to contain terminal neuraminic acid residues attached to penultimate galactosyl residues¹². Inhibitory effects of lectins on the antiviral action of interferon therefore could be caused by binding to the interferon molecule or to the cell surface. Table 1 suggests that both phenomena are possible.

There was pronounced inhibition of the antiviral activity when only the cells were preincubated with PHA, whereas little inhibition resulted from preincubation of IF-Sepharose alone. In contrast, inhibition by con A was more pronounced after preincubation of IF-Sepharose than after preincubation of the cells, although in the latter case appreciable inhibition was also observed. Inhibition by lotus lectin seems to be analogous to that by con A. The reversal of the interferon inhibitory action of PHA and con A by lectin-specific glycoprotein or saccharide solutions further supports the interpretation that the phenomena observed are due to the carbohydrate-binding properties of these substances.

It has become more apparent that certain peptide hormones exert their effects by interaction with the cell surface without penetration into the cell, chiefly because of Cuatrecasas' work with Sepharose-bound insulin¹³. In the case of insulin, receptor sites on the cell surface have been shown to be carbohydrate-containing macromolecules. These receptor sites bind to specific plant lectins; binding of lectin to the cell surface before addition of insulin blocks or modifies the hormone's action¹⁴. There is a striking parallel between the action of insulin and interferon: both diffuse from the site of formation through the blood stream to distant sites; both remain active when attached to an insoluble support, and both are inhibited by pretreatment of target cells with plant lectins. Although it is conceivable that the interferon inhibitory action of PHA is due entirely to specific binding of the lectin to interferon receptor sites on the cell surface, interpretations based on non-specific steric effects or electrostatic charge effects produced by the bound lectin are possible. Whether interferon-specific receptor molecules can be isolated from the cell membrane of interferon-sensitive cells and whether these molecules indeed bind to PHA and related lectins, remains to be established.

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¹ Isaacs, A., and Lindenmann, J., *Proc. R. Soc. London*, **B147**, 258-267 (1957).

² Isaacs, A., Cox, R. A., and Rotem, Z., *Lancet*, **ii**, 113-116 (1963).

³ Grossberg, S. E., *New Engl. J. Med.*, **287**, 1-19 (1972).

⁴ Ankel, H., Chany, C., Galliot, B., Chevalier, M. J., and Robert, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2360-2363 (1973).

⁵ Chany, C., Ankel, H., Galliot, B., Chevalier, M. J., and Gregoire, A., *Proc. Soc. exp. Biol. Med.* (in the press).

⁶ Craighead, J. E., and Shelokov, A., *Proc. Soc. exp. Biol. Med.*, **108**, 823-826 (1961).

⁷ Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).

⁸ Ahmed, A. I., Osuga, D. T., and Feeney, R. E., *J. biol. Chem.*, **248**, 8524 (1973).

⁹ Sage, H. J., and Green, R. W. in *Methods in Enzymology* (edit. by Ginsburg, V.), **28**, 332-339 (1972).

¹⁰ Osawa, T., and Matsumoto, I. in *Methods in Enzymology* (edit. by Ginsburg, V.), **28**, 323-327 (1972).

¹¹ Bourgeade, M. F., and Besancon, F., *C. hebd. Seanc., Paris*, **277**, 2833-2835 (1973).

¹² Dörner, F., and Scriba, M., and Weil, R., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1981-1985 (1973).

¹³ Cuatrecasas, P., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 450-451 (1969).

¹⁴ Cuatrecasas, P., *J. biol. Chem.*, **248**, 3528-3534 (1973).

Actinomycin D-induced breakage of human KB cell DNA

ACTINOMYCIN D is a potent inhibitor of RNA synthesis both *in vitro* and *in vivo*^{1,2}. Its mode of action involves the intercalation with DNA at G-C regions and prevention of polymerase movement along the template^{3,4}. Actinomycin D has also been shown to inhibit DNA synthesis, cell division and colony formation in mouse L cells⁵. We have studied the effects of this drug on the DNA of human KB cells in culture. Extensive DNA breakage was induced even at quite low concentrations of the drug.

When human KB (carcinoma of the nasopharynx) cells were treated with different concentrations of actinomycin D for 2.5 h, the rate of DNA synthesis, as measured by the incorporation of ³H-thymidine was greatly reduced at a concentration as low as 0.01 $\mu\text{g ml}^{-1}$ (Fig. 1). At concentrations greater than 10 $\mu\text{g ml}^{-1}$, DNA synthesis was inhibited completely.

To determine the effects of the drug on DNA breakage, cells were prelabelled with ³H-thymidine and treated with actinomycin D for 3 h. The cells were then lysed and the DNA was analysed by alkaline sucrose gradient centrifugation⁶. Figure 2 shows that fragmentation of DNA was induced by the drug at a concentration as low as 0.01 $\mu\text{g ml}^{-1}$. Moreover, the size of the fragments decreased with increasing concentrations of the drug. Figure 3 shows that increasing the treatment time with 10 $\mu\text{g ml}^{-1}$ of actinomycin D resulted in more extensive fragmentation of the DNA.

When fragmentation of DNA occurred the treated cells were

completely attached to the surface of tissue culture flasks. Less than 1% of the cells survived, however, at concentrations higher than $0.1 \mu\text{g ml}^{-1}$ of the drug as measured by colony formation (Table 1). It is possible that fragmentation of the DNA contributes to inhibition of DNA synthesis and loss of cell viability.

The possibility of DNA repair after removal of the drug was examined next. Cells prelabelled with ^3H -thymidine were treated with actinomycin D for 3 h, washed extensively with phosphate buffer saline, and incubated at 37°C for 10 h in growth medium in the absence of the drug. The DNA was then analysed by alkaline sucrose gradient centrifugation (Fig. 4). A comparison

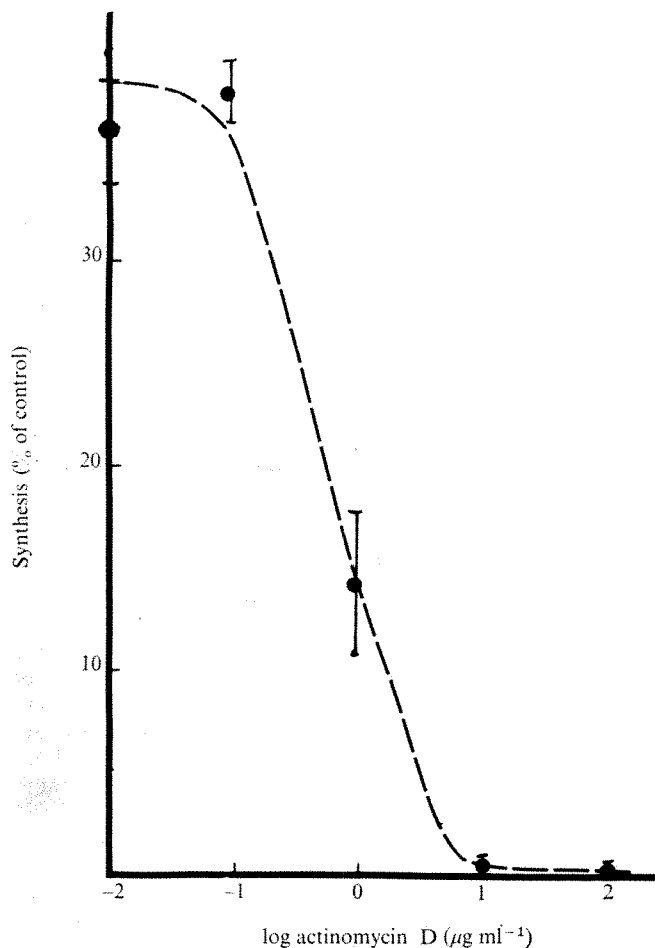


Fig. 1 Human KB cells grown on monolayers were trypsinised and 10^6 of them in 5 ml of alpha minimal essential medium (flow laboratories) + 10% foetal calf serum were seeded in 25 cm^2 Falcon tissue culture flasks and allowed to attach for 1 h at 37°C . Actinomycin D (Merck, Sharpe and Dohme) was then added. After 2 h, ^3H -thymidine (5 Ci mmol^{-1} , Amersham) was added to give a final concentration of $1 \mu\text{Ci ml}^{-1}$ and incubated for a further 30 min. The medium was then removed and the cells were lysed with 1% sodium dodecyl sulphate after washing with phosphate-buffered saline (PBS). The lysate was treated with cold 5% trichloroacetic acid (TCA) and the precipitate collected on to nitrocellulose filters. The radioactivity was determined by liquid scintillation counting. The amount of radioactivity incorporated into the control cells was 1.05×10^5 c.p.m. per 10^6 cells.

of Fig. 4 with Fig. 2 indicated that there was no DNA repair under the conditions of our experiments. Instead, the DNA was fragmented further at concentrations greater than $0.1 \mu\text{g ml}^{-1}$ of actinomycin D. These data suggest that breakage of DNA by the drug at concentrations greater than $0.1 \mu\text{g ml}^{-1}$ is irreversible. This irreversibility could, however, be due to persistence of the drug in these cells since no information on the elution profile of actinomycin D from these cells is available.

The mechanism of cellular DNA breakage by treatment with actinomycin D is not known. It is possible that *in vivo* binding of this drug to DNA induces distortion in the helix⁷ such that attack by nucleases is facilitated. This suggestion is compatible

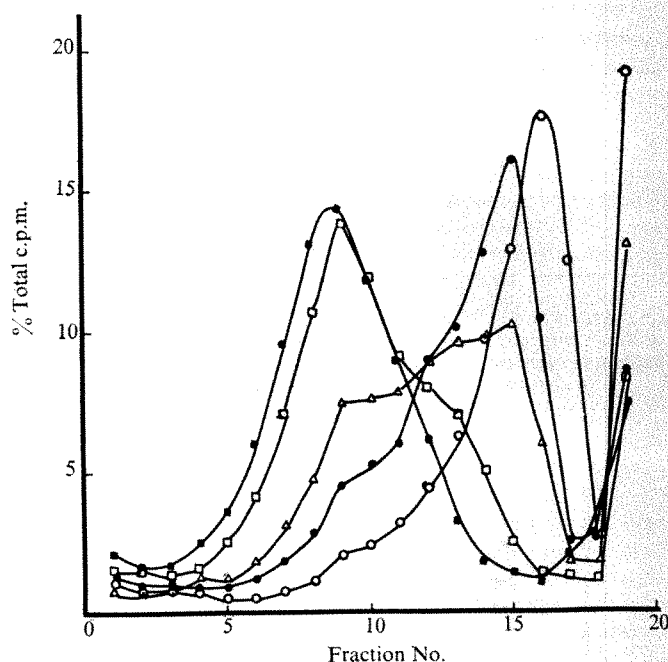


Fig. 2 Human KB cells prelabelled with ^3H -thymidine ($0.5 \mu\text{Ci ml}^{-1}$) for 12 h were treated with actinomycin D for 3 h as described in Fig. 1. The cells were trypsinised, washed and resuspended in PBS. About 2×10^4 – 4×10^4 of them were layered on to a 0.5 ml lysing solution containing 0.5 M NaOH, 0.2% SDS, and 0.01 M ethylenediaminetetraacetic acid (EDTA) on top of a 5–20% alkaline sucrose gradient containing 0.3 M NaOH, 0.01% SDS, 0.001 M EDTA. They were left to lyse for 12 h, and then centrifuged using a SW27.1 rotor in a Beckman ultracentrifuge at 20,000 r.p.m. for 6.5 h at 20°C . One-hundred-drop fractions were collected from the bottom of the tube. Radioactivity in each fraction was determined after cold TCA precipitation. Actinomycin D concentration ($\mu\text{g ml}^{-1}$): \circ , 0; \bullet , 0.1 (0.1 also similar to 0.01); Δ , 1; \square , 10; \blacksquare , 100. (Direction of centrifugation from left to right.)

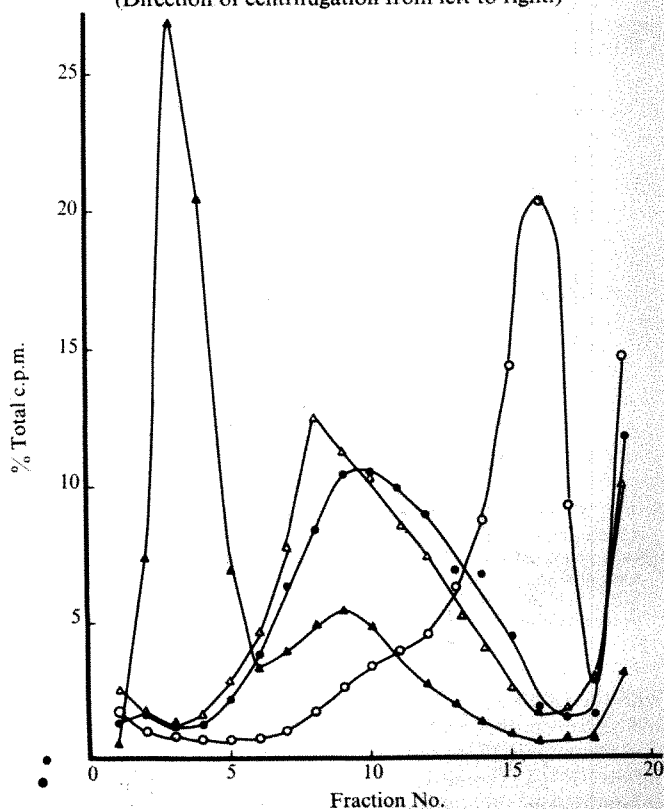


Fig. 3 The cells were prelabelled as described in Fig. 2 and incubated in the presence of actinomycin D at a concentration of $10 \mu\text{g ml}^{-1}$ for the indicated times. The DNA was analysed by alkaline sucrose gradient centrifugation as in Fig. 2. Duration of treatment (h): \circ , 0; \bullet , 1; Δ , 2.5; \blacktriangle , 10.

Table 1 Effect of various concentrations of actinomycin D on cell survival

Actinomycin D ($\mu\text{g ml}^{-1}$)	Average no. of colonies per plate
Control	150
.01	57
.1	0
1	0
10	0
100	0

Triplicate aliquots of 250 cells in 3 ml of α MEM plus 10% FCS were seeded in 60 \times 15 mm Flacon tissue culture dishes and allowed to attach and grow overnight. The medium was replaced by fresh medium containing the given concentration of actinomycin D. After 3 h of incubation, the cells were washed and growth medium was added. The number of colonies per plate was determined after 6 d of growth.

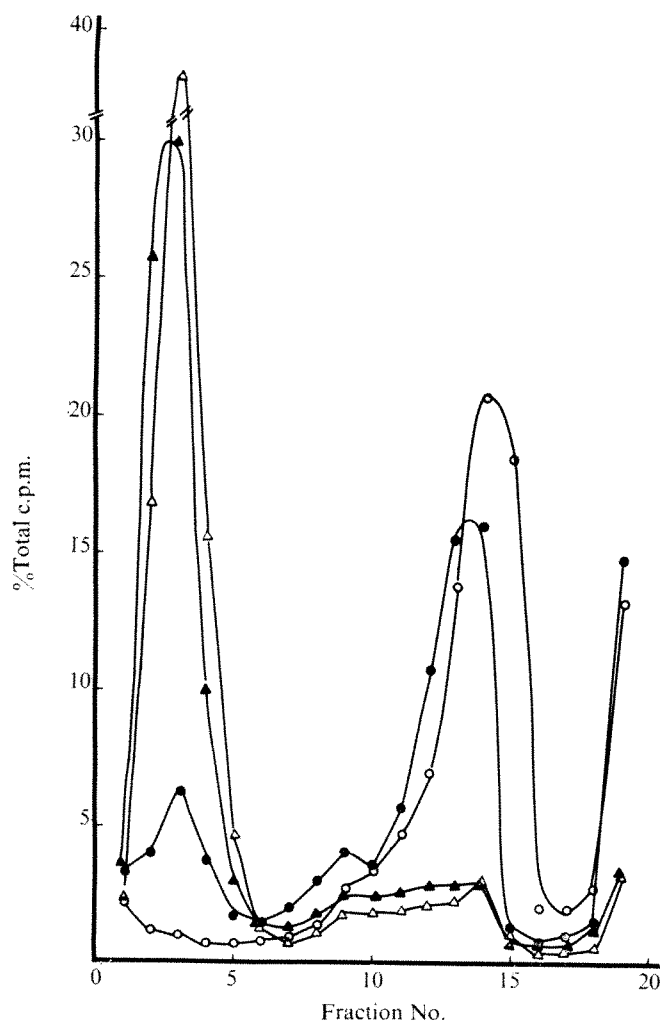


Fig. 4 The cells were prelabelled as described in Fig. 2 and incubated in the presence of actinomycin D for 3 h. The medium was removed and the cells were washed with 2×10 ml medium every 30 min for 2.5 h and incubated for 10 h further. The DNA was then analysed by alkaline sucrose gradient centrifugation. Actinomycin D concentration ($\mu\text{g ml}^{-1}$): \circ , 0; \bullet , 0.1; Δ , 1; \blacktriangle , 10.

with the fact that the DNA breakage induced by actinomycin D has not been observed *in vitro*^{8,9}.

Actinomycin D, at various concentrations, has been used to inhibit RNA synthesis and to examine the effects of this inhibition on other cellular functions. Data presented here and elsewhere¹⁰ suggest that caution is necessary in the interpretation of results obtained in such studies and that the fragmentation of the genome and its effects on cellular functions must be considered concomitantly. Furthermore, it appears that this drug

interferes with DNA as well as RNA metabolism in mammalian cells. Recently, it has been reported that initiation of protein synthesis is also inhibited¹¹. Thus, this drug may have more diverse effects on metabolic processes than is generally assumed.

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- ¹ Reich, E., and Goldberg, I. H., *Proc. Nucleic Acid. Res.*, **3**, 183-234 (1964).
- ² Perry, R. P., and Kelley, D. E., *J. Cell Physiol.*, **76**, 127-139 (1970).
- ³ Sobell, H. M., *Proc. Nucleic Acid. Res.*, **13**, 153-190 (1973).
- ⁴ Richardson, J. P., *J. molec. Biol.*, **21**, 83-114 (1966).
- ⁵ Bacchetti, S., and Whitmore, G. F., *Biophys. J.*, **9**, 1427-1445 (1969).
- ⁶ Paleic, B., and Skarsgard, L. D., *Int. J. Radiat. Biol.*, **21**, 535-544 (1972).
- ⁷ Muller, W., and Crothers, D. M., *J. molec. Biol.*, **35**, 251-290 (1968).
- ⁸ Kresten, W., Keresten, H., and Szybalski, W., *Biochemistry*, **5**, 236-244 (1966).
- ⁹ Waring, M., *J. molec. Biol.*, **54**, 247-279 (1970).
- ¹⁰ Elkind, M. M., *Int. J. Radiat. Biol.*, **22**, 313-324 (1972).
- ¹¹ Goldstein, E. S., and Penman, S. J., *J. molec. Biol.*, **80**, 243-254 (1973).

Potential of phytomitogens action by neuraminidase and basic polypeptides

TREATMENT of lymphocytes with neuraminidase enhances their blastogenic response to some antigens and mitogens¹, increases their lectin-induced agglutinability^{2,3}, antigenicity and immunogenicity^{4,5}, alters their homing properties⁶ and renders the cells highly susceptible to the cytolytic effects of alloantibody and complement^{7,8}. These changes might result from the exposure of new sites on the cell surface^{2,3,9}, a reduction in the net surface charge of the cells or a combination of the two effects. Polycations agglutinate different cell types¹⁰⁻¹² and markedly reduce their anodic electrophoretic mobility¹⁰. In order to study the effect of cell surface charge on lymphocyte blastogenic response to phytomitogens the effect of polycations on this process was compared with that of neuraminidase.

The stimulation of ³H-uridine incorporation into RNA in rat lymph node lymphocytes, induced by con A (Fig. 1) and by phytohaemagglutinin (PHA) (Fig. 2), was enhanced after treatment of the cells with neuraminidase. In the experimental conditions, outlined in the legend to Fig. 1, 2.2 μg of sialic acid is released per 10^8 cells. This is equal to the total amount of sialic acid which can be released by neuraminidase action. The enhancing effect of neuraminidase was more apparent upon treatment of the cells with low concentrations of the phytomitogens. Like neuraminidase, basic polypeptides were also found to enhance blastogenesis induced by phytomitogens. Poly-L-ornithine and Poly-D-lysine were used in this study as they are resistant to degradation by proteolytic enzymes¹³. As shown in Fig. 3a, poly-L-ornithine enhances the stimulation of ³H-uridine incorporation into RNA in rat lymph node lymphocytes induced by con A. A similar effect was obtained upon addition of poly-D-lysine (Fig. 3b). Poly-L-ornithine also enhanced the stimulation of ³H-uridine incorporation into RNA in rat lymphocytes induced by PHA (Fig. 4). The stimulation of ³H-thymidine incorporation into DNA in rat lymph node lymphocytes, induced by con A and by PHA was also enhanced by poly-L-ornithine. The extent of ³H-thymidine

incorporation (c.p.m. \pm s.e.) into control, con A ($0.5 \mu\text{g ml}^{-1}$) or PHA ($0.5 \mu\text{g ml}^{-1}$)-treated rat lymphocytes, after incubation for 48 h, was 800 ± 79 , $32,590 \pm 3,170$ and $33,710 \pm 6,010$, respectively. ^3H -thymidine incorporation into similar cultures incubated in the presence of poly-L-ornithine ($1 \mu\text{g ml}^{-1}$) was 742 ± 44 , $62,970 \pm 7,190$ and $80,260 \pm 5,680$, respectively. The basic polypeptides alone did not stimulate the cells. In the experimental conditions outlined in Fig. 3a, poly-L-ornithine did not enhance the response of neuraminidase-treated cells to con A. As it seemed possible that the effects caused by neuraminidase and by the basic polypeptides might result from an increase in the binding of the lectins to the lymphocytes, I investigated this possibility. Rat lymph node lymphocytes ($5 \times 10^6 \text{ ml}^{-1}$ Dulbecco's modified Eagle's medium, supplemented

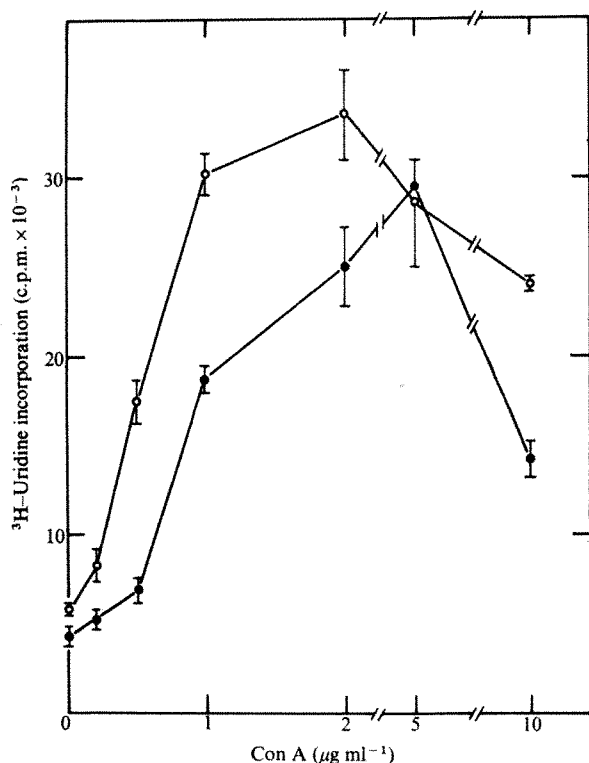


Fig. 1 Effect of neuraminidase on the response of rat lymphocytes to con A. Wistar rat lymph node lymphocytes ($10^6 \text{ cells ml}^{-1}$ PBS) were incubated with *Vibrio comma* neuraminidase (Behringwerke AG) (50 U ml^{-1}) for 30 min at 37°C . The cells were then washed and suspended ($5 \times 10^6 \text{ ml}^{-1}$) in Dulbecco's modified Eagle's medium, supplemented with horse serum (2%) and incubated as previously described²³. ^3H -Uridine incorporation was determined after incubation for 24 h. Con A, twice crystallised (Miles-Yeda) was used. Results are expressed as the mean \pm s.e. of triplicate cultures. \circ , Neuraminidase-treated cells; \bullet , control cells.

with horse serum (2%)) were incubated with ^{63}Ni -con A ($1 \mu\text{g ml}^{-1}$) in the absence and presence of poly-L-ornithine ($2 \mu\text{g ml}^{-1}$) at 37°C for 2 h. The specific binding of ^{63}Ni -con A to the cells was determined as previously described¹⁴. In these conditions, 48,000 molecules of con A were bound per cell in the control experiment and neither neuraminidase nor poly-L-ornithine had any effect on the extent of lectin binding.

In contrast to our finding that neuraminidase enhances the response of rat lymph node lymphocytes to PHA, other investigators have found that neuraminidase does not change the response of human lymphocytes to PHA¹ and inhibits PHA-absorptive capacity and PHA response of mouse spleen cells¹⁵. The discrepancies between the data obtained by different groups may be due to species differ-

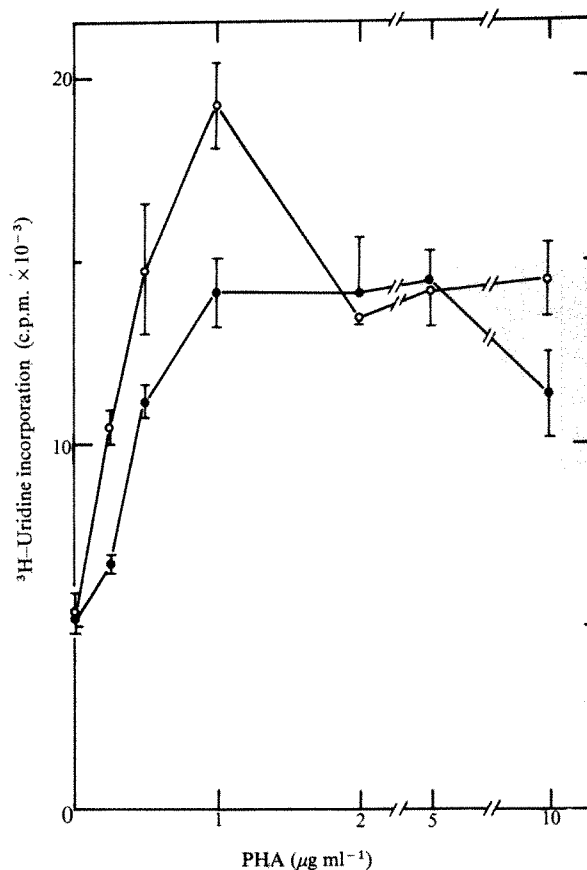


Fig. 2 Effect of neuraminidase on the response of rat lymphocytes to PHA. For experimental details see legend to Fig. 1. Purified PHA from Wellcome was used. \circ , Neuraminidase-treated cells; \bullet , control cells.

ences and to the different doses of neuraminidase and PHA used in the various studies. It has been suggested that cross linkage and aggregation of specific membrane sites may be involved in the triggering of lymphocytes to undergo blastogenesis¹⁶⁻¹⁸. Aggregation of membrane sites (most probably glycoproteins) induced by lectins, occurs when the balance of forces favouring aggregation overcomes those which oppose it, like charge repulsion forces. The overall net sur-

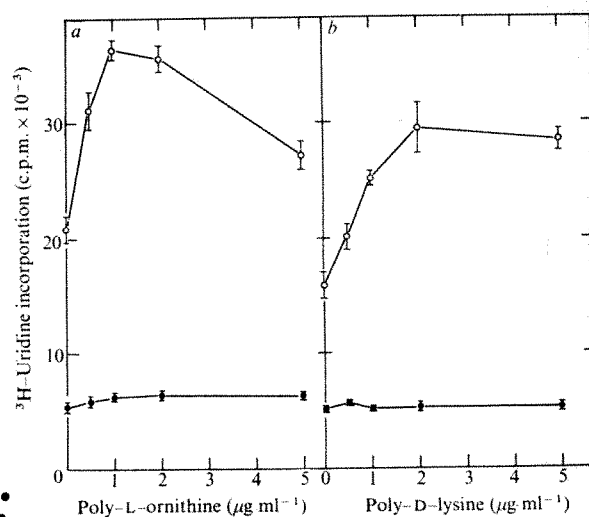


Fig. 3 Effect of basic polypeptides on the response of rat lymphocytes to con A. Cells were cultured in the presence of **a**, poly-L-ornithine (average degree of polymerisation (DP) = 340) or **b**, poly-D-lysine (DP = 380). \circ , In the presence of con A ($1 \mu\text{g ml}^{-1}$); \bullet , control.

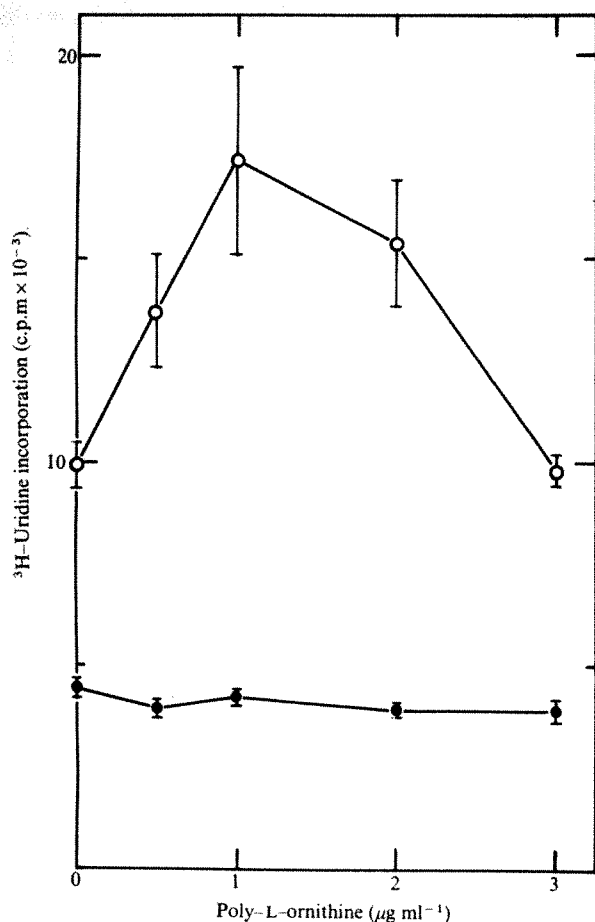


Fig. 4 Effect of poly-L-ornithine on the response of rat lymphocytes to PHA. O, In the presence of PHA (0.5 $\mu\text{g ml}^{-1}$); ●, control.

face charge of the lymphocyte is negative¹⁹ but little is known about the net charge of individual glycoproteins of the lymphocyte membrane. Glycophorin is a glycoprotein isolated from red cell ghost which carries the receptors for the plant lectins PHA and wheat germ agglutinin and contains 60% carbohydrate and 25% sialic acid by weight^{20,21}. If the lymphocyte receptors for con A and PHA were of a similar chemical nature, it might be expected that both neuraminidase treatment and polycations would facilitate their lectin-induced aggregation, by reducing charge repulsion. The enhancing effect of neuraminidase and basic polyamino acids on phytomitogens-induced transformation might also result from an increase in cell aggregation induced by either of these agents. Cell contact has been

shown to increase mitogen-stimulation of lymphocytes²². Neuraminidase treatment has a marked effect also on some cellular properties of non-lymphoid cells^{23,24}. It might be of interest to compare the effect of neuraminidase with that of polycations in a variety of biological systems. This approach might help to elucidate whether an observed effect induced by neuraminidase results primarily from a reduction in the surface charge or results from exposure of new sites on the cell membrane.

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- ¹ Han, T., *Clin. exp. Immun.*, **13**, 165-170 (1973).
- ² Nicolson, G. L., *J. natn. Cancer Inst.*, **50**, 1443-1451 (1973).
- ³ Novogrodsky, A., and Katchalski, E., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2515-2518 (1973).
- ⁴ Bagshawe, K. D., and Currie, G. A., *Nature*, **218**, 1254-1255 (1968).
- ⁵ Lundgren, G., and Simmons, R. L., *Clin. exp. Immun.*, **9**, 915-926 (1971).
- ⁶ Woodruff, J. J., and Gesner, B. M., *J. exp. Med.*, **129**, 551-567 (1969).
- ⁷ Schlesinger, M., and Amos, B. D., *Transpl. Proc.*, **3**, 895-897 (1971).
- ⁸ Ray, P. K., Gewurtz, H., and Simmons, R. L., *Clin. exp. Immun.*, **11**, 441-460 (1972).
- ⁹ Hammarström, S., Hellström, U., Perlmann, P., and Dillner, M., *J. exp. Med.*, **138**, 1270-1275 (1973).
- ¹⁰ Katchalsky, A., Danon, D., and Nevo, A., *Biochim. biophys. Acta*, **33**, 120-138 (1959).
- ¹¹ Moroson, H., *Cancer Res.*, **31**, 373-380 (1971).
- ¹² Duksin, D., Katchalski, E., and Sachs, L., *Proc. natn. Acad. Sci.*, **67**, 185-192 (1970).
- ¹³ Katchalski, E., Sela, M., Silman, I., and Berger, A., in *The Proteins*, II (edit. by Neurath, H.), 521-602 (Academic Press, New York, 1964).
- ¹⁴ Novogrodsky, A., and Katchalski, E., *Biochim. biophys. Acta*, **228**, 579-583 (1971).
- ¹⁵ Adler, W. H., Osunkoya, B. O., Takiguchi, T., and Smith, R. T., *Cell Immun.*, **3**, 590-605 (1972).
- ¹⁶ Fanger, M. W., Hart, D. A., Wells, J. V., and Nisonoff, A., *J. Immun.*, **105**, 1484-1492 (1970).
- ¹⁷ Greaves, M., and Janossy, G., *Transpl. Rev.*, **6**, 87-130 (1972).
- ¹⁸ Lotan, R., Lis, H., Rosenwasser, A., Novogrodsky, A., and Sharon, N., *Biochem. biophys. Res. Commun.*, **55**, 1347-1355 (1973).
- ¹⁹ Nordling, S. L., Andersson, L. C., and Häyry, P., *Science*, **178**, 1001-1002 (1972).
- ²⁰ Marchesi, V. T., Segrest, J. P., and Kahane, I., in *Membrane Research* (edit. by Fox, C. F.) 41-51 (Academic Press, New York, 1972).
- ²¹ Wintzler, R. J. in *Glycoproteins*, part B (edit. by Gottschalk, A.), 1269-1293 (Elsevier, Amsterdam, 1972).
- ²² Peters, J. H., *Exp. Cell Res.*, **74**, 179-186 (1972).
- ²³ Vaheri, A., Ruoslahti, E., and Nordling, S., *Nature new Biol.*, **238**, 211-212 (1972).
- ²⁴ Editorial, *Lancet*, **i**, 523-524 (1972).
- ²⁵ Novogrodsky, A., and Katchalski, E., *Biochem. biophys. Acta*, **215**, 291-296 (1970).

Erratum

In the article "Dynamic evidence on massive coronas of

galaxies" by J. Einasto, A. Kaasik and E. Saar (*Nature*, **250**, 309; 1974) Tables 1 and 2 were inadvertently omitted.

Table 1 Parameters of galactic populations (individual galaxies)

Galaxy	L_s ($10^{10} L_\odot$)	M_s ($10^{10} M_\odot$)	M_v ($10^{10} M_\odot$)	R_{90} (kpc)	$-\log p_v$ (g cm^{-3})
NGC224	2.0	17	> 35	> 14	24.58
NGC300	0.45	2.0	> 2.9	> 6	24.60
NGC598	0.32	1.5	> 2.8	> 6	24.54
NGC3031	1.8	12	> 21	> 11	24.52
IC342	4.9	12	> 34	> 13	24.55

Table 2 Parameters of galactic populations (pairs of galaxies)

Type of primary galaxy	$\langle L_s \rangle$ ($10^{10} L_\odot$)	$\langle M_s \rangle$ ($10^{10} M_\odot$)	$\langle M_v \rangle$ ($10^{10} M_\odot$)	$\langle R_{90} \rangle$ (kpc)	No. of pairs
Spiral (intermediate)	3.8	38	350	25	33
Spiral (bright)	15	150	1,600	47	32
Elliptical	12	250	> 1,500	> 46	40

matters arising

Blocking one-way maternal-foetal MLR

SIR,—The results of animal experiments^{1,2} show that enhancing or blocking antibodies may be at least partly responsible for the apparent lack of maternal immunological response to the paternally derived histocompatibility factors of the foetus. Youtananukorn and Matangkasombut³ used peripheral blood from normal *post partum* women to demonstrate that migration inhibition of maternal leukocytes occurred in the presence of pooled placental antigens. This response was completely blocked in the presence of autologous plasma but was unaffected by plasma from unrelated *post partum* women. We have used the one-way mixed lymphocyte reaction (MLR) between maternal (responding) and mitomycin-treated (stimulating) foetal cells obtained from cord blood to investigate the possible blocking effect of autologous maternal plasma.

Peripheral venous blood samples were collected at the end of labour from 12 women who had normal pregnancies and labours. Samples of cord blood were obtained at the same time, taking care to avoid contamination with maternal blood. Lymphocytes were separated from the heparinised blood samples by erythrocyte sedimentation in Plasmagel (Roger Bellon Laboratories). Cord blood lymphocytes were incubated at a concentration of $10 \times 10^6 \text{ ml}^{-1}$ in mitomycin C ($25 \mu\text{g ml}^{-1}$) for 20 min at 37°C and then washed three times in Eagle's MEM medium⁴. MLR

was then performed using the method previously described⁵. Results are summarised in the Table (figures in parentheses are standard deviations).

Autologous maternal plasma produced a significant inhibition of maternal lymphocyte transformation to stimulation by foetal lymphocytes in 3 out of 12 cases, while no significant effect was produced in control cultures containing homologous maternal plasma. In the presence of pooled male serum, a mixed lymphocyte reactivity of more than two occurred in only four out of twelve cases. This suggests the possibility that previous exposure of maternal lymphocytes to blocking factors *in vivo* might be partly responsible for their low responsiveness to foetal lymphocytes *in vitro*.

This might explain why autologous maternal plasma only produced a significant inhibition of response in those cases with a high mixed lymphocyte reactivity. It would also account for the apparent discrepancy between our findings and those of Youtananukorn and Matangkasombut³, since there might be different mechanisms whereby blocking factors produce their effects on lymphocyte transformation and leukocyte migration inhibition.

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² Kirby, D. R. S., *Transplantation*, **9**, 1005 (1968).

³ Youtananukorn, V., and Matangkasombut, P., *Nature new Biol.*, **242**, 110 (1973).

⁴ Bach, F. H., and Voynow, N. K., *Science*, **153**, 545 (1966).

⁵ Jones, E., Curzen, P., and Gaugas, J. M., *J. Obstet. gynec. Br. Commonw.*, **80**, 603 (1973).

A reply will be published in a forthcoming issue.

On the origins of molecular biology

SIR,—We wish to respond to the *Nature* supplement of April 26 entitled, "Molecular Biology Comes of Age". This gives the misleading impression that a specific date¹ can be placed on the birth of molecular biology. We suggest there are earlier origins for a true molecular biology.

In 1867 Spencer wrote in *Principles of Biology*:

"We have seen it to be a necessary inference from various orders of facts, that organisms are built up of certain highly-complex molecules which we distinguish as physiological units—each kind of organism being built up of physiological units peculiar to itself."

Forerunners of present concepts of molecular biology were proposed in the period 1860–1910 by Karl Nageli

Table 1 Results of the one-way MLR

One way MLR in pooled male serum	Mean 10 min count in pooled male serum	Mean 10 min count in autologous maternal plasma	Effect of autologous maternal plasma (%)	Probability	Mean 10 min count in homologous maternal plasma	Effect of homologous maternal plasma (%)	Probability
2.2	1,200 (249)	459 (74)	38	0.01–0.02	773 (198)	64	NS
0.8	387 (70)	550 (173)	142	NS	526 (24)	136	NS
0.7	520 (72)	627 (72)	121	NS	909 (495)	175	NS
9.8	3,089 (719)	3,193 (1,058)	103	NS	2,530 (91)	81	NS
1.1	361 (89)	421 (36)	117	NS	379 (108)	104	NS
2.0	502 (125)	548 (170)	109	NS	252 (72)	50	NS
1.0	478 (57)	491 (72)	103	NS	398 (51)	83	NS
1.1	338 (75)	316 (29)	94	NS	421 (109)	124	NS
0.8	283 (54)	294 (37)	104	NS	272 (79)	96	NS
1.4	703 (121)	344 (63)	49	0.02–0.05	619 (272)	88	NS
0.8	524 (147)	346 (46)	66	NS	536 (148)	102	NS
3.8	2,007 (601)	572 (227)	29	0.02–0.05	1,242 (1,194)	62	NS

3.25×10^6 maternal (responding) and 3.25×10^6 foetal (stimulating) cells were cultured in 3 ml of Eagle's MEM medium (pH 7.2) enriched with 1 ml of 2 mM L-glutamine per 100 ml, for 7 d. Test cultures (in triplicate) contained 15% complement-inactivated autologous maternal plasma. Two sets of control cultures (each in triplicate) contained 15% complement-inactivated homologous maternal plasma, and 15% pooled male serum respectively. Maternal and cord blood lymphocytes were cultured separately in triplicate, to assess spontaneous transformation. Mixed lymphocyte reactivity is expressed as the ratio of the mean of the triplicate counts of the mixed cultures to the mean of the triplicate counts of the separate maternal and foetal cell cultures. The effects of autologous and homologous maternal plasma are expressed as percentages of the ratio of the mean triplicate counts of the mixed cell cultures in autologous and homologous plasma respectively, to the mean of the triplicate counts of the mixed cell cultures in pooled male serum. NS, Not significant.

(quoted in ref. 3), August Weismann⁴, Edmund Wilson⁵ and Hugo de Vries⁶, among others.

The early origins of molecular biology discussed by Gunther Stent⁷ and others emphasise the desire to apply laws much like those of physics to biology. In this regard, Erwin Schrodinger's book *What is Life?*⁸ was influential. Fifteen years before the publication of Schrodinger's book, the well-known biologist Edmund Wilson wrote a book with a similar intention entitled *The Physical Basis of Life*⁹.

Whereas a preoccupation with physics may have motivated the so-called "phage group"¹⁰ there was a separate origin for the application of physicochemical methods to studies of the structures of biological molecules¹¹. W. Astbury, an X-ray crystallographer, clearly played a major part in this, and indeed was the first to coin the term molecular biology¹². In addition, it is not generally realised that in 1949 Sven Furberg, a Norwegian graduate student in the laboratory of J. D. Bernal, was the first to propose a helical structure for DNA, albeit single-stranded, on the basis of X-ray studies on nucleosides¹³.

We thus conclude that it is somewhat difficult to set a precise date for the origin of molecular biology, but we presume that by April 25, 1953, molecular biology had already come of age.

Yours faithfully,

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¹ Watson, J. D., and Crick, F. H. S., *Nature*, **171**, 737 (1953).

² Spencer, H., *Principles of Biology*, 2, 8 (Appleton, New York, 1867).

³ Coleman, A., *Proc. Am. Phil. Soc.*, **109**, 143 (1965).

⁴ Weismann, A., in *Essays upon Heredity and Kindred Biological Problems* (edit. by Poulton, E. B., Schonland, S., and Shipley, A. E.), 8 (Clarendon Press, Oxford, 1889).

⁵ Wilson, E. B., *The Cell in Development and Inheritance*, 358 (Macmillan, New York, 1900).

⁶ De Vries, H., *Intracellular Pangenesis*, 6, 48 (Open Court, Chicago, 1910).

⁷ Stent, G., in *Phage and the Origins of Molecular Biology* (edit. by Cairns, J., Stent, G., and Watson, J. D.), 3 (Cold Spring Harbor, 1966); *Science*, **160**, 664 (1968).

⁸ Schrodinger, E., *What is Life? The Physical Aspects of the Living Cell* (Cambridge University Press, Cambridge, 1944).

⁹ Wilson, E. B., *The Physical Basis of Life* (Yale University Press, New Haven, 1928).

¹⁰ Delbruck, M., in *Phage and the Origins of Molecular Biology* (edit. by Cairns, J., Stent, G., and Watson, J. D.), 9 (Cold Spring Harbor, 1966).

¹¹ Kendrew, J. C., *Scient. Am.*, **216**, 141 (1967).

¹² Astbury, W. T., *Nature*, **190**, 1124 (1961).

¹³ Furberg, S., thesis, London University (1949); *Acta chem. Scand.*, **6**, 634 (1952).

DNA synthesis in plants

SIR,—Buchowicz¹ reports "that incorporation of thymidine into DNA of germinating wheat seeds begins only after some preliminary activation of RNA synthesis is completed, implying an RNA-dependent DNA synthesis".

I do not agree with this conclusion. Indeed, it has only been demonstrated that RNA synthesis precedes DNA synthesis^{2,3} and that inhibition of protein synthesis between 0 and 9 h results in a complete suppression of DNA synthesis. These essential proteins are probably coded for by pre-formed mRNA^{4,5}. Thus, it is not at all evident that DNA synthesis is dependent on RNA in this way. Thymidine is not incorporated into DNA before a lag period in germinating wheat because, as in other plants, the phosphorylating enzymes are lacking in dry seeds^{6,7}. They become detectable a few hours before the onset of DNA synthesis^{7,8}. In contrast, uridine can be phosphorylated by extracts of dry seeds^{8,9}.

I wish to draw your attention to my interpretation of Dr Buchowicz's results. A prerequisite to check whether the cytoplasm may be an early site of nuclear DNA synthesis is to obtain a cytoplasmic fraction free of nuclear contamination. I have frequently obtained significant ¹⁴C-thymidine labellings in similar 'cytoplasmic fractions' with radish seedlings, but all the electron microscope controls I have performed have revealed that nuclei were broken in the 1,000g pellet, and that successive 15,000g and 27,000g pellets still contained chromatin. The difficulty of isolating unbroken plant nuclei is well known amongst plant biochemists, and this is the reason why I believe that most of the radioactivity detected by Dr Buchowicz in his cytoplasmic fraction is of nuclear origin.

The higher specific activity of the cytoplasmic fraction can be explained if we assume¹⁰ that newly synthesised, short, DNA fragments are probably more easily released from broken nuclei than highly polymerised DNA from chromatin. One way to check whether the cytoplasmic fraction is free of nuclear contamination would

be to assay this fraction for nuclear enzymatic activities such as RNA polymerase.

Yours faithfully,

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¹ Buchowicz, J., *Nature*, **249**, 350 (1974).

² Rejman, E., and Buchowicz, J., *Phytochemistry*, **10**, 2951 (1971).

³ Chen, D., Schultz, G., and Katchalski, E., *Nature new Biol.*, **231**, 69 (1971).

⁴ Mory, Y., Chen, D., and Sarid, S., *Plant Physiol., Lond.*, **49**, 20 (1972).

⁵ Chen, D., Sarid, S., and Katchalski, E., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 902 (1968).

⁶ Delseny, M., Got, A., Julien, R., and Guillon, Y., *C. r. Acad. Sci., Paris*, **277**, 2361 (1973).

⁷ Wanka, F., Vassil, I. K., and Stern, H., *Biochim. biophys. Acta*, **85**, 50 (1964).

⁸ Delseny, M., and Julien, R., *9th Int. Cong. Biochem., Stockholm*, Abstract B 3m47 (1973).

⁹ Wanka, F., and Walboomer, J. M., *Z. Pflanzenphysiol. Bd.*, **55**, 458, (1966).

DR BUCHOWICZ REPLIES: I was interested to learn that Dr Delseny had observed similar labelling of cytoplasmic DNA in radish seedlings.

The possibility of nuclear contamination of the 'cytoplasmic fraction' has not been overlooked in my letter¹. Instead, it was found insignificant, as the cytoplasmic radioactivity dropped when the possible source of contamination, radioactivity of nuclear DNA, increased. The comments concerning the dependence of DNA synthesis on thymidine kinase activity are obviously important. Nevertheless, the DNA synthesis would proceed without the participation of thymidine kinase (deriving TMP from UMP reduction and methylation) if not limited by other factors. It is known, however, that other precursors as well as thymidine are not incorporated into DNA at early germination stages, in spite of the fact that enzymes catalysing UMP synthesis² and reduction³ are active. It seemed, therefore, justified to point out that the initiation of DNA synthesis in awakening wheat embryo may depend on the appearance of a newly synthesised RNA fraction.

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¹ Buchowicz, J., *Nature*, **249**, 350 (1974).

² Mazus, B., and Buchowicz, J., *Phytochemistry*, **11**, 77 (1972).

³ Müller, H., Wahl, R., and Follmann, H., *9th Int. Cong. Biochem., Stockholm*, abstract 187 (1973).

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reviews

Scientific laws and logic

The Structure of Scientific Inference. By Mary Hesse. Pp. vii+309. (Macmillan: London and Basingstoke, April 1974.) £5.95.

IN this book Dr Hesse develops a logic of science. She is concerned for the most part with inference. One might expect however, that some account of scientific theories would be adopted as a starting point because theories are involved in most scientific inference. The account of theories will to some extent determine the characteristic patterns of inference. For example, if it is held that theories are partially interpreted deductive systems, then inference from theory to observable consequence will be deductive. In common with an increasing number of contemporary writers Dr Hesse rejects this formalist view. As an alternative a network model of theories is proposed (page 4):

"Briefly, the model interprets a scientific theory in terms of a network of concepts related by laws, in which only pragmatic and relative distinctions can be made between 'theoretical' and 'observational'. Some lawlike statements of the theory can be tested relatively directly by observation, but which these are may depend on the current state of the whole theory and whether an observation statement is accepted as 'true' or 'false' in any given case may also depend on the coherence of the observation statement with the rest of the currently accepted theory."

This implies that the acceptance or rejection of a given observation statement may depend on current theoretical considerations. These may change, and so we may come to reject as false a statement previously accepted as true. Dr Hesse mentions that the network model owes much to Quine and Duhem. This is clearly true.

The Quine-Duhem thesis and related views are by no means unproblematical. Suppose we want to reject as false the observation statement "The individual x_1 is red" which we had previously accepted. If x_1 and all those other individuals x_2, x_3, \dots which were previously said to be red still retain the same colour as before, then if x_1 is no longer red then neither are x_2, x_3, \dots , on the assumption that all the individuals have the same colour, hence nothing is red. If necessary some new word could be invented to describe this colour. Dr Hesse is not committed to this trivial view because on her account of how we learn to use words such as

red we recognise that individuals are similar to each other to an unspecified degree. Thus theoretical considerations may suggest that after all x_1 is not sufficiently similar to x_2, x_3, \dots to be called red. But if 'similar' is always taken to be 'similar in a given respect', as it is by Popper, then this implies that there are no degrees of similarity and also that 'similar' is transitive. There are of course, differences within the class of red objects in that there are shades of red, but degrees of redness in this sense does not imply degrees of similarity among red objects. Dr Hesse discusses similarity at some length; in particular she defends her account against a Popperian view. It is unfortunately not possible to do justice to her arguments in a short review.

Dr Hesse's account of theories leads her to discuss inductive and analogical inference; this involves a discussion of arguments from particular to general and from particular to particular. In the context of a logic of science these concern the confirmation of theories and predictions by evidence. Dr Hesse does not attempt to justify non-deductive inference, but rather she gives an explication of the intuitive inductive rules used by scientists. An explication is the process of formulating precise rules of use, possibly expressed in a formalised system, for an imprecise concept which is used in some domain of discourse. The explication may suggest revisions in the use of the concept in question or in the use of related concepts. Dr Hesse sees the problem of induction as falling into two parts: (1) to formulate a set of inductive rules; (2) to formalise these. She concentrates on (2). In the light of the network model she suggests that the axioms of probability are best adapted to explicate inductive practices. Probability is interpreted in the personalist sense as degree of rational belief. Thus the answer to this problem of induction is an acceptable probabilistic confirmation theory.

At least two problems confront a proponent of a probabilistic confirmation theory. First, it is necessary to show that some adequate resolution of the paradoxes of confirmation is possible within the theory. Dr Hesse takes the so-called transitivity paradox to be one of the most general and powerful paradoxes. It suggests that confirmation is not always transitive. We are not, therefore always able to assume that the degree of confirmation of a

theory by certain evidence is passed on to the predictions of the theory. In order to account for our confidence in predictions Dr Hesse interprets theoretical inference as inference from evidence to prediction bypassing theory. The inference is taken to be analogical and the role of the theory is to make explicit the analogy between evidence and prediction. This is a most interesting suggestion.

Second, if a probabilistic confirmation theory is to account for the confirmation of laws, it may be thought necessary to justify the restriction of the scope of laws to finite domains. This restriction, which is a consequence of the explication, is necessary because, given that the language is infinite and that there are no *a priori* reasons for assigning finite initial probability to any finite subset of possible universal generalisations, then no finite amount of evidence will raise the probability of such a generalisation above the initial value of zero required by the probability axioms. Popper and Lakatos have taken this to be a fundamental objection to Carnap's confirmation theory. But Dr Hesse argues that laws are expressed by generalisations of finite scope because it is unreasonable to believe that unrestricted generalisations have any chance of being true and hence that zero probability is appropriate for such generalisations. If restricted generalisations may be assigned non-zero probability, then we would expect that the reason why unrestricted generalisations have zero probability concerns the fact that their scope is unrestricted. But according to Dr Hesse (page 181):

"This is not so much because such a belief covers an infinite number of instances, but rather because it may not be reasonable to believe that U [a Universal generalisation] states a law accurately even in one instance."

If this is true, then it seems to follow that it is unreasonable to believe that a finite generalisation expresses a law accurately. It is not however, obvious that it is reasonable for scientists to be convinced (probability=1) that all universal generalisations are false. If it is held that some laws are expressed by universal generalisations, then we may be inclined to reject an explication which has as a consequence that these laws have zero probability.

Although I think that it is possible to give further arguments in favour of these criticisms of Dr Hesse's logic of science, this is not to say that Dr

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Hesse's book is not an interesting and original contribution to the philosophy of science. It is therefore to be recommended to the advanced student, though to one who is not familiar with the subject it may prove a little difficult.

JOHN FORGE

Television tomorrow

Television: Technology and Cultural Form. By Raymond Williams. Pp. 160. (Fontana/Collins: London, 1974.) 45p.

WHEN Raymond Williams was television critic of *The Listener*, he tried to appraise television as a phenomenon in spite of the convention among reviewers that what counts are programmes, as isolated units. In this book he is able to stand back and take a very long view. Television—what is it? Technology, or social force? What does the experience of watching television consist of? How did the institutions of broadcasting develop out of the technology and what kinds of institutions may arise to serve up television in its new forms—video cassettes, cable television, international broadcasting?

The author (now reader in drama at Oxford) concludes that there was nothing inevitable in television's emergence as a form of mass entertainment consumed by individuals in the privacy of their homes. He does not swallow the argument that "I Love Lucy" was as inherent in the vacuum tube as some believe the atomic bomb was in the splitting of the atom. Television, he maintains, emerged in its present form because it was under the control of the broadcasting institutions that had developed radio. And radio's growth was determined, even forced, by the equipment manufacturing industry. But between the two media (it is possible to use the word in the plural) there is a profound difference. Radio is cheap. Television is expensive. And the contradiction remains. No country, even the United Kingdom, has satisfactorily solved it. How to recover the enormous costs of television from a mass audience of individual viewers?

Mr. Williams devotes a long chapter to the comparative incidence of various types of television programmes on five television channels—three British and two American—during a single week. The results are hardly surprising. (BBC1 led the pack in public affairs discussions, with 8.3 hours, while Channel 7 in San Francisco was tops in commercials, with 18.4 hours.) A mild point made by the author is that the formal category of programme matters less than its manner. A serious documentary may be trivialised. A panel quiz game may illuminate rela-

tionships between husband and wife.

The most interesting thesis of Mr Williams's book, to my mind, is the idea that television is the whole package. He calls it "flow". The programmes, news and weather breaks, commercials, trailers for later programmes, the lot. By this standard, the BBC is full of commercials—for itself. He is amusing when he recounts the difficulty, in Miami, in trying to follow the plot of an old movie. Not only were the advertisements inserted more frequently as the viewer became hooked on the programme but there were added as well trailers of two other old films, to be shown on subsequent nights. He also observes that on the San Francisco channel, the news that some pharmaceutical companies had been accused of false advertising claims was part of the same news bulletin that contained a commercial for a pain killer.

Looking ahead, Mr Williams is worried by the paradox presented by the new technology. He sees the promise of cable (multi-channelled, wired) television and international satellite broadcasting as that of democratising communications, of bringing a new universal accessibility to television. Yet he sees (by looking back) that these developments could be stifled by existing broadcasting organisations, by international advertisers and governments. At the same time he sees that uncontrolled development of cable television could destroy what is good in national broadcasting. His main suggestion is that there should be more independent television production companies. These could diminish, without destroying, national broadcasting while providing content for cable systems to transmit. Cable he would have as a national utility.

There are a few quibbles one must make. Cable television will not be universally available in the United States by 1980. And it is unlikely to reach 30% of television homes in Britain by that time either. Its growth has been stopped dead, by restrictive rules and by shortage of investment capital. Similarly, the author's recommendation that proper international agreements on satellite television should not involve bodies with weighted voting (like Intelsat) is unrealistic. The fact is that, as the United Nations' experience has shown, a system of one vote to a country is a form of weighted voting itself, for it allows small countries a disproportionate influence from sheer numbers.

It is a pity that this book is not more readable for there is much in it that will interest media buffs. On the future of British Broadcasting, Lord Annan's committee should find it stimulating.

BRENDA MADDOX

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Recent advances in genetics, biology, and medicine have important social, ethical, and legal implications as well as being of great scientific interest. This book, which is based on a study by a British Association working party, has its origin in a series of discussions among scientists, doctors, surgeons, lawyers, theologians, M.P.s, and social scientists. It is concerned with some of the most significant topics: artificial insemination and fertilization of humans; genetic screening and selective abortion; organ transplantation, genetic engineering and cloning. The authors have set out to present the issues dispassionately in a form that will be intelligible to the general public. £4 paper covers £1.25 *forthcoming*

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Ian Varcoe

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Molecular Reaction Dynamics

R. D. Levine and R. B. Bernstein

This book deals with the molecular-level mechanisms of elementary chemical reactions, emphasizing the important role of binary collisions. The goal is an understanding of chemical and physical rate processes from the fundamental, microscopic point of view. Primary attention is devoted to the physical phenomena and their conceptual interpretation rather than to the details of experimental techniques or theories. Topics discussed in some detail include the dynamics of molecular collisions, potential-energy surfaces, reaction cross-sections, molecular and ion beam scattering, 'direct' and 'complex' modes of reaction, photo-fragmentation, energy partitioning, energy transfer, chemiluminescence, and chemical lasers. £5 *forthcoming*

Doctor meets patient

Therapeutics: From the Primitives to the 20th Century, with an Appendix—History of Dietetics. By Erwin H. Ackerknecht. Pp. x+194. (Hafner, Macmillan: New York; Collier Macmillan: London, January 1974.) £6.25.

MEDICAL historians have all too often shown greater interest in what doctors have written than in how they have treated their patients. They have studied medical ideas and theories rather than the ways these ideas have been applied. Certainly, theory fascinates more than praxis. Yet for the patient at least, praxis is the key medical activity, patients being far more concerned with what their physicians can do than with what they know.

Therapeutics is the point where doctor and patient meet. Nevertheless, such has been the neglect of this subject by medical historians that the last general history of therapeutics was published in 1877—a fact which makes Professor Ackerknecht's lucid and stimulating volume all the more welcome. First published in German in 1970, it has now been translated by the author, formerly Director of the Institute of Medical History at the University of Zürich. The book charts the therapeutic programmes and rationales of medicine from the Egyptians to the twentieth century. Ackerknecht's method involves analysis of the therapeutic writings of major medical figures in each particular age: the Hippocratics, Galen, Paracelsus, Sydenham, Laennec, Ehrlich, and so on. In addition, he refers frequently to the works of less well known doctors whose activities and thoughts were equally significant in defining the medical texture of each of his historical portraits. The result is a monograph which is crammed with facts and brimming with ideas.

It is difficult to summarise a book which is so wide ranging. Nevertheless, two recurrent themes should be mentioned. First, as Ackerknecht repeatedly demonstrates, medical theories have been far more flexible than medical therapeutics. Bloodletting, for instance, has been employed in many different settings. The primitives used it. The Greeks recommended it, as did the Indians. Bloodletting was practised in the Middle Ages, the Renaissance and the Enlightenment. In fact only in the middle of the nineteenth century did physicians begin to look critically at this traditional form of therapy. The therapy itself remained essentially unchanged, yet the rationale for this therapeutic procedure could be different for each particular age or culture setting. The same may be said for many other traditional therapies, such as purgatives, cathartics and emetics.

The second historical insight which Ackerknecht brings to bear on his subject concerns the nature of medical experience. In therapeutics, each doctor should be able to form independent evaluations of the effectiveness of his armamentarium. Unfortunately, experience often being no more than 'pseudo-experience', doctors have found that any particular therapeutic regimen yields precisely the results they expected it to. Patients rarely disappoint their physicians if they can help it.

Professor Ackerknecht explores the historical ramifications of these and many other themes in this splendid little book. A full appreciation of it requires some prior acquaintance with the general features of the history of medicine. This can be pleasantly acquired through Ackerknecht's *Short History of Medicine*, published in 1955 but still the best short introduction to the subject available. The remarkable range of his interests may be seen from some of his other contributions to the history of medicine: a biography of Rudolf Virchow, a study of French medicine in the first half of the nineteenth century, a monograph on malaria in the Upper Mississippi Valley, a short history of psychiatry, and a series of classic papers on primitive medicine. These works mark Erwin Ackerknecht as a true cosmopolitan and one of the outstanding medical historians of our time.

W. F. BYNUM

Rejecting behaviourism

The Psychology of Consciousness. By Robert E. Ornstein. Pp. xxi+247. (Freeman: San Francisco, 1972.) \$3.50.

THIS book represents a current swing away from behaviourist psychologies, in which consciousness is ignored or even denied. It criticises Western science, as being over-limited to sequential processes of reasoning at the cost of immediate intuitive understanding, associated with the Eastern approach to studying mind represented by the Chinese I Ching and the meditative and rhythmic exercises of yoga. Dr Ornstein tries to relate East and West, with the neurological notion that (in right handed people) the right cerebral hemisphere serves the artist and dreamer, while the left hemisphere serves sequential logical thinking and language. For this he refers to the important 'split brain' experiments of Roger Sperry, in which the fibres of the corpus callosum, normally joining the hemispheres, are sectioned in serious cases of epilepsy.

Ornstein suggests that the right-hemisphere Eastern culture and the left-hemisphere Western culture can be combined—to make better use of the brain as a whole. Is this a neurological alliance of C. P. Snow's Two Cultures?



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Grassland Ecology and Wildlife Management

E. DUFFEY, M. G. MORRIS, J. SHEAIL,
L. K. WARD, D. A. WELLS and T. C. E. WELLS

July 1974: 0 412 12290 1: 304 pages:
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This book describes the distribution and ecology of lowland grasslands in Britain with special reference to their flora and fauna, history, and management for wildlife conservation. The maintenance and manipulation of grasslands for agricultural, scientific, conservation and recreational purposes requires an extensive knowledge of the responses of plants and animals to different treatments and disturbance factors. The book examines these in relation to the range of variation in lowland grassland ecosystems and to the known land-use history.

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Ornstein refers also to recent experiments on 'biofeedback', in which he has himself made interesting contributions on the control of automatic functions not normally under conscious control. There are also references to anthropological data on different ways that people categorise, describe and see things as evidence for the relativity and perhaps arbitrariness of what is accepted as knowledge.

The book starts by referring to Kuhn's scientific paradigms, as both giving stability to science and belief (analogous to the perceptual constancies) and also as limiting what we can see and understand. To Ornstein, behaviourism's rejection of consciousness, and logical positivism's rejection of statements not susceptible of strict 'scientific' verification as not meaningful, are examples of paradigms so narrow that important understanding is blinkered and lost. Although one may sympathise with Ornstein's dissatisfaction with behaviourism as a paradigm for psychology (however useful it may have been to concentrate experiments on technically feasible enquiry) he does not lay siege with much fact or rigour, except indeed to point to consciousness as a fact that it is absurd to ignore. He seems in places to follow Aldous Huxley in regarding the nervous system as doing not much more than filtering experience—quoting with approval Huxley's remark: "Mind at large has to be funnelled through the reducing valve of the brain and nervous system. What comes out at the other end is a measly trifle of the kind of consciousness which will help us to stay alive on the surface of this particular planet". This is, however, a view far opposed to current theories of perception, as active construction from limited data signalled by the senses—but which Ornstein also espouses. There seems to be a lack of consistency in the book on these questions; but its aim is evidently to loosen what Ornstein regards as constraining bonds of current theories of brain function, rather than attempt at this time to produce a conceptual synthesis, or a consistent working paradigm for psychology. It does have the merit of asking some awkward questions, with suggestions that answers might be found in strange places.

Here we come to the nub of the problem: to the Western reader without experience of 'meditation', the descriptions of conscious states and so on are difficult to comprehend or even to take seriously; but is this because of restricting limits of our experience, and paradigms of what should constitute science? Ornstein puts the matter cogently (page 6): "Science as a mode of knowing involves a limitation on enquiry. The essence of good experiment is successful exclusion". And

later (page 100), reversing the case from a non-technological to a Western society: "Our peasant . . . cannot see why, if it is indeed possible (as we claim) to fly to the planets, he cannot do it now in his own terms . . . When he fails, he will likely come to believe that 'space flight' is really an impossibility, that any one who claims it to be possible is simply gullible, 'unscientific', or even a liar . . . It is similar tendencies in ourselves which we, as Western students of psychology, may need to overcome in investigating an area which is so new, so spectacular, and so unknown to us . . . We should not ignore 'Eastern science' because of its imbalances, or because of the misinterpretations heaped around it". This book presents a case with honesty and with learning which is nevertheless in parts very difficult to comprehend, let alone judge. But if the case were valid, surely this is just what we might expect!

RICHARD L. GREGORY

Highly strung

Development and Regeneration in the Nervous System. Edited by R. M. Gaze and M. J. Keating. Pp 105-193. (British Medical Bulletin Vol. 30, No. 2.) (British Council: London, May 1974.) UK £2.25; elsewhere £2.50.

THE title of this bulletin is most appropriate for the two thirds concerned with studies on the nervous systems of animals. Research on nerve specificity and regeneration using histology, electron microscopy, surgery and electrophysiology is described. The rest of the bulletin is concerned with medical and social topics to which the physiological studies should be relevant: the epidemiology of spina bifida, the effect of malnutrition on intelligence, the educability of the subnormal. Though the gap between the two groups is to some extent bridged by Balács's description of metabolic influences on brain development in both animals and men, the difference in treatment is marked. This is inevitable as long as the relation between nervous connections and learning is unknown. Only Cragg speculates on this, discussing his work on synaptic patterns in mentally deficient mice.

The physiological articles are mostly excellent reviews of areas of research. Gaze's article on neuronal specificity in goldfish is particularly lucid. I found the other articles slightly less satisfactory, because of the relative paucity of facts. An exception is Bower's fascinating review on the innate abilities of newborn babies and how these develop. The bulletin is well presented and will be invaluable for those interested in the wide fields it covers.

GILLIAN MOORE

$$G = \frac{Mmc}{r^2}$$

**a NEW ROAD TO RELATIVITY—
DISCOVERED by MORRIS REDMAN SPIVACK**
4th Edition, 1974. © by M. R. Spivack 1967, '68 & '72.

G—gravitational attraction. **M** is one mass, in grams, **m** is another. **c** is the velocity of light (cm/s). **r**—radius between **M** and **m**.

In this revolutionary formula (the velocity of light being identical with the velocity of transmission of the gravitational impulse) **c** represents the gravitational field, while **Mm** represents the inertial field. Thus I bring Einstein's principle of equivalence into Newton's law of gravitation! (**c** = 2 in the numerator) (but also cm/s)

ABSTRACT

1. In my formula $G = \frac{Mmc}{r^2}$, the $\frac{c}{r^2}$ gives the energy of transmission of the gravitational impulse between the masses **M** and **m** — it is the velocity "**c**" that puts the energy value of the Einstein field into Newton's inverse square law. That is to say the velocity "**c**" is already involved in the Newtonian gravitational potential, but this potential (as Einstein puts it) is only one half of the total potential.

Consider a body situated at the surface of a mass. The radius of the gravitational field (**r**) will coincide with the radius (**r**) of the volume of the given mass. The energy value of the field will be exactly equal to the Newtonian potential at the surface of the mass — a geometrical principle is involved — the Newtonian potential at the surface is doubled. This is the situation in the test of general relativity for the bending of a ray of light passing the limb of the sun, also in the test for the gravitational red-shift of a ray of light issuing from a massive body like the sun or the Companion of Sirius.

The radius of the gravitational field can never be less than the given radius of the volume of the attracting mass, furthermore in this situation the energy contribution of the field, which starts at the centre of the attracting mass, will be at its highest possible level.

Since in my formula, c/r^2 represents the energy of the field, we can readily see that the contribution of the factor "**c**" (3×10^{10} cms) will be greatest in these cases, that is where the radius of the field (**r**) coincides with the radius (**r**) of the attracting mass. So my formula coincides with Einstein's famous doubling principle and gives the identical results in the above cited two tests.

The doubling principle may be understood by analogy of the structure of the gravitational field with the electro-magnetic field as consisting of TWO wave trains — INERTIA and GRAVITATION crossing each other orthogonally.

The FIELD (4 terms) preserves the REIMANNIAN indices. The DOUBLING arises from the SYMMETRY of the FIELD.

This structure of the universe is shown in my chart "VITAL CORRELATIONS" (Bayonne, N. J., 1946). This interpretation is also supported by Einstein's "equivalence principle".

Starting from this fundamental result, we can be guided by the ratio r'/r for all cases where the distance between the two bodies is greater than the radius of the volume of the given attracting mass.

And we draw the conclusion that the energy value of the factor "**c**" (as indeed the Newtonian attraction also) will decline relative to the maximum possible value (at the surface of the attracting mass) as r' becomes greater than r .

2. Applying the above findings to the problem of the excess motion of the perihelion of Mercury, we have:

Radius of the sun = 432500 miles. Mean distance of Mercury from the sun = 36 million miles.

Ratio — 1/83.

Ratio for mean distance to surface of sun = 1/82.

But in order to include the rotation of the ELLIPSE of Mercury around the sun we must double the energy contribution of the field — which is to say the additional

The above is an abstract of **A NEW ROAD TO RELATIVITY** (7 pp.) which can be obtained gratis (loose-leaf) by sending minimum postage for sea or air mail to the author and publisher: **M. R. SPIVACK**, poste restante 12, Upsala, Sweden. Bound copies (limited) £4

contribution of the velocity factor "**c**" (as energy of the field) will be 1/41 of the Newtonian potential at the surface of the sun, or 1/41 **c** (equivalence principle) (see above).

$1/41 \times 3 \times 10^{10}$ (velocity of light in centimeters per second, in the cgs system) = 7.317×10^8 .

According to Newcomb, in the American Ephemeris, the perihelion of MERCURY has a motion not accounted for by NEWTON which can be found by multiplying the MEAN MOTION by 8.06×10^{-8} .

The MEAN MOTION of Mercury appears (rounded out) as $5\ 381\ 016'' \times 100$ per century. Multiplying this figure by 8.06×10^{-8} gives $43.37''$ of arc excess motion of the perihelion per century.

As above, I derive the factor 7.317×10^8 directly as inversion from the velocity of light as multiplier of mass in Newton's inverse square law.

$7.317 \times 10^8 \times 5\ 381\ 016'' = 39.38''$ of arc (per century), which leads to a value of $42.96''$ for the total excess motion.

$3.7317 \times 10^8 \times$ mean motion, includes within the terms only 1/2 of that part of the excess motion which is due to increase of mass (with increase of velocity) of Mercury as it moves thru the perihelion.

The other half is due to increase in energy of the field, with velocity (equivalence principle). Put in other words, mass increase due to velocity increase must be added to the **Mm** in $G = Mmc/r^2$ both for the inertial and gravitational fields.

Now the Newtonian line-element ds^2 is related by the special law of relativity to the ratio v^2/c^2 in such a way that increase in the velocity **v** may, in the case of Mercury, lead to mass increase by a factor of as much as $1/2\ v^2/c^2$.

The unamended Newtonian line-element, however, can only give values equal to 1/2 of the combined effect of the inertial field and the gravitational field — and consequently only 1/3 of the non-periodic advance of the perihelion of a planet, and as we lack only 1/2 of the mass increase — a factor of $1/4\ v^2/c^2$ — this amounts to 1/12 of the total excess motion of the perihelion. To make up 1/12, we add 1/11 to $39.38 = 42.96$ seconds of arc per century. This is within the modern observed value $43.10 \pm .44$ seconds of arc per century.

In terms of **c**, we have $1/37.585$ of **c** instead of $1/41\ c$, or $1/37.585 \times 3 \times 10^{10}$ cm/s instead of $1/41 \times 3 \times 10^{10}$.

* The doubling of the value of **Mm** in Mmc/r^2 is actually a result of the transinteraction of the inertial and gravitational fields. Therefore **c** must be expressed as a multiple in the formula even if the result of the transinteraction is additive. A notation in the form $Mm + c$ (each side taken as unity) would fail to express the dynamics of the transinteraction.

In the extraction of the excess motion of the perihelion of Mercury from the mean observed motion, the velocity of light is used in centimeters per second as a multiple (inverted to deal with the line-element).

The line-element $3ds^2$ used in this section is dependent on **c** = 2 in the numerator of the formula.

Thus both forms **c** = 2 and **c** = 3×10^{10} cm/s are used as required, and are actually interdependent.

This proves that the value **c** = 2 truly derives from and represents the velocity of light.

$G = Mmc/r^2$ IS THE NEW ROAD TO RELATIVITY.

(For MARS my formula gives circa 1.3 seconds of arc per century, excess motion.)

MORRIS REDMAN SPIVACK

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Human Settlements: The Environmental Challenge. (A Compendium of United Nations Papers Prepared for the Stockholm Conference on the Human Environment, 1972.) Pp. xvi+209. (Macmillan: London and Basingstoke, March 1974.) £5.95.

Man, Materials, and Environment. National Academy of Sciences: National Academy of Engineering. Pp. xviii+236. (MIT: Cambridge, Massachusetts and London, 1973.) \$3.95.

Topophilia: A Study of Environmental Perception, Attitudes and Values. By Yi-Fu Tuan. Pp. x+260. (Prentice-Hall: Englewood Cliffs, New Jersey, January 1974.) \$8.95 cloth; \$4.95 paper.

THE United Nations Conference on the Human Environment held in Stockholm 1972 may well prove one of the most significant events of the decade. Consequently a volume of collected papers from the conference is likely to form a valuable reference to any research group working in this area. One feature of economic growth has been the enormously rapid development of cities, particularly in developing countries, and the fifteen background papers prepared on this issue around the theme "The Planning and Management of Human Settlements for Environmental Quality" are brought together in this work. The papers are not intended to be original. What they attempt to provide is a thorough and coordinated review and analysis of current problems in each subject area. Included are general commentaries on comprehensive development planning, population growth and distribution, rural development, a case study on the environmental impact of Polish industry on Warsaw central city, transport and communication, waste disposal and sewage and lastly, a relatively short account of social, cultural and aesthetic factors. An appendix lists those recommendations of the conference concerned with settlement and urbanisation.

The main purpose of *Human Settlements* is to demonstrate the importance of the careful planning and management of human settlements and that this should be recognised as a major means of assuring an appropriate environment for human survival and development. The book emphasises the point that pollution need be by no means an unavoidable consequence of economic development. The suggestions made in the various chapters (written by different authors although not individually named here) are wide ranging but suffer in that they tend to be over-generalised and lack a certain coherence of view; possibly inevitable in a set of collected papers. Nevertheless, the work contains much of

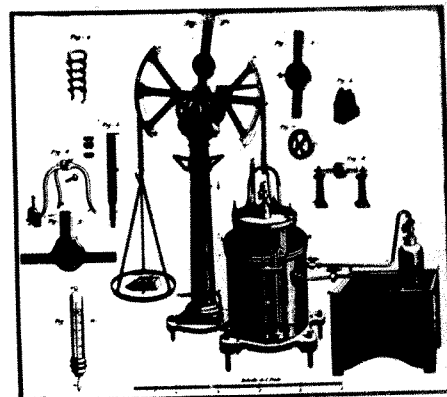
interest to those who would argue that it is in towns and cities, where most people spend the bulk of their lives, that the environment matters most. Two factors mitigate against wholeheartedly recommending it as a purchase for the individual researcher; the first is that two years have elapsed since the conference in June 1972; the second is an exceedingly high price for what, after all, is a summary.

By comparison, *Man, Materials and Environment*, a report on materials policy in the United States in relation to environment, has been produced with laudible rapidity. Using the formula of direct replication of authors' typescript has kept the cost down to a reasonable level and resulted in a book sturdy enough to withstand its presumably limited life (in this rapidly developing research area). The book represents the findings of a number of authoritative study groups contracted by the National Academy of Sciences, one of whose roles was to evaluate the effects on US materials policy of the suggestions of the Stockholm Conference. The aim of the study groups was to identify major issues and propose some positive actions with respect to public policy and research. The detailed findings of the teams are conveniently summarised in the first chapter. The second chapter, on the economic implications of seeking a high environmental quality, sets out the arguments for and against reduction in the overall rate of economic growth. The problems of metallic and non-metallic mineral extraction, fuels and forest products and the relations of domestic policy for international trade are tackled in turn and included for good measure is a comparative case study of environmental resource problems and policies in Japan. Particular attention is paid to the operation of the 'polluter pays' principle in a mixed economy and this method is advocated for the United States (and has now also been accepted by the European Commission for inclusion into the Community Environment Protection Programme). Emission taxes rather than subsidies are reckoned to be the socially least expensive but in a number of areas, such as packaging, the suggestion is that experimentation will lead to the best allocation of charges. In the main, this volume is entirely constructive, suggesting lines of research and policy methods to safeguard the future of the natural environment. One may jibe at some of the emphases but in doing so must accept that the research proposed will help to clarify these areas of uncertainty. A book certainly to be recommended to any serious researcher in this field.

Finally, Tuan's book *Topophilia*, easily compensates for the absence of

discussion about cultural aspects of the environment found in the other volumes. Topophilia is the affective bond between people and place. In this enjoyable and stimulating book Tuan sets himself the task of describing a complicated synthesis and he has succeeded admirably. The book has Tuan's distinctive style and communicates well both his subject and his own love of it. He approaches the questions: What are our views on the physical environment, natural and man made? How do we perceive, structure and evaluate it? What have been and what are our environmental ideals? How do economy, life style, and the physical setting affect environmental attitudes and values? What are the links between environment and world views? He traces and compares the answers to these questions from the early Greeks to the modern American city, the Aivilik Eskimo and the Hopi Indian, between humans and animals. By contrasting the perceptions and activities of man, the city dweller with that of his forbears, Tuan exposes both the richness and the poverty of city life. He builds an image of 'civilised' man's perception of nature, especially the 'countryside', as a reaction to the city which itself was originally evolved as an escape from the dangers of the wilderness. Thus, Tuan describes the city as a symbol, even tracing the history and function of city nicknames. For example, of the four American cities with the largest number of nicknames, New York (the Big Apple, the Babylonian Bedlam) boasts its world status, Washington its political supremacy, Chicago projects civility and San Francisco elegance. Of a different

Dispensing gases



Lavoisier's gasometer, taken from his *Traité élémentaire de Chimie*. The gasometer supplied a stream of gas flowing at a steady rate to a reaction chamber and was used in the demonstration of the quantitative composition of water; it was introduced to Holland by Martinus Van Marum. From *Martinus Van Marum: Life and Work*; vol. 4, Van Marum's Scientific Instruments in Teyler's Museum. By G. L'E. Turner and T. H. Leveres (Noordhoff: Leyden, 1973, Dfl. 60).

culture, Tuan describes how the rain-forest environment of the pygmy with its lack of horizon and landmarks, without pattern, has resulted in a world view in which the sense of distance and perspective is "distorted" and a sense of time is curtailed.

But to dip into the book, despite the inherent interest of every page, is not to do it justice because it must be emphasised that Tuan has composed a coherent and systematic thesis, analysing those innate aspects of our values and perceptions which we seem to share with other life forms, from those which mark us as individuals, or as members of a certain culture or subgroup. Possibly, and this might be taken as an anticipation rather than an omission from an otherwise comprehensive work, Yi-Fu Tuan's thesis could have led into a deeper discussion of the evolving nature and complexity of contemporary society in relation to concepts such as "post-industrial society" and "future shock", but perhaps this is for another volume. What he has achieved is to extend a way of looking at the natural and physical environment which demonstrates and calls for an appreciation of our own and other people's values and way of life at all levels of planning. The book is inexpensive and well indexed and should provide a useful and thought provoking basic text for both the casual reader and university courses dealing with urban geography, man in relation to the environment or the humanities.

H. S. D. COLE

Acoustic orientation

Echolocation in Animals. By E. S. Airapet'yants and A. I. Konstantinov. (Translated from Russian.) Pp. vi+309. (Academy of Sciences of the U.S.S.R. Joint Scientific Council on Physiology of Man and Animals.) (Israel Program of Scientific Translations: Jerusalem; Wiley: Chichester; January 1974.) £10.50.

THIS book was first published in the Soviet Union in 1970. It reviews Russian literature up to 1969 and Western literature up to 1968 so that many important discoveries made within the past five years are not covered. The major part of the book is devoted to studies on bats. This includes sections on the biology of bats, the nature of sound waves and methods of studying bat sounds. The signals emitted by bats are described and methods of sound production and radiation are discussed. Studies on both the auditory system of bats and their echo location ability are dealt with in some detail. Echo location in birds and small mammals is also discussed, but very briefly. The chapters on Cetacea include descriptions of their biology, mechanisms

of sound production and reception, a review of the different signals emitted by these animals and a consideration of their echo location performance. The final chapter of the book is devoted to echo location in pinnipeds.

The main value of the book is that it draws together much recent Russian literature on echo location. Russian work is described in some detail, particularly in the sections on hearing in bats and on the echo location abilities of both bats and dolphins. Although this is useful for non-Russian readers, it often results in considerable imbalance in the treatment of different topics; a more comprehensive treatment of some topics would have been useful. For example, the authors describe their own equipment to illustrate methods of studying bat signals. But this excludes a consideration of equipment used by other workers, such as portable bat detectors and sound spectrographs, both of which are important in the study of bat signals. Displays from the latter, taken from the literature, are presented later in the book with no explanation and occasionally without adequate labelling.

The authors express the hope that the book will appeal to readers of many professions, but very little help is given to interdisciplinary readers or to newcomers to the subject. For instance the theoretical considerations of various aspects of echo location are often difficult to follow and many of the equations are given without any hint of derivation or a source reference. Also, descriptions of the anatomy of the larynx and the auditory pathways are given as a string of latin names, without adequate explanations or diagrams. The absence of an index is also a great drawback and makes the book difficult to use as a reference text.

On the whole the English translation from the Russian is very readable but there is some awkwardness of expression in which the exact meaning is often not clear. Some apparently literal translations have been given, for example 'humeral girdle' and 'cathode repeater' when these could have been fairly easily replaced by their more commonly accepted equivalents, here 'pectoral girdle' and 'cathode follower' respectively.

Some points of production are irksome. The latin names of species and genera are not italicised (this is a reversal from the Russian text) and the references given in the text and in the bibliography are not always accurate or consistent.

Because the literature on echo location is expanding so rapidly, a new comprehensive, critical review would be welcome. This book is disappointing both in the treatment of the material and in its presentation and does not

fulfil the requirements of such a review. But because of its account of Russian work, the book will be a useful, though expensive, addition to literature on bioacoustics.

GILLIAN D. SALES

Plant ecology in Africa

East African Vegetation. By E.M. Lind and M. E. S. Morrison. Pp. xvii+257. (Longman: London, April 1974.) £6.

THE appearance of this work will be hailed by those interested in tropical Africa as an ecological milestone, for in spite of excellent herbaria and taxonomic works, biologists have had to work in these regions without the aid of a general description of the vegetation. Indeed in many cases, large mammal research schemes have been devised without reference to botanical studies.

The authors have avoided the difficulties of describing vegetation with long lists of plant names by referring to dominant species and community structure, and relating this information to details of microclimate, water relations, soils and productivity. Additionally, they have succeeded in relating botanical information to simple descriptions of animal communities.

Vegetation types are dealt with in considerable detail and include forest habitats, rangelands (grassland and wooded grassland), inland aquatic vegetation, coastal vegetation (including intertidal and mangrove swamps), and high altitude vegetation. Considering the difficulties of describing local conditions existing at altitudes ranging from sea level to 19,000 foot, the authors have achieved a good balance between purely descriptive material and broader ecological information.

Part two discusses the influence of water availability and temperature on plant form, structure and growth followed by a short chapter on soils: the description of catenary soil associations and their vegetation types is particularly valuable.

Alan Hamilton contributes a useful chapter on vegetational history, in which he examines evidence derived from macro fossils, pollen analysis and plant geography. Of particular interest is the author's comparison of the apparently impoverished African forests with those of Malaysia and South America. He concludes that the flora of these areas have been depauperated at a time when the climate was very dry.

This valuable addition to the tropical literature will be of great value to research workers, teachers and students at all levels, and with luck will provide a new impetus for field botanical studies in Africa.

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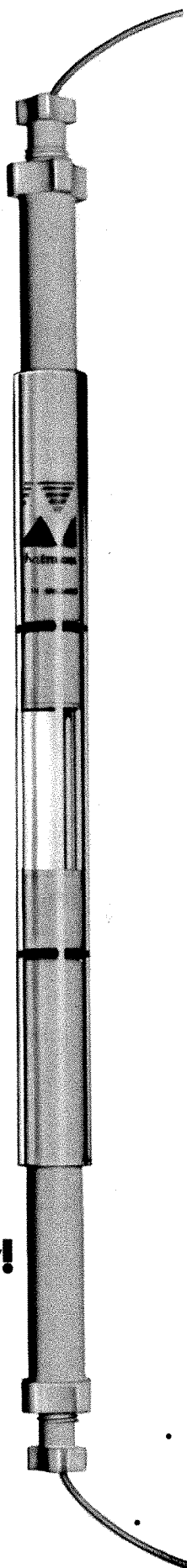
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H. G. J. MOSELEY The Life and Letters of an English Physicist, 1887-1915

J. L. HEILBRON

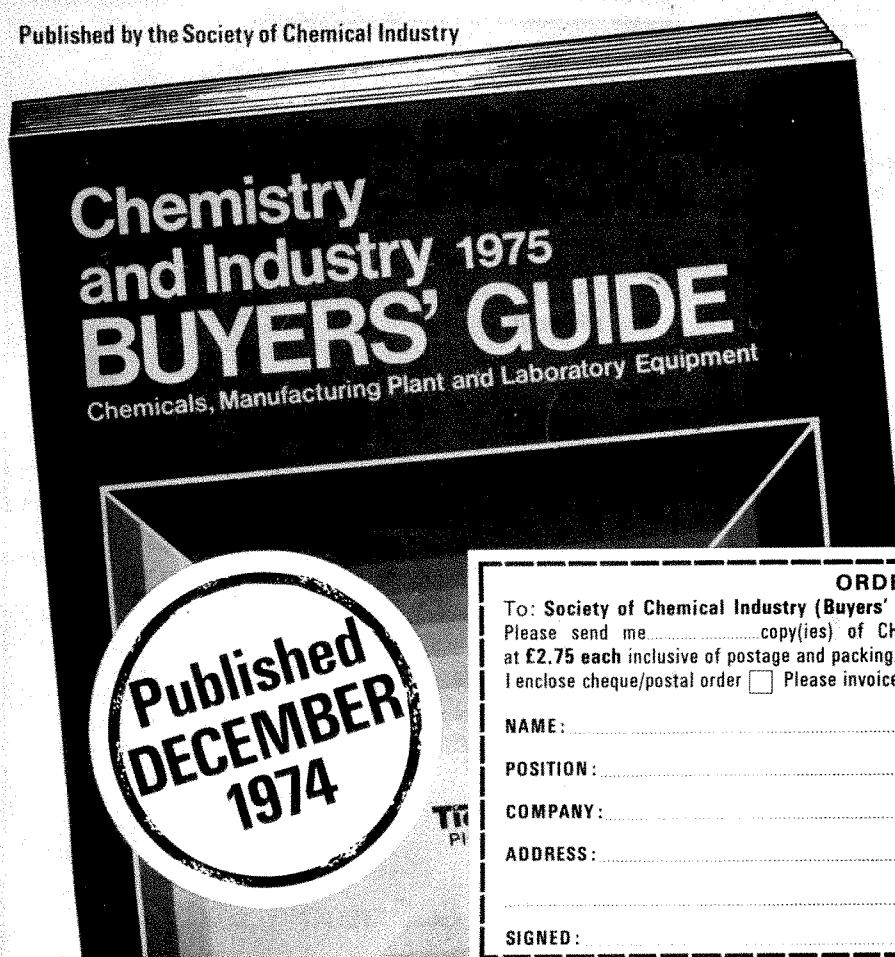
H. G. J. Moseley (1887-1915), the son and grandson of distinguished English scientists, a favorite student of Rutherford's and a colleague of Bohr's, completed researches of capital importance for atomic physics just before the outbreak of World War I. He was urged to devote himself to scientific war work in England, but his duty as he saw it was to join the battle. He procured himself command of a signal section in the Royal Engineers, a speedy trip to Gallipoli, and death in the bloody battle for Sari Bair.

In this work the author presents a full record of Moseley's brief and brilliant career. It gives instructive detail about Eton, which, as Mr. Heilbron shows, offered more opportunity for acquiring a foundation in science than its emphasis on Greek and games would suggest; about Oxford, a scientific backwater in Moseley's time; and about Rutherford's thriving laboratory at the University of Manchester. It describes in detail Moseley's apprenticeship in experimental physics, his growth under the tight supervision of Manchester, and his classical independent work on X rays, which almost certainly would have brought him the Nobel Prize. An epilogue sketches the chief results secured by others in the decade after his death and in the research lines he opened.

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Hormones to kill insects

Insect Hormones and Bioanalogues. By K. Sláma, M. Romanuk and F. Sorm. Pp. ix+447. (Springer-Verlag, Vienna and New York, 1974.) \$45.90.

WITH its various sections written by an insect physiologist and by two organic chemists, this is primarily a chemical study of two groups of insect hormones, the juvenile hormone and the moulting hormone (ecdysone), and of the numerous synthetic materials with similar physiological activities. The natural juvenile hormone in the body of the insect they call consistently the corpus allatum hormone (CAH) and all isolated chemicals, whether natural or synthetic, which show the same activities in greater or less degree, they call 'juvenoids'. Likewise the natural moulting hormone in the body is named the prothoracic gland hormone (PGH) and all natural and synthetic chemicals with the same activities they call 'ecdysoids'. This is a useful convention which avoids much unnecessary argument.

The chemical structure and the various syntheses devised for natural juvenoids and ecdysoids are described in great detail with clear and consistent structural formulae throughout, and at the end of the book a table of some 350 compounds gives structural formulae and the approximate dosage required for equivalent activities of each, in a range of a dozen or so insects which have been extensively used for assay purposes; and a further table gives an alphabetical list of the same 'juvenoids' according to their chemical derivation. Sorm and Romanuk have worked extensively in this field of organic chemistry in relation to both juvenoids and ecdysoids, and the book contains much previously unpublished information from their laboratory. But published work from elsewhere is well covered; although doubtless much more information remains in the hands of commercial firms which have been active in this field.

The table of activities provides a useful basis for reflections and speculations about the chemical factors concerned in the relative activity of juvenoids—but the figures must, of course, be interpreted with caution, since a 15-fold increase in activity of a given compound may result if it is applied to the cuticle at a dilution of 1:1,000 or more in a non-volatile oil, as compared with the usual method of application in a volatile solvent (such as acetone or octane) which leaves an undiluted residue of the active principle on the surface. And a similar increase in activity results if the application is spread in the form of small doses throughout the sensitive period in the

life of the insect, as compared with a single application at the outset.

The authors are principally concerned with the possibility of juvenoids (and to a less extent of ecdysoids) being used as insecticides: to render insects non-viable by disturbance of their normal metamorphosis; to arouse dormant insects from diapause at some inclement season of the year; to sterilise females by adverse effects on their developing ovaries; or to produce non-viable embryos or teratological injuries to young larvae by applications to pregnant females or to newly laid eggs. As a background for such considerations Sláma provides a very full review of the normal hormonal physiology of insects and of the effects of ecdysoids and juvenoids.

The endocrinology of insects is beset with numerous pseudo-problems. What are the chemical effects of ecdysoids? Do they induce DNA synthesis, or RNA synthesis? When renewed growth results in cell multiplication (as it commonly does) DNA synthesis of course occurs; but if sufficient cells already exist it has long been known that moulting (with or without metamorphic change) can take place without DNA synthesis. Renewed growth commonly means renewed protein synthesis; it has long been known that within an hour or so of ecdysoid treatment RNA is increasing in the affected cells—but the same happens in many other cells without the need of ecdysoids. An early effect of ecdysoids at metamorphosis in some caterpillars is conversion of tryptophane into red ommochrome pigments; in fly larvae, the conversion of tyrosine into quinones to sclerotise the puparium. But all such effects are episodes in the growth process which ecdysoids initiate; they are not direct effects of the hormone. The juvenile hormone was originally described as 'inhibiting' metamorphosis; and that is a fair enough description. Unfortunately this was interpreted to mean an antagonistic relation between this hormone and the moulting hormone; an idea which has led to much unprofitable controversy. The earliest suggestion on the nature of the 'inhibitory action' of the juvenile hormone was that it accelerated the deposition of the new cuticle and thus arrested 'differentiation toward the adult form'. This process was clearly demonstrable; but many years elapsed before it was realised that it is due to excessive amounts or precocious timing of moulting hormone supply and not to the juvenile hormone. Finally, it is often very difficult to distinguish between direct effects of a hormone and feedback or homeostatic effects resulting from its action elsewhere in the body. By and large Sláma adopts the rational view, in dealing with these

problems; but he gives a conscientious account of much of the literature, which tends to obscure the rationality of his approach.

V. B. WIGGLESWORTH

Liquid physics

Liquid State Physics: A Statistical Mechanical Introduction. By Clive A. Croxton. Pp. x+421. (Cambridge University: London, May 1974.) £10.

DR CROXTON'S intention in writing this book was to provide a guide to the uninitiated. On the whole, I think he has achieved this aim, although the book is not in any sense an elementary one. The material covered in the first chapter deals with the theory of imperfect gases and forms a sound basis for the latter sections. The method of correlation functions is introduced in the second chapter and is applied to the problems of the liquid state in the way associated with the names of Kirkwood, Rice, Percus and others. Chapters 3 and 5 aim to discuss the numerical side of the subject and to make a comparison between theory on the one hand and experiment or computer simulation on the other. Here the difficulty that faces all authors in writing textbooks on subjects which are developing rather rapidly is that some of the material necessarily is out of date even before the book is published. There are statements which doubtless Dr Croxton would now wish to modify in the light of recent work.

Chapter four is, to my mind, the most worthwhile in the book. The nature of the surface of liquids has been sadly neglected in the past both from an experimental and a theoretical point of view. Dr Croxton brings the special properties of the surface into the context of distribution functions and contact is made between theory and measurement by special reference to the surface tension. This is a field in which the author himself has made a major contribution and this fact is reflected in the clarity with which difficult ideas are explained. The chapter concludes with a short but very useful exposition of the way that the basic theory must be modified to handle surface properties of quantum liquids.

The final chapter is concerned with transport processes, and this is a subject which has been covered quite adequately in the past in a number of review articles. There is perhaps room for a little more comparison between the neutron diffraction work and theory, but this is compensated by the very comprehensive list of up-to-date references which the author attaches to the chapter.

J. E. ENDERBY

Weapons and laws of war

The Problem of Chemical and Biological Warfare, Vol 2: CB Weapons Today; vol. 3: CBW and the Law of War. Stockholm International Peace Research Institute. Vol 2: Pp. 420; vol. 3: pp. 194. (A study of the Historical, Technical, Military, Legal and Political Aspects of CBW, and Possible Disarmament Measures.) (Almqvist and Wiksell: Stockholm; Humanities: New York; Elek: London, 1973.) Vol. 2: Sw.kr.75; \$16.50; £7.50; vol. 3: Sw.kr. 40; \$10; £5.

THESE two latest volumes in the SIPRI series maintain the scholarly distinction and absorbing interest of their predecessors (volumes 1, 4 and 5, published in 1971, were reviewed in *Nature*, 236, 355; 1972.) Volume 2 now takes the historical survey of CB Weapons, defences, policies and programmes beyond 1945 where volume 1 left off. It brings together in a masterly way the available information on contemporary CBW technology and examines as far as is known the policies of each state towards the use of CBW, including tables indicating continent by continent states' formal attitude towards the 1925 Geneva Protocol and the 1972 Convention on Biological and Toxin Weapons, which is not yet in force.

This volume, written by Julian Perry Robinson, with the assistance of Carl-Göran Hedén and Hans von Schreeb, is essentially a work of information, endowed with more than 1,600 references in addition to footnotes. Special attention is paid, as one would expect in a SIPRI publication, to the importance of research and development programmes and the relationships thus engendered between the military and scientific communities (pages 325-332). Specific R and D programmes, such as that concerned with 'binaries', are also described.

Volume 3, written by the Danish peace researcher Anders Boserup, addresses itself to the question: what does international law, and specifically the international law of war, have to say about CBW? One might think this a straightforward question, the answer to which could scarcely deserve a volume to itself: a question in any case less interesting than whether the law is 'strong' enough, and if not what can be done to strengthen it, subjects on which SIPRI had already had much to say in volume 5. One might therefore expect volume 3, being "only concerned with the legal issues in the narrow positivist sense of determining what the law says" (page 42), to be uncontroversial to the point of boredom.

Not so. For there happens to be considerable disagreement over the state of the law. Most of the responsibility for the present uncertainty must

be laid at the door of those governments which have made it their business to discover and exploit supposed ambiguities in the Versailles Treaty formula for defining chemical weapons. This 1919 formula—"asphyxiating, poisonous or other gases and all analogous liquids, materials or devices"—was also embodied in the Washington Treaty of 1922, which did not enter into force, and the Geneva Protocol of 1925, which did. More than 90 states are now parties to the protocol.

What is agreed is that there exists a legal prohibition on the use of chemical weapons and biological methods of warfare. But is the chemical ban as absolute as the biological? Is the content of the prohibition in customary international law more extensive than the treaty prohibition binding only parties to the Geneva Protocol? Is retaliatory use of CBW as firmly banned as first use? And what is the legal effect of the reservations made by many states, including Britain, upon ratifying the protocol, reserving the right to employ CBW against not only the state which violates the protocol but equally against that state's allies?

The SIPRI study examines these and other questions but deals most fully with two matters of bitter controversy: harassing agents (gases such as CN and CS) and chemical herbicides. The controversy is first resolved, systematically, into four questions: (1) Is the use in war of harassing agents prohibited, on condition of reciprocity, among parties to the Geneva Protocol? (2) Is the use in war of herbicides likewise prohibited under the protocol? (3) Is the use in war of harassing agents prohibited nowadays by a peremptory norm (*jus cogens*) of customary international law, binding (by definition) on all states irrespective of their attitude to the protocol? (4) Is the use in war of herbicides likewise prohibited by a peremptory norm?

SIPRI's treatment of these four key questions is thorough, well informed and tightly argued. In the end we find that the answer given to each of the four is yes, although it is admitted (pages 137-8) that (4), the customary prohibition of herbicides, is less conclusively proved than the first three. This is partly because *jus cogens* is a vague concept, with no such precise apparatus of proof as the rules of treaty interpretation provide; partly because evidence of state practice and belief is relatively slender.

So SIPRI settles for the most extensive view of the state of the law, incidentally supporting U Thant and the United Nations General Assembly in the views which they separately expressed in 1969 and from which few states other than the United States

have ever deviated. Some of the restrictive interpretations suggested seem to have been based on misunderstandings or ignorance of international law, whereas of others the kindest thing that can be said is that they "can only arise from a highly specious reading of the Protocol" (page 43). Both comments apply in full measure to the notorious British policy statement purporting to exclude CS gas from the scope of the protocol: a policy as legally untenable (and unnecessary) now as when it was first announced in 1970. SIPRI once again condemns the British statement (pages 59-62) but, as in previous volumes of this study, attributes it to improbable causes, overlooking the clues contained in its very ineptitude of expression.

In conclusion this volume can be warmly commended, but with two qualifications. Its author did not have time to take adequate account of the implications of states' signature and ratification of the 1972 convention. Indeed, the reference (page 149) to this partial disarmament treaty is marred by a faulty argument regarding the significance of the withdrawal clause which assumes, mistakenly, that use of BW and toxins was among the activities banned by the convention. (It should have been; but on that point Britain was obliged, by Sweden and the East European states, to give way in the autumn 1971 round of negotiations, so that the Convention ended up with no ban on use as such). Less excusable on grounds of time is the failure (pages 62-3) to distinguish between interpretations of the Geneva Protocol and statements of intent for future CW agreements. The 1970-71 policy declarations of Canada, Norway and the Netherlands were clearly of the second type and should not have been adduced as evidence supporting the extensive interpretation of the protocol.

N. A. SIMS

Geochemistry handbook

Handbook of Geochemistry Vol. 2/3. K. H. Wedepohl (executive editor) and C. W. Correns, D. M. Shaw, K. K. Turekian and J. Zemann (editorial board). Pp. iv+845. (Springer-Verlag: Berlin and New York, 1974.) DM 258; \$99.40.

THE *Handbook of Geochemistry* has been taken a further substantial step towards completion with the publication of this section. Chapters for sixteen new elements are introduced; of these only that for barium is supplied in entirety, although substantial parts of the chapters on nitrogen, fluorine, gallium, selenium, indium, tellurium and thallium are also provided. Eleven

other sections relate to incomplete chapters which have already been published and these allow the completion of another nine chapters (lithium, sodium, potassium, zinc, rubidium, cadmium, cesium, platinum and bismuth). Now that more than half the text, in both number of elements and pages, is complete (chapters for more than fifty elements are now complete, or virtually so), much of the frustration caused by gaps is removed. The standard of production remains high.

D. G. MURCHISON

Putting viruses together

Morphogenesis of T-Even Bacteriophages. By B. F. Poglazov. Pp. vi+105. Monographs in Developmental Biology, Vol. 7. (Karger: Basel, London and New York, 1973.) 54 Sw.fr.; £7.85; \$16.75.

In a monograph which might better be entitled "T4 structural proteins and their assembly", the author has attempted to integrate the extensive physical and chemical studies on the proteins of bacteriophage T2 from his laboratory with the genetic analysis of phage development which has come out of laboratories in Europe and the United States in the past ten years. The product is a review of the results of roughly 100 papers arranged according to the part of the phage structure whose assembly is being considered.

Of the seven chapters, the two principal ones are devoted to assembly of the head and tail of T4 and T2. The discussion of head morphogenesis is devoted to the inferences on form determination which can be drawn from genetic analyses of mutants in T4 head genes and the interesting experiments from Poglazov's own laboratory on structure formation from solubilised phage T2 head protein. Unfortunately, little mention is made of the work which has been published to elucidate the role of DNA in phage head formation, a subject of considerable recent controversy.

The assembly of the phage tail receives the longest treatment—fully half the book. Again most space is devoted to the work of Poglazov and his collaborators on dissociation and reassociation of tail tube and sheath protein.

A short and extremely dense chapter is devoted to tail fibre assembly. The brevity is unfortunate because the fibre assembly pathway is certainly the best example of a morphogenetic pathway in T4 assembly, and beautifully illustrates the complementary approaches of genetics, immunology, electron microscopy and biochemistry to problems of structure formation. But enough references are given to

enable the interested reader to fill in the details for himself.

In his devotion to detail, Poglazov obscures some of the larger problems of phage morphogenesis. There is hardly any mention of the timing of phage development, regulation of quantities of various components synthesised, or of possible mechanisms of length or form determination.

Generally, the author's policy is to present observations and results without comment. The lack of synthesis will make for difficult reading for the uninitiated, and the lack of evaluation of published results will at times be misleading for those not already familiar with the difficulties of experimentation in the field.

MICHAEL K. SHOWE

Weather satellites

Climatology from Satellites. By E. C. Barrett. Pp. xii+418. (London Methuen: London, May 1974.) £7.90.

THE idea of this book, if I have divined its origins aright, is to liven up the teaching of climatology in schools—and, perhaps, to geographers in universities—by introducing the subject through the medium of satellite pictures, backed up by the analysis of other kinds of satellite data. Or it may be meant just to give a fresh view of the subject in this way. The 41 satellite photographs are the book's main attraction. Among the more impressive ones are several which show the frontal cloudsheets of the northern and southern hemispheres trailing till they meet in the equatorial convergence and one (plate 19), a half-month composite, which shows the cloudless zone along the equator in the Pacific extending from the Americas as far as 170°E. It is a pity that the minimum brightness composite (plate 9) which gives a sample of the rendering of ice and snow and persistent cloud was chosen from the Antarctic summer.

The book is written for geographers; others may find it useful as a handy small guide to the early history of weather satellites, the data and issues which are now available, the symbols in common use on map analyses of satellite information and so on. Among its features are a table (on page 21) of the spatial resolution and frequency of various kinds of satellite observation, the list of routinely available average, minimum and maximum brightness maps and their uses (on pages 50-51), the long table (pages 87-92) on identification of cloud types from satellite photographs, the samples of infrared techniques for deriving surface and cloud-top temperatures, mean cloudiness and relative humidities and the

introduction (on pages 111-7) to the derivation of precipitation from satellite surveys. More could have been said about the inadequacy of precipitation measurements at the surface, with the insoluble problems of representative catchment of the precipitation at sea and of snowfall anywhere, and the potential virtue of uniformity in derivations, however difficult and indirect, from satellite observations. We learn (page 124) that most cloud bands in all latitudes are aligned with the surface wind; we learn also, however, the complications and limited possibilities of accuracy in deriving wind speeds and cloud-top heights.

But whether climatology can be taught in the way this book attempts it may be doubted. The effect is rather like that of walking into a cinema in the middle of the film: the order of presentation is strangely jumbled. We meet the gradient wind on pages 120-3, the sun as the source of energy on page 147 and are rewarded for patience with a nice set of pole-to-pole profile diagrams of the various components of the energy budget on page 159.

I hope not to be unfair in saying that I found this author's style foreign and difficult; for example the ambiguous statement on page 71 that: "the amplitudes and locations of the maxima and minima of incoming radiation are seasonally variable" (within a given season? from season to season? or when we compare the same season in different years?). The waves in the upper westerly winds are introduced (page 196) with the statement that they "undulate meridionally". The stratospheric circulation is explained (page 209), only too briefly, as "a huge standing wave related to the zone of maximum insolation": in this the book clearly attempts too much. Finally, the odd statement is made that an atlas of cloudiness might seem uninteresting except in relation to programmes of space research and Earth Resources Technology Satellites, though we are persuaded that the knowledge is needed for computer modelling and for tracing the general circulation of the atmosphere: surely the last named is the average viewer's first exciting impression of satellite cloud photography.

The author is an enthusiast for the use of satellite observations and has contributed valuable studies of the circulation and cloud development over the tropics, which occupy much of the regional climatology section of the book. Despite the irritating awkwardnesses, the book succeeds in communicating this enthusiasm and may therefore succeed also in stimulating the general reader to try out the use of this fascinating tool himself and seek further understanding. H. H. LAMB

Group theory

Classical Groups for Physicists. By Brian G. Wybourne. Pp. xvi+415. (Wiley: New York and London 1974.) £10.60.

THIS is a fine introduction to the group theoretical method in modern physics. During the past twenty-five years, the significance of symmetry transformations in describing physical phenomena has come to be recognised more and more and with it the use of group theory—and particularly the theory of Lie groups, due to the work of Wigner, Racah and others. This volume gives an exposition of Lie group theory for physicists following the traditional treatment of Weyl and Cartan as modified by the work of Dynkin and the Russian School.

The book divides into two major parts. The first presents a detailed exposition of the principal features of Lie groups and algebras and their topological structures, leading up to the Cartan–Weyl classification. Diagrammatic techniques of Dynkin are used to construct simple Lie algebras, and concepts of weights, Casimir invariants, and Clebsch–Gordon coefficients are discussed in detail. The discussion is mostly carried through for compact groups though there is a brief section on the non-compact $SU(1, 1)$. This portion of the book is written very much from a physicist's point of view—that is to say, principal concepts are introduced, results stated though not always proved with the emphasis being very much on ideas and the methodology rather than on rigour. I thoroughly approve of the approach—I only wish this part of the book was longer.

The second part treats the group theory of three topics in detail—the isotropic harmonic oscillator, the hydrogen atom and the shell structure in nuclear theory. There is an excellent bibliography.

I would recommend the book for postgraduate students in nuclear theory and high energy physics as a self-contained introduction to the advanced ideas and techniques.

ABDUS SALAM

Intelligent beings

The Development of Mind. By A. J. P. Kenny, H. C. Longuet-Higgins, J. R. Lucas and C. D. Waddington. Pp. 152. (Gifford Lectures 1972/1973.) (Edinburgh University: Edinburgh, April 1974.) £2.25.

THE traditional Gifford Lecture, with some notable person putting forward a synthesis of his views on natural theology, is a well established part of the British intellectual scene. For two recent winters, however, something

different has been tried: a group of four people have shared the lectures, and at each session one person gave an opening statement, and there was then discussion between the four. Although each man is widely talented, they could be labelled as specialists in artificial intelligence, philosophy, theology, and biology respectively: and this volume reports the second series of such lectures.

On the whole, the title is taken as referring to the appearance of minds in the course of evolution: not in the other possible sense of the development of a mind from that of the baby to that of the Gifford Lecturer. There are some cursory references to the latter problem, but on the whole the assumption is taken that "there is overwhelming evidence that our most primitive skills . . . are inborn". The discussion concentrates mostly therefore on the view of the world we ought to have if evolution has given rise to creatures possessing our capacities: problems in fact not too dissimilar from those handled by Monod in *Chance and Necessity*, and that book is mentioned several times. By contrast with a book like Monod's, one naturally does not get a unified and single point of view in all its complexity: the main merit of this kind of attack is rather that any overemphasis by one lecturer can instantly be picked up by another. Thus for example the argument that language is difficult to explain by natural selection, because it is only of benefit when more than one person has it, is immediately countered by the argument that we only have to explain a series of small steps in improving communication, rather than the sudden appearance of a complete complex syntax. For those who want the balance and interaction of the various points of view represented, this will in some ways be a more satisfying book than that of Monod. Probably one should not lay down that all future Gifford Lectures should be of this type: but it was worth the attempt on this occasion.

Reaction to lectures on natural theology is bound to be a personal matter: but in two ways this volume fails to speak to my condition. First, the emphasis is on the processes which produce mind and not upon its nature, and this is linked with a silent assumption that all human beings have the kind of minds which can be heard discussing in Oxford and Edinburgh common rooms. Yet the outburst of studies of the behaviour of the developing child, and for that matter of adults with a rather different type of mind from the academic one, make one a bit doubtful of the generality of this kind of discussion. Our ancestors looked at the species which now exist

and could see no way to explain them other than by special creation: and there is something of the same flavour about these discussions which take the mind as an unanalysed given, without considering development within the individual. To psychologists therefore, whose professional blinkers forbid them to take for granted what the mind is and what it does, this kind of discussion is bound to seem curiously old-fashioned and irrelevant.

This kind of reaction leads on to another at a more human level. The pressing problems of natural theology seem to some psychologists to lie, not in the origin of species including ourselves, but in the problems which a human being may face in achieving a stable and satisfactory way of organising his mind, in the existence of alternative ways of doing so, in the need to change from one way to another as circumstances change, and in the difficulties in doing so once some mode of reaction has been established in an earlier situation. In the older, and doubtless outworn, language, these are the problems of the consciousness of sin and the experience of redemption, rather than those of the creation of the world, and perhaps they might be regarded as outside the field of the Gifford Lectures. They can, however, be the subject of rational discussion, quite apart from the particular solutions offered to them, and some of us would like to read a similar volume on that line even more than this one.

DONALD E. BROADBENT

Where sediments build up

Depositional Sedimentary Environments, with Reference to Terrigenous Clastics. By H. E. Reineck and I. B. Singh. Pp. xvi+439. (Springer-Verlag: Berlin and New York, 1973.) DM108; \$44.30.

IN recent years there has been a spate of books published in the field of sedimentary geology. These have included general texts on the origin and petrography of sedimentary rocks and processes of sedimentation, and many dealing with specific sedimentary environments. The authors of this volume are to be congratulated on compiling and analysing all the major modern sedimentary environments in which terrigenous clastic sediments are deposited. The potential scope of the subject is so vast, yet the authors have succeeded in summarising it in a most readable manner. To this end, most of the information can otherwise only be seen in journals, so the book should provide both professional geologists and students with a commendable review of depositional environments.

The first part of the book deals

Second skin



Sloughing palmate gecko from the Nambi desert in south-west Africa. From *The World of Reptiles and Amphibians* by Maurice Burton, (Orbis: London, February 1974.) £2.50.

specifically with primary sedimentary structures and textures, commencing with a brief summary of the major hydrodynamic factors controlling the formation of certain types of structure. This section is similar in its approach to other monographs on the subject. One of its assets is that, as well as being descriptive, the authors have attempted to summarise the varied opinions on the genesis of sedimentary structures. It is perhaps unfortunate that more attention was not paid to the hydrodynamic significance of sedimentary structures, and that the importance of chemical and mineralogical parameters should have been restricted to just over two pages. Also, this section does get involved at times with rather laborious classification schemes.

In the second and most important part of the book the emphasis is placed on modern environments and includes an encyclopaedic treatment of glacial, desert, lake, fluvial, delatic, coastal, shelf and lagoon, tidal flat and ocean basin environments. The authors have, where possible, based their discussions on studies published within the last decade. Consequently, the book provides an up to date, and at times stimulating, appraisal of the subject. Certain topics have been treated to greater depths than others, due in part to a combination of the particular expertise of the authors and a current lack of detailed knowledge on some modern environments. But, in all chapters, the writers have been objective in their efforts to present the processes and characteristic structures and sequences typifying given sedimentological regimes. A pleasing feature is that, from the choice of examples, it is clearly demonstrated that more than one facies model is often required for a particular environment. Furthermore, the importance and application of biological parameters is stressed throughout.

The book is lavishly illustrated with

579 diagrams and photographs, mostly of high quality, but often rather repetitive. It is in fact a little irritating at times to find, due to their large number, that many of the figures are out of phase by several pages with the text. Editorial standards are high, and both printers' and geological errors are at a minimum. The reference list is most comprehensive and should prove to be invaluable for literature searches.

BRIAN WAUGH

Stellar surface

Cosmic Gas Dynamics. By Evry Schatzman and Ludwig Bierman. Pp. xv+291. (Wiley: Chichester, March 1974.) £8.40.

PROBLEMS of gas dynamics are of importance in most branches of theoretical astrophysics, and a comprehensive treatise on cosmic gas dynamics would be vast indeed. A glance at this volume immediately indicates its specialised nature in spite of its general title. Roughly two thirds of the book is taken up by Schatzman's article on stellar hydrodynamics. The remainder is devoted to a study of the physics and dynamics of the solar wind written by Biermann. There is no attempt to link the two articles and they can be read independently of one another.

Schatzman's article is concerned largely with hydrodynamic and hydro-magnetic phenomena occurring in the outer layers of main sequence stars. Stars of spectral types A to G possess extensive outer zones in which the energy transport is largely by convection. The properties of convection zones are not well understood, and their structure is generally described by the phenomenological approach of mixing length theory. The convective motions act as a source of pressure waves which may propagate outwards into the surrounding radiative zone. The study of these waves and their effects are of great

importance in establishing the properties of the stellar corona. A large section of the article deals with these problems. Various aspects of stellar surface activity, such as solar flares, are discussed and the article ends with a brief discussion of the possible role of stellar mass loss in the angular momentum decrease which must occur as stars contract onto the main sequence. Although well written, the article makes few concessions to the reader, and a fairly good background of hydrodynamics and magnetohydrodynamics is necessary to follow the discussion.

The existence of the solar wind is a direct consequence of the types of subsurface stellar phenomena referred to earlier. Biermann first briefly reviews the properties of the solar corona, and then describes in more detail the gross dynamics of the corona and solar wind. A logical progress from simple inviscid hydrodynamics to two-temperature models is followed. The conditions under which the flow may be described by macroscopic equations are discussed and reference is made to solar wind models in which the ion properties are approached by way of the Vlasov equation. The interplanetary magnetic field and the angular momentum of the wind are briefly treated, and the article ends with a short discussion of the interface of the wind and interstellar medium. I was rather disappointed to find no account of the interaction of the wind with planetary bodies and comets.

Both authors are well known for their extensive contributions to the work they describe and write with considerable authority. I have two criticisms. The diagrams have been taken directly from the literature without any attempt to relate their size to the amount of information contained in them. The caption arrangements are very poor. Captions are separate from the figures in some cases, two figures are captioned merely as "universally familiar", and one caption is in French. The second criticism is the very long delay between the writing of the articles and the publication date. The contents are based on lectures given by the authors in 1968 with slight amendments made in 1970. This delay has been particularly unfortunate for the otherwise excellent article by Biermann. Since the time of writing, satellite observations have given a great deal of information directly bearing on the subjects in the article. Considerable progress has been made theoretically and the extensive reviews in the *Asilomar Conference Proceedings* are available in print. The publication delay has not affected the stellar hydrodynamics section as much, but then £8.40 is a great deal to pay for 176 pages of lecture notes. J. E. DYSON

obituary

Vannevar Bush

Dr Vannevar Bush died on June 28 at his home in Belmont, Massachusetts. He was 84. He had been Professor, Vice-President and Dean of Engineering at the Massachusetts Institute of Technology in the 1920s and 1930s, and President of the Carnegie Institution in Washington, DC, from 1939 to 1955. While there, he served as science adviser to President Roosevelt. He was Chairman of the Corporation of MIT from 1957 to 1959 and Honorary Chairman from 1959 to 1966.

Bush received BSc and MSc degrees from Tufts College in 1913. With money for only one year's study, he entered MIT and earned a doctorate in electrical engineering in that single year. In 1916, the DEng was awarded to him jointly by MIT and Harvard.

After the entry of the United States into the First World War he did anti-submarine research, developing a magnetic device for detecting submarines. In 1919 he returned to MIT as associate professor of power transmission.

In studies of power lines, he found that traditional mathematical methods were inadequate for the analysis of increasingly complex systems. In 1925 he set several graduate students to work designing an analogue machine called the Product Integrator to grapple with such problems. It was the first in a series of machines which were precursors to modern computers.

His Differential Analyser was completed in 1931 and was so successful that it served as a prototype for machines built elsewhere in Europe and the United States. This led to the construction of a 100-ton giant known as the Rockefeller Differential Analyser, which had 2,000 tubes, 200 miles of wire and 150 motors.

In 1939 he began meeting with a small group to discuss what might be done to prepare for a massive technological programme which was thought to be essential if the United States entered the war. They concluded that the nation was "... pathetically unprepared from the standpoint of new weapons".

In early 1940, Dr Bush went to President Roosevelt with the group's plan for the establishment of the National Defense Research Committee. The President approved, and appointed Dr Bush as its chairman. Compton took charge of radar research, Conant chemistry and explosives, Jewett com-



Dr Bush with the differential analyser (Courtesy: MIT).

munications and Tolman armour and ordnance. Research activity became so extensive that by 1941 the Office of Scientific Research and Development was established, with Dr Bush as director.

In 1941, after preliminary studies indicated the feasibility of developing an atomic bomb, Dr Bush secured the President's approval to proceed and gave Dr Conant the responsibility for the programme. When the project was ready for large-scale construction, it was turned over to the Corps of Engineers, which established the Manhattan Engineering District to carry the enterprise to completion. A Military Policy Committee, to function as a kind of board of directors, was formed, with Bush as chairman and Conant as his deputy. Following the death of President Roosevelt, it was Dr Bush who gave the first detailed information about the bomb to President Truman. Bush, Conant and Compton were members of the Interim Committee which, after careful deliberation, recommended to the President that the bomb be used.

Of that recommendation, Dr Bush wrote in *Pieces of the Action*: "I knew that Japan would succumb within a matter of months even if the bomb were not used. But I also knew that an invasion of Japan was already being mounted, that it involved several hundred thousands of estimated casualties, and that once rolling, it could not be stopped in its tracks. I also felt that use of the bomb, far less terrible in my mind than the fire raids on Tokyo, if it brought a quick end to the war, would save more Japanese lives than it snuffed out."

"But there was another aspect of this heavy subject. By that time I knew that civilisation faced an utterly new era,

and I felt that it might as well face it squarely. I knew that nerve gases, delivered in a dozen different ways, could be as terrible as an A-bomb. And I had no illusions about the potential power of biological warfare. When science became really applied to warfare, which occurred only during the Second World War, it presented humanity with two alternatives. Either it could refrain, formally or informally, from use of weapons of mass destruction—not only the bomb but also gases and bacteria and viruses—or it could thrust itself back into the dark ages. Over twenty years have passed, and the world has understood and has thus far refrained. If for no other reason I would justify the use of the bomb at Hiroshima and Nagasaki because it was the only way in which the dilemma could be presented with adequate impact on world consciousness."

At the same time he was responding to Roosevelt's request for a comprehensive report on postwar scientific research policy. Published in 1945 under the title *Science the Endless Frontier*, it contained the recommendations of committees (which Dr Bush had organised) to make studies, with respect to health, education, unemployment and other areas in which science would play a part. Dr Bush wrote: "... without scientific progress no amount of achievement in other directions can insure our health, prosperity and security as a nation in the modern world ... Basic scientific research is scientific capital. Moreover, we cannot any longer depend upon Europe as a major source of this scientific capital."

The report was a stimulus for growth in science and technology with an impact on virtually every aspect of American life. The National Science Foundation was established as a major source of Federal support for research. The Office of Naval Research was formed to move far beyond the limits of traditional military research. When the Department of Defense was organised Dr Bush became chairman of its Research and Development Board.

A justifying typewriter was one among a number of Dr Bush's inventions. While still at MIT he invented a high speed library research machine called the rapid selector which was taken over by the Navy for cryptanalysis. He obtained his first patent in 1912 while he was still in college, for a machine mounted on two bicycle wheels which could be used in surveying land. He was granted dozens of patents and built many unpatented devices, ranging from a birdfeeder which discriminated against pigeons and bluejays to hydrofoil boats.

Endless Horizons and *Modern Arms and Free Men* were two of Dr Bush's popular books.

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(934)

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Mr D. Sweetman
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(885)

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An application form and further particulars may be obtained from the Personnel Officer, Sunderland Polytechnic, Chester Road, Sunderland SR1 3SD, Tyne and Wear. (939)

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Applications (2 copies) stating age, qualifications etc., together with the names and addresses of two referees should be submitted by September 24, 1974, to the Secretary and Registrar, University College of North Wales, Bangor LL57 2DG, from whom further particulars may be obtained. (900)

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Applications (3 copies) including names and addresses of three referees should be sent as soon as possible to Dr J. M. Robertson, Electrical Engineering Department, King's Buildings, Edinburgh EH9 3JL, from whom further details can be obtained. Please quote reference number 5044. (908)

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In reply please quote Ref. No. 3536M. (917)

CSIRO**AUSTRALIA****DIVISION OF PROTEIN CHEMISTRY
PARKVILLE, VIC.****POSTDOCTORAL
FELLOW****POSITION NUMBER 462/397**

The Commonwealth Scientific and Industrial Research Organisation has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 6,500 employees—2,100 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

FIELD: PROTEIN CHEMISTRY

GENERAL: The Division of Protein Chemistry is located at Parkville, Victoria and has a research staff of some 60 organic and physical chemists, biochemists and biophysicists. Research in the Division deals mainly with the structure and chemistry of the wool fibre and its constituent proteins and comparative studies on related keratins. It also includes studies on leather manufacture and on muscle proteins, seed proteins, enzymes, virus proteins and allergens. There is also a nucleus of work on the biology of hair growth using cell and tissue culture techniques.

DUTIES: To investigate some of the factors controlling hair growth and especially the possible existence of an antimetabolic factor (chalone). The ultimate aim of the work is to facilitate controlled biological (chemical) defleecing of sheep.

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**POSTDOCTORAL
FELLOW****POSITION NUMBER 462/396****FIELD: BIOCHEMISTRY**

GENERAL: The Division of Protein Chemistry is located in Parkville, close to Melbourne University, and has a research staff of about sixty scientists. A wide range of physical techniques is available in the Division including an analytical ultracentrifuge, u.v., i.r. and fluorescence spectroscopy; e.s.r., n.m.r. and mass spectrometry. Facilities are also available for the large scale preparation of proteins, for amino acid analysis and sequencing and for gas and other forms of chromatography.

BACKGROUND: During the biosynthesis of keratin fibres such as wool and hair the cell membranes undergo a transformation in the latter stages of development which involves profound structural changes. The transformed membranes exert an important influence on the surface properties of the fibre and also form a barrier to the penetration of various reagents.

DUTIES: To study the nature of the lipid component of the membranes and to investigate the form of the binding between the lipid and protein components. This information could be of great importance in binding macromolecules and other reagents at the fibre surface.

QUALIFICATIONS: A Ph.D. degree, or equivalent qualification, in an appropriate discipline together with a demonstrated ability for original research. Experience in the fields of physical organic chemistry or biochemistry would be advantageous.

SALARY: Appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A9,698 to \$A14,706 p.a.

TENURE: These positions are available for a fixed term of three years.

APPLICATIONS: Applications stating full personal and professional details, the names of at least two professional referees, and quoting the appropriate Reference Number should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
64-78, Kingsway,
LONDON WC2B 6BD

by the 27th September, 1974.

Applications in U.S.A. and Canada should be sent to
The Counsellor (Scientific),
Embassy of Australia, 1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C. 20036, U.S.A.

(897)

**HARP LAGER BREWERY
(SOUTHERN) LTD****CHEMIST/BIOCHEMIST**

Harp Lager has a vacancy for a young Chemist or Biochemist, preferably between 22 and 27 years of age, to work in the Production Control Laboratory of their Brewery at Alton. The commencing salary will be in the range of £2,250 to £2,600 a year, in addition there is currently a threshold payment.

The work entails responsibility for both non-routine analyses, for which a knowledge of G.L.C. and other instrumental techniques will be necessary, and the supervision of routine Quality Control analyses. Experience in analytical development and/or relevant industrial experience would be an advantage.

Applicants, male or female, should have good graduate or other recognised equivalent qualifications in Chemistry or Biochemistry.

Applications, which will be treated in strict confidence, should include details of qualifications, experience, age and other relevant particulars and be addressed to:

The Administrative Manager,
Harp Lager Brewery (Southern) Ltd.,
Manor Park,
Alton,
Hants. GU34 2PS. (912)

BIOLOGIST WANTED

A Programme Director is wanted to be in charge of the North American Salmon Research Centre at St Andrews, New Brunswick, Canada, and, in particular, of a study of the genetics and selective breeding of Atlantic Salmon. Under a three-way contract the International Atlantic Salmon Foundation is financing the construction of a hatchery and rearing station in which initial multiple crosses of Atlantic salmon stocks are being made in the autumn of 1974. The administration of the programme is by the Huntsman Marine Laboratory, its operational costs by the Canadian Government Fisheries and Marine Service. The scientific planning to date was by an eminent geneticist. Courses in aquaculture technology are planned in association with the salmon genetics programme; provision is planned for ancillary research by scientists from universities or other agencies.

This unique and developing programme is expected to contribute to the improvement of North American Atlantic salmon stocks. To direct it a biologist is wanted with broad interests and experience, especially in genetics, nutrition, disease or other fields related to its objectives. Education should be at a doctoral level. Salary is negotiable in the range \$18,000 to \$24,000. Availability should be not later than May 31, 1975.

Applications, with curriculum vitae, should be sent to Dr A. W. H. Needler, The Huntsman Marine Laboratory, St Andrews, New Brunswick, Canada, and will be accepted to October 1, or until the position is filled. (919)

**WESTFIELD COLLEGE
(University of London)**

Two year Postdoctoral Appointment (Lectureship Scale) to study the fate of P.C.B. pollutants in shallow marine ecosystems. Experience with G.L.C., suspension feeding in bivalves or meiofauna/sediment relationships pertinent but not essential. Apply at once with the names of two referees to Dr W. A. M. Courtney, Department of Zoology N, Westfield College (London University), Kidderpore Avenue, Hampstead NW3 7ST. (896)

**UNIVERSITY OF GLASGOW
POSTDOCTORAL RESEARCH
APPOINTMENT**

Applications are invited from Organic Chemists for a Postdoctoral appointment to carry out research on the chemistry of substances produced by infection of potato tissue with fungal pathogens. The appointment is for three years from October 1, 1974, at an initial salary of £2,247 rising to £2,412 and £2,580 per annum. F.S.S.U.

Further details may be obtained from Dr N. J. McCorkindale, Department of Chemistry or Dr D. D. Clarke, Department of Botany, the University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 3534M. (914)

UNIVERSITY OF LONDON
INSTITUTE OF NEUROLOGY
 (QUEEN SQUARE)
TECHNICIAN

required to do quantitative polyacrylamide electrophoresis of spinal fluid proteins. Initial appointment for one year. Write giving details of previous experience and names of two referees to The Secretary, Institute of Neurology N, Queen Square, London WC1 3BG. Salary on University Scale £1,557 plus £228 London Weighting. (895)

THE MACAULAY INSTITUTE
FOR SOIL RESEARCH

DEPARTMENT OF SPECTROCHEMISTRY

Applications are invited for a physical or inorganic chemist to undertake investigations into the trace element status of soils, plants and other biological materials by spark-source mass spectrometry. The work will involve the development of analytical and diagnostic techniques.

Candidates should possess a First or Upper Second Class Honours Degree in Chemistry, or a Higher Degree, and have an aptitude for experimental research.

The appointment will be in the Scientific Officer (£1,931 to £2,675 per annum) or Higher Scientific Officer (£2,461 to £3,371 per annum) grade, according to qualifications and experience, at least two years relevant post-qualifying experience being required for appointment as H.S.O. Superannuation under F.S.S.U., with a non-pensionable allowance to offset personal contributions.

Forms of application and further particulars may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, to whom they should be returned before September 13, 1974. (Quote Ref. 74/19). (890)

NATIONAL ORGAN MATCHING
AND DISTRIBUTION SERVICE

A vacancy exists for a computer scientist in the National Organ Matching Service at the Regional Transfusion Centre, Southmead, Bristol BS10 5ND.

The Service makes use of computers in several facets of its work and the appointee will be expected to play a major role in the rationalisation and development of this work. Candidates should therefore have a strong interest in development of information storage and retrieval systems for specialised medical data as well as for scientific analysis. The appointment will be initially for a two year period and will then be subject to review.

Salary scale, Junior Scientific Officer, commencing at £1,497 rising to a maximum of £2,694.

Application forms and further details of the post may be obtained from the Administrative Officer at the above address. (915)

ENTOMOLOGIST

Applications are invited for the post of Editorial Assistant on Entomology Abstracts. Candidates should have a degree in zoology, with specialisation in entomology, or qualification in entomology. Working knowledge of French or other European language an advantage. Starting salary £1,721 p.a. Applications to: Dr E. S. Krudy, Information Retrieval Limited, 1 Falconberg Court, London W1V 5FG.

Applications are invited for the post of Assistant Editor and Editorial Assistant. On Entomology Abstracts, candidates should have a degree in Zoology, with specialisation in Entomology, or qualification in Entomology.

Work knowledge of French or other European language an advantage.

Starting salary £1,912 p.a. and £1,721 p.a. respectively. Applications to Dr E. S. Krudy, Information Retrieval Ltd, 1 Falconberg Court, London W1V 5SG. (916)

UNIVERSITY OF BATH

Applications are invited for the post of

PROFESSOR OF PHYSICS

which falls vacant due to retirement on September 1, 1975.

Further particulars may be obtained from Personnel Officer, The University, Bath BA2 7AY, quoting reference 74/156. Closing date: October 4, 1974. (918)

UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF BIOCHEMISTRY

Research Associate/Senior Research Associate or Research Fellow is required for an investigation of the mechanism of stimulation of lipid-metabolising enzymes in synaptic membranes of the central nervous system. The post will be supported by a grant from the Medical Research Council for three years. Enquiries and applications to Dr C. E. Rowe, Biochemistry Department, University of Birmingham, P.O. Box 363, Birmingham B15 2TT. (931)

Imperial College

Division of Life Sciences

Electron Microscopy Unit

Vacancy for suitably qualified person to take charge of a new Electron Microscopy unit which will provide a service to staff and students of the division of life sciences.

Duties include sole responsibility for the running and maintenance of the Electron Microscopes in the unit including transmission and scanning, development of additional equipment and techniques and advice and assistance to users of the microscopes.

Salary in the range £3,045—£3,429 plus threshold Superannuation, four weeks leave plus additional days at Christmas and Easter: excellent staff amenities.

Further details from, and applications to: Mr R. Adams, c/o Botany Department, Imperial College, London SW7 2AY by September 30, 1974. (904)

UW Wageningen

LANDBOUWHOGESCHOOL WAGENINGEN
THE NETHERLANDS

Applications are invited for the vacant chair in
PHYSICAL BIOLOGY

Preference will be given to a biologist, more specifically to a botanist with outstanding research experience in either photobiology or physiology of membrane functions, or both.

The appointee will be responsible for the programs in teaching and research of the Department of Physical Biology (formerly known as the Laboratory of Plant Physiological Research).

He will supervise the existing research programs of the department and stimulate new applications of physical methodology to the study of the biology of plants, animals and microorganisms.

He will be expected to contribute proportionally to the teaching obligations of the Landbouwhogeschool, together with the staff of the Department.

Applicants and those who want to make suggestions as to qualified persons, are invited to address to the Cabinet of the Faculty of the Agricultural Sciences, Salverdaplein 11, Wageningen, the Netherlands, within 4 weeks after the insert of this advertisement, supplying detailed information and mentioning the number 74-87 on the envelope. (909)

ST BARTHOLOMEW'S HOSPITAL
WEST SMITHFIELD, LONDON EC1A 7BE
RESEARCH ASSISTANT

Graduate or qualified technician required to join a small research team on the Surgical Professorial Unit investigating the immunosuppressive effect of cancer. Present studies are directed towards the effect of gastrointestinal cancer on human lymphocyte function. Biochemical or immunological background preferred. Opportunity may arise for successful candidate to study for further degree.

Commencing salary (according to qualifications) between approx. £1,750 p.a. and £2,500 p.a.

Applications plus curriculum vitae and two names or reference to Personnel Department (quoting R/4687/N by September 16, 1974. (928)

UNIVERSITY OF LEEDS
RESEARCH TECHNICIAN (Grade 3)

A Research Technician is required to assist experimental studies of the effect of Radiation and Chemotherapeutic agents on mammalian cells. Salary scale: £1,650 to £1,920 plus threshold payments. Applicants should have experience of tissue culture techniques and an interest in work with experimental animals. Applications stating age, qualifications, experience and present salary together with the names of two referees, should be sent to: Professor C. A. Joslin, the University Department of Radiotherapy, Cookridge Hospital, Leeds LS16 6QB, from whom further details may be obtained. (949)

CANADA

Several vacancies exist with the Department of Mineral Resources in Saskatchewan for Geologists II and I Geophysicist. Qualifications required range from a good Honours Degree in Geology to a PhD.

Salary Range from Oct 1st, 1974:—

GEOLOGIST II with Ph.D.
\$15,000 to \$19,128
GEOLOGIST II and GEOPHYSICIST II
\$14,292 to \$18,216
GEOLOGIST I
\$12,492 to \$15,924

Closing date for applications October 1 1974.
Application Forms may be obtained from The Public Service Commission, 1820 Albert Street, Regina, Saskatchewan S4P 2S8 and, details of positions, etc., from D. R. Francis, 2708 12th Avenue, Regina, Saskatchewan, S4S 0B1, Canada.

(893)

THE ROYAL FREE HOSPITAL HAMPSTEAD

E.E.G. TECHNICIAN

(Part-Time)

Half-time E.E.G. Technician or Neuriphiological Technician with E.E.G. experience to work in the Department of Psychological Medicine. Experience of telemetry or computer based techniques would be an advantage, but is not essential.

The appointment will be at the New Royal Free Hospital, Hampstead, following a short initial period at the existing Liverpool Road Branch.
Further information from the Department of Psychological Medicine. Tel: 226 3043 Ext: 147. Application forms (to be returned by September 11) from the Personnel Department, 21 Pond Street, Hampstead NW3 2PN. Tel: 794 0431 Ext: 2. (923)

UNIVERSITY OF RHODESIA CHAIR AND HEADSHIP OF THE DEPARTMENT OF GEOGRAPHY

This post will be vacant from March 1, 1975 and applications for it are invited.

Salary Scale (Approx. Stg. equivs.): £7,557 by £281 to £8,962.

Family passages and allowance for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia. Sabbatical and biennial visits with travel allowance. Superannuation and medical aid scheme.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth etc), qualifications, experience and publications and giving names and addresses of three referees, should be submitted by **October 7, 1974** to the Registrar, University of Rhodesia, P.O. Box 2702, Salisbury, Rhodesia, from whom further particulars may be obtained. Overseas applicants please send a copy of their applications to Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. (952)

OXFORDSHIRE AREA HEALTH AUTHORITY (TEACHING) ASSISTANT IN IMMUNOPATHOLOGY

Applications are invited from Science Graduates for the post of assistant in Immunopathology. Although it is not essential that applicants should have had previous experience in this field, they should be familiar with general biological laboratory technique. This post offers good opportunities for training in a full range of immunological techniques and participation in research investigations.

It is hoped that the successful applicant will take up the post in September.

The salary scale will be that for Biochemists, Physicists and Scientific Officers in the National Health Service (Probationary Period £1,497 to £2,259. Post probationary period £1,953 to £2,674). The starting point will depend on qualifications and experience.

Applications with details of training and experience should be submitted to Dr M. M. Pickles, Immunopathology Unit, Gibson Laboratories, Radcliffe Infirmary, Oxford from whom further particulars can be obtained.

Closing date September 6. (947)

Imperial College

Department of Biochemistry

An experienced technician is required to work in the field of Nucleic Acid research. The work will involve Purification of Proteins and Nucleic Acids. Radioactive Techniques, high voltage Electrophoresis. Gel Electrophoresis and some Micro-analytical techniques. The successful candidate should have experience in some of these fields.

The appointment is supported by an M.R.C. grant and is tenable for 3 years, commencing on October 1, 1974.

Salary in the range £2,007 to £2,157 plus £228 per annum London Weighting and threshold payments. Applications to Departmental Superintendent, Department of Biochemistry, Imperial College, London SW7 2AY. (905)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE SCIENTIFIC OFFICER

Applications are invited for a post as SCIENTIFIC OFFICER in the Chemistry Division to assist with research in a small group concerned with the chemistry of sulphur compounds in relation to food. Candidates should possess a degree, H.N.C., or equivalent professional qualification in chemistry with an interest, and preferably experience in, the synthesis of organic compounds.

Salary: In the scale £1,592 to £2,675, with starting salary depending upon qualifications and experience. The post is pensionable under a non-contributory superannuation scheme.

Application forms and further particulars from the Secretary, Food Research Institute, Colney Lane, Norwich NOR 70F, quoting Vacancy No: 74/12. (930)

Imperial College DEPARTMENTS OF BIOCHEMISTRY CHEMICAL ENGINEERING

Applications are invited from graduate Microbiologists or Chemical Engineers for the post of research officer for studies concerning Aeration + Agitation Phenomena in Conventional Stirred Fermenters in the presence of Viscous Non-Newtonian Culture Fluids. The Department possesses a comprehensive semi-industrial scale fermentation pilot plant with unique facilities for this type of work. The project is part of a collaborative research programme between the departments of Biochemistry and Chemical Engineering for a 3 year period.

Salary in the range £1,485 to £3,498 plus threshold payments, with F.S.S.U. Applications to be sent to Departmental Superintendent, Biochemistry Department, Imperial College, London SW7 2AY. (903)

Imperial College

Department of Biochemistry

M.R.C. sponsored postdoctoral research assistant required to work on the structure and recognition of messenger RNAs. Experience in TB field of Nucleic Acid research and/or Prote Synthesis is desirable but not essential. The appointment is tenable for 3 years, commencing on October 1, 1974.

Salary range £2,331 to £2,625, with F.S.S.U. applications including curriculum vitae and the name of two referees should be sent to Dr M. Szekely, Department of Biochemistry, Imperial College, London SW7 2AY.

(906)

AGRICULTURAL RESEARCH COUNCIL SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER

required to join multidisciplinary research team studying new areas in the physiology and biochemistry of growth and development of meat producing animals. An interest in the metabolism of hormones and drugs would be an advantage.

Minimum qualifications: Pass degree, H.N.C. or equivalent (preferably physiology, chemistry, or related subjects).

SALARY: Scientific Officer scale £1,592 to £2,675; Higher Scientific Officer scale £2,461 to £3,371 depending on qualifications and experience, a minimum of 5 years post qualifying experience being required for appointment in the H.S.O. grade. Non-contributory pension scheme.

Application forms (quote ref. BP.39): Secretary, Meat Research Institute, Langford, Bristol BS18 7DY. (963A)

UNIVERSITY OF MALAYA RESEARCH ASSISTANTSHIP FOR I.D.R.C. PROJECT (FOOD FERMENTATION)

Applications are invited from University graduates who are Malaysian citizens for posts in research in food and fermentation microbiology and animal nutrition.

Two scholarships, available for approximately 2½ years, sponsored by the International Development Research Centre for research in methods of solid substrate food fermentation and biological evaluation of the products as enriched animal feedstuffs.

One post requires a candidate with an Honours degree (minimum Second Upper) or equivalent in nutritional biochemistry or animal nutrition with a basic background of biochemistry, bacteriology, and/or food science; the other post requires a candidate with an Honours degree (minimum Second Upper) or equivalent in the field of microbiology with a basic knowledge in biochemistry, bacteriology and/or food science.

This project is at present in progress. The preliminary findings are encouraging and should give the successful candidate a challenging opportunity for original research. This scholarship carries a stipend of M\$700 per mensem for the first 12 months followed by M\$800 per mensem beyond that period.

The successful candidates will be required to enrol for study leading to higher degrees (M.Sc. or Ph.D.) at the University of Malaya.

Applications should be made on prescribed forms (9 copies) and sent to the Registrar (Recruitment Section), University of Malaya, Kuala Lumpur 22-11, Malaysia, together with a copy of transcript of the academic record of the candidate. Application forms and further information about the positions, including conditions of appointment, may be obtained from the above address. Closing date for applications is **October 26, 1974**. The University does not pay travelling or any other expense to candidates to attend interviews. Eligible candidates who may be overseas at the time of advertisement may be exempted for interview subject to the location and qualification of the applicants. Ref: UM.1118 Pt. XI (237). (953)



IIRS
IRELAND

DIRECTOR OF SCIENCE DIVISION

The Institute for Industrial Research and Standards is an agent of the Irish Government charged with the responsibility for assisting in the industrial development of the country by providing technological support to industry.

The post of Director of the Science Division is a challenging post in the senior management of the Institute. The Division has 140 staff in Departments of Chemistry, Physics, Food Technology, Forest Products and Chemical Engineering which provide a wide range of services to various sectors of industry. Among the activities of the Division are services in air and water pollution abatement, minerals and inorganic materials technology, chemical analysis and computing. The types of services range through testing, field advisory work, technical consultancy and applied research and development. The Director will ensure that the services are responsive to the needs of industry. He will maximise the effectiveness of all services and the income earned for them from industry. He will be responsible in particular for the management of both contract and Institute-sponsored applied research and development programmes/projects and his effectiveness in this context will be measured against the criterion of commercial success.

The successful candidate will be a scientist of high academic attainment with proven management ability and at least ten years' experience in industry, much of which should have been in applied research and development. The salary is in a range rising to a maximum of £6,600.

Further particulars concerning the post and application forms are available from:

**THE PERSONNEL MANAGER,
INSTITUTE FOR INDUSTRIAL RESEARCH AND
STANDARDS,
BALLYMUN ROAD, DUBLIN 9, IRELAND,
who should receive completed forms not later
than September 30, 1974.**

(910)

ROTHAMSTED EXPERIMENTAL STATION HARPENDEN, HERTS. AL5 2JQ HIGHER SCIENTIFIC OFFICER/ SENIOR SCIENTIFIC OFFICER

required for a two-year appointment as a BIOLOGIST to work in the Nematology Department on nematodes of arable crops. Good honours degree in Botany, Zoology or other branch of Biology required. At least two years' postgraduate experience for appointment as H.S.O., and four years as S.S.O. Experience in Plant Nematology is essential for appointment as S.S.O.

Starting salary according to qualifications and experience:

H.S.O.: £2,461 to £3,371.
S.S.O.: £3,157 to £4,441.

plus threshold payments. Superannuation with a contribution of 1½% for family benefits (male staff).

Applications giving full personal details, naming two referees and quoting Reference No. 2382 to The Secretary ●, September 21, 1974. (950)

SENIOR TECHNICIAN DRUG METABOLISM

An experienced Technician is required to work in the Drug Metabolism Section of Boots Research Department, whose modern well-equipped laboratories are situated in Nottingham.

The successful candidate will have H.N.C. or H.N.D. or an equivalent qualification, and will be concerned primarily with in vitro techniques used in the study of drug metabolism.

Experience in tissue fractionation, enzymology and the use of radioactive materials would be an advantage.

The conditions of employment are good, including a profit earning bonus and contributory pension schemes.

Please write, giving brief details of age, qualifications and experience to: Mrs E. M. Durrance, Employment Services, The Boots Company Limited, Station Street, Nottingham NG2 3AA. (948)

CSIRO

AUSTRALIAN NUMERICAL METEOROLOGY RESEARCH CENTRE

MELBOURNE, VICTORIA

OFFICER-IN-CHARGE

Applications are invited for the position of Officer-in-Charge.

GENERAL: The Centre is a combined activity of the Department of Science and the Commonwealth Scientific and Industrial Research Organisation and is located within the Bureau of Meteorology premises in Melbourne. The main objective of the Centre is to conduct research into numerical methods for improving the accuracy and timescale of weather forecasting and for understanding the global climate and its variations. It includes the interpretation and use of new forms of observational data. The Centre will effectively continue the work of the Commonwealth Meteorology Research Centre which has been in existence for five years. During this period the CMRC has worked on schemes of numerical analysis for the Australian region and the southern hemisphere and on prognosis models of two types, grid-point and spectral, which are respectively in, and close to, operational use. General circulation models are currently being used to investigate relevant climatic mechanisms.

DUTIES: The Officer-in-Charge will be appointed by CSIRO after consultation with the Secretary, Department of Science, and will be responsible for the direction of the scientific programme of the Centre and its administrative control. The Officer-in-Charge will be supported by about ten professional meteorologists from the Bureau of Meteorology, about ten research scientists from CSIRO, together with appropriate computer, programmer, administrative, and clerical support.

SALARY: Appointment will be made within the salary ranges of Senior Principal Research Scientist or Chief Research Scientist Grade 1: \$A19,319 to \$A21,295.

TENURE: This position is available for an indefinite period and Australian Government Superannuation benefits are available.

Applications should state full personal and professional particulars and the names of at least three professional referees, and should reach:—

The Chairman,
Environmental Physics Research Laboratories,
P.O. Box 77, MORDIALLOC,
VICTORIA, AUSTRALIA, 3196

by September 28, 1974

They should include a full statement of the applicant's experience and achievements in numerical meteorology or closely cognate fields, and his views on present prospects and new orientations within the broad objective of the Centre. Intending applicants may obtain a copy of the formal agreement between the Department of Science and CSIRO, under which the Centre will operate, and other relevant material by writing to the above address. (957)

THE UNIVERSITY OF THE WEST INDIES—TRINIDAD EXECUTIVE DIRECTOR

PROPOSED CARIBBEAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE

Applications are invited for the post of Executive Director of the Caribbean Agricultural Research and Development Institute (C.A.R.D.I.) which is to be established by the member Governments of the Caribbean Community as the successor organisation to the Regional Research Centre of the Faculty of Agriculture of the University of the West Indies. The Institute has been established to serve the research and development needs of the region and will have its headquarters at the St Augustine, Trinidad, campus of the University of the West Indies.

Applicants should possess a good degree in agriculture or one of its related fields. A higher degree, though not essential, would be a distinct advantage.

The person appointed will have had considerable experience in tropical agriculture or agricultural research and development and/or be an administrator of exceptionally high calibre. Experience with the region would be an advantage.

The Executive Director will be responsible for the day to day control, management and administration of the Institute.

The appointment will be on contract for five years in the first instance. Salary will be negotiable based on qualifications and experience. Other allowances are payable. A gratuity in lieu of pension will be paid. Unfurnished accommodation will be provided at a cost of 10% of salary. Alternatively a housing allowance of 20% salary will be paid in lieu of accommodation. Four weeks annual leave will be granted. Up to five full passages will be provided on appointment and on normal termination.

The person appointed would be expected to assume duties as soon as possible and preferably by October, 1974.

Applications giving full details of date of birth, marital status, qualifications and experience and the names and addresses of three referees should be sent by airmail by September 13, 1974 to the Secretary, University of the West Indies, St Augustine, Trinidad, from whom further particulars can be obtained. (961)

ROYAL POSTGRADUATE MEDICAL SCHOOL

TECHNICIAN (Organic Chemist)

required in the peptide chemistry section of the Endocrine Unit, to assist with the synthesis of biologically active peptides. Qualifications: Relevant 'A' Levels, O.N.C. or H.N.C. in chemistry. Starting salary and grade according to qualifications and experience.

Applications to the Secretary, R.P.M.S., Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting ref. no. 2/322N. (926)

THE UNIVERSITY OF ADELAIDE

invites applications for appointment as

LECTURER IN ANATOMY AND HISTOLOGY

A medical qualification registrable in South Australia is desirable but not essential. The appointment will be made as soon as the person concerned is able to assume duty.

The Department of Anatomy and Histology teaches University students of Medicine, Dentistry and Science, and in addition Physiotherapy and Occupational Therapy students of the South Australian Institute of Technology. Research fields are neuroendocrinology, human and comparative neurology, cell biology, experimental embryology, and clinical anatomy. Persons with an interest in clinical anatomy or gross anatomy are especially invited to apply.

Salary Scale: \$A9,002 by \$A479(4) by \$A478(3) to \$A12,352 (plus a loading for registrable medical qualifications at the rate of \$A833 a year) with superannuation provision.

Further Information, including list of particulars required in an application, is available from the Registrar of the University or from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should reach the Registrar, G.P.O. Box 498, Adelaide, South Australia 5001, not later than September 16, 1974. (929)

UNIVERSITY OF THE WITWATERSRAND

JOHANNESBURG, SOUTH AFRICA

SENIOR LECTURER/LECTURER IN PLANT TAXONOMY

Applications are invited for appointment to the above post in the Department of Botany and Microbiology. Duties are to be assumed on January 1, 1975 or as soon as possible thereafter.

Reference will be given to those interested in modern systematics (chemosystematics, numerical systematics or experimental biosystematics), but with a training in classical taxonomy.

Salary will be in the following ranges:

Senior Lecturer R7,245 to R9,315.
Lecturer R5,520 to R7,935

The initial salary to be determined according to qualifications and experience. Benefits include an annual bonus, pension and medical aid facilities, and a housing subsidy, if eligible.

Intending applicants should obtain the information sheet relating to this post from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications should be lodged not later than September 27, 1974. U.K. applicants may obtain the information sheet from the London Representative, University of the Witwatersrand, 278 High Holborn, London, W.C.1 to whom a copy of the application should be sent. (945)

ROYAL HOLLOWAY COLLEGE (University of London)

EGHAM HILL, EGHAM, SURREY LECTURER IN ZOOLOGY

Lecturer required in the field of Invertebrate Zoology from January 1, 1975. Entomology is the preferred specialty but other fields of terrestrial Invertebrate Zoology are not excluded. Interest in quantitative biology an advantage. Salary up to £2412 plus F.S.S.U. Further details from the Personnel Officer (N). Applications (6 copies please) including a curriculum vitae and the names and addresses of 2 referees should reach her by September 16, 1974. (944)

**LA TROBE UNIVERSITY
MELBOURNE, AUSTRALIA
LECTURESHIP/SENIOR
LECTURESHIP IN
MICROBIOLOGY
(ONE OR TWO POSITIONS)**

Candidates should have experience in any field of microbiology, but there is a need for an appointment in virology. Preference will be given to applicants who have research and teaching experience.

The Department of Microbiology is a new department in the School of Biological Sciences and will offer a widely-based course in general microbiology leading to B.Sc. ordinary and honours degrees. Teaching will commence in March 1975 with an introductory course in microbiology given to second-year science students. Informal enquiries about the proposed development of the department can be sent to the Chairman of the Department, Professor J. S. Waid, whose address until the closing date is Department of Botany, University of Canterbury, Christchurch, New Zealand.

Salary: Senior Lecturer \$A12,643(1) by \$A417(4) by \$A416 to \$A14,724; Lecturer \$A9,002(4) by \$A479(3) by \$478 to \$A12,352.

Further information and application forms are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar, La Trobe University, Bundoora, Victoria, Australia 3083.

Applications close on **October 14, 1974.** (943)

RESEARCH TECHNICIAN (Grade 4)

required by Department of Anatomy. To assist in Research Studies on Brain Structure and Central Nervous System. Experience in Electron Microscopy or Neurohistological techniques essential. H.N.C. or equivalent qualification. Salary within scale £1,848 to £2,163 plus £228 London Weighting plus Threshold. Application form from Personnel Officer, (Technical Staff FB24), University College London, Gower Street, London WC1E 6BT. (942)

**University of New South Wales
WOLLONGONG UNIVERSITY
COLLEGE**

TO BECOME THE

**UNIVERSITY OF WOLLONGONG
JANUARY 1, 1975**

LECTURER IN BIOLOGY

Flexibility in the initial academic structure of the Department of Biology will enable applications to be considered from persons competent in the area of Energy Metabolism. The appointee would share responsibility for an instructional unit in Energy Metabolism and in the development of the Biology course as a whole.

Further information may be obtained from The Professor Elect, Assoc. Professor A. D. Brown, School of Microbiology, University of N.S.W., P.O. Box 1, Kensington, N.S.W. 2033, Australia.

Commencing salary according to qualifications and experience, within the range \$A9,002 to \$A12,352.

Applications close **October 4, 1974.**

Conditions of appointment and application procedure from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. (962)

**UNIVERSITY OF BIRMINGHAM
DEPARTMENT OF PHYSICS
RESEARCH ASSOCIATE**

Applications are invited for a Research Associate-ship in the Applied Nuclear Science Group to undertake a theoretical investigation of the behaviour of neutrons in the breeder blankets of controlled thermo nuclear reactors. A higher degree and/or experience in neutron physics and computing are desirable.

This three-year U.K.A.E.A. supported programme carries a salary on the Research Associate scale:

£1,758 to £2,412 (exceptionally £2,931) plus F.S.S.U. and threshold payments. Initial placing according to age and experience.

Applications (3 copies), naming 3 referees, should be submitted to the Assistant Registrar(S) P.O. Box 363 Birmingham B15 2TT by September 27, 1974. Please quote ref. NP4. (963)

INFORMATION SCIENTIST

Central Research is the organisation responsible for all research in Pfizer. At Sandwich, Kent, over 450 staff are engaged in research and development of compounds for use in human medicinal and veterinary fields.

We now require to increase the staff in our Research Information Services department by appointing a young graduate. Ideally the man or woman we need will have two or three years' experience in information work and the possession of an M.Sc. (Information Science), postgraduate Diploma in Information Science, or an equivalent qualification would be an added advantage.

The person appointed will be involved in the development and maintenance of information resources, including the exploitation of computer-based information systems. He/she will work in very close collaboration with research project teams. Our conditions of employment include flexible working hours, assistance with relocation expenses where appropriate and free pension and death benefit schemes.

Pfizer

Applications, giving brief details of age and experience, should be addressed to:

**D. W. Sells,
Personnel Manager,
Central Research,
Pfizer Limited,
Sandwich, Kent.**

(959)



Wellcome

Entomologist

The Wellcome Foundation Limited, requires an Entomologist at their Research Laboratories at Berkhamsted Hill, preferably having postgraduate experience in economic entomology and in the biological testing of insecticides. Requirements include an ability to work in a team and to develop initiative in applying knowledge to laboratory and field practice in the U.K. and overseas.

We offer excellent conditions of employment, including help with re-location expenses where necessary, and real career prospects. Our laboratories are modern and well equipped, situated in pleasant countryside close to Berkhamsted, which is a small country town about 35 minutes from Euston, London.

If you are interested please write or telephone for an application form quoting reference PA 62 to:

**R. P. Woolridge, Senior Personnel Officer,
The Wellcome Foundation Limited,
Ravens Lane, Berkhamsted, Herts HP4 2DY.
Tel: Berkhamsted 3333.**

(955)

**THE NATIONAL RADIOLOGICAL
PROTECTION BOARD**
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BIOLOGY DEPARTMENT
OF THE
RESEARCH AND DEVELOPMENT DIVISION

The successful applicant will assist senior scientists in the solution of a wide variety of problems in theoretical dosimetry and epidemiology.

The work currently being undertaken includes the interpretation of biological experiments, the calculation of doses from internally incorporated radionuclides, the assessment of doses received from occupational exposure, and the interpretation of epidemiological data. Good computer facilities are available.

Candidates should possess a first or upper second class degree in mathematics, physics or a biological science coupled with experience in computation.

Salary on the scale £1,705 to £2,865 per annum.

Write or telephone for further information and an application form to:

The Personnel Officer (Ref. A.57),
National Radiological Protection Board,
Harwell,
Didcot,
Oxfordshire OX11 0RQ.

Telephone: Rowstock (023-583) 600 Extn. 216.

Closing date for applications October 4, 1974.

(960)

UNIVERSITY OF DUNDEE
**DEPARTMENT OF PHARMACOLOGY
AND THERAPEUTICS**
RESEARCH STUDENTSHIP

Applications are invited for one M.R.C. studentship for training in research methods. The studentship, which is tenable for up to three years, can be taken up on October 1 1974 or as soon as possible thereafter. The training programme involves studies of the effect of drugs on cerebral histamine metabolism. The Department, which is located in the new Ninewells Hospital and Medical School, is fully equipped for these studies. The successful applicant may register for a higher degree and will receive a stipend in accordance with the rates for M.R.C. students.

Applicants, who should have a first or upper second class honours degree or equivalent in pharmacy, pharmacology, biochemistry or related subject, should write to Professor J. Crooks, Department of Pharmacology and Therapeutics, Ninewells Hospital, Dundee, DD2 1UD, giving their qualifications and the names of two referees. The closing date for applications is September 14, 1974.

(935)

LIVERPOOL POLYTECHNIC
SCHOOL OF PHARMACY
**S.R.C. STUDENTSHIP IN
MECHANICAL CHEMISTRY**

Applications are invited from candidates with a good honours degree in Pharmacy or Chemistry, or Grad. R.I.C., to work for a Ph.D. degree on the project "4-phenylpiperidine derivatives as potential non-addicting analgesics". The work will involve organic synthesis and stereochemical studies. The project is a C.A.S.E. award and the co-operating body is Allen and Hanburys Ltd., usual S.R.C. stipend applies which may be supplemented by payment for teaching duties and income during the industrial period.

Enquiries to: A. F. Casey, D.Sc., F.P.S. School of Pharmacy, Byrom Street L3 3AF. (889)

UNIVERSITY OF NOTTINGHAM
**DEPARTMENT OF AGRICULTURE
AND HORTICULTURE**

The National Environment Research Council is prepared this year to offer to a suitable candidate a Studentship tenable at the University of Nottingham School of Agriculture. The value of the N.E.R.C. awards in 1974 will normally be £880 per annum.

Candidates should have first or upper second class honours degrees. The field of research will be Seed Production of Amenity Grasses.

Applications, marked 'Agriculture' and giving the names and addresses of two referees, should be sent to the Secretary, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough LE12 5RD within two weeks of the appearance of this advertisement. (940)

**ST. MARY'S HOSPITAL
MEDICAL SCHOOL**

(University of London)

PADDINGTON, LONDON W2 1PG

Applications are invited from candidates with an Honours Degree in Chemistry, Biochemistry, Pharmacology or related discipline wishing to work for a Ph.D. degree, for a studentship to work on the metabolic fate of artificial sweetening agents. Apply, with curriculum vitae and names of two referees to Professor R. T. Williams, Department of Biochemistry. (898)

UNIVERSITY OF HULL
DEPARTMENT OF PLANT BIOLOGY

Applications are invited for the post of Post-doctoral Fellow in Plant Biochemistry, to work with Dr D. R. Threlfall on the biosynthesis of isoprenoid quinones and chromanols.

The post is financed by the S.R.C. and is tenable for one year from October 1, 1974, or as soon as possible thereafter.

Salary (excluding threshold payments) will be on the scale £2,226 by £114 to £2,340 by £72 to £2,412, plus F.S.S.U. benefits.

Applications (three copies) giving details of age, qualifications and experience together with the names of two referees should be sent by September 16, 1974 to the Registrar, The University of Hull HU6 7RX from whom further particulars may be obtained. (922)

UNIVERSITY OF CAPE TOWN
SENIOR LECTURESHIP IN ENVIRONMENTAL STUDIES

Applications are invited for the newly created post of Senior Lecturer in Environmental Studies. Appointment, according to qualifications and experience, will be made on the salary scale R6,300 by R300 to R8,100 per annum plus a pensionable allowance of 15% of basic salary. This salary scale does not include improvements under consideration.

Applicants for the post must have interest and experience in at least one aspect of environmental study, should desire to participate in inter-disciplinary teaching and research, and should have a Ph.D. or its equivalent.

Applicants should submit a curriculum vitae, stating present salary, research interests and publications, when available if appointed, and the names and addresses of three suitable referees.

Further information concerning the post and general conditions of service should be obtained from the Registrar, University of Cape Town, Private Bag, Rondebosch, 7700, South Africa, by whom applications must be received not later than November 1, 1974.

Appointment will be subject to a satisfactory medical examination. The University reserves the right to appoint a person other than one of the applicants or to make no appointment. (932)

**THE QUEEN'S UNIVERSITY
OF BELFAST**
**FACULTY OF AGRICULTURE AND
FOOD SCIENCE**
**RICHARDSONS ULSTER
STUDENTSHIP**
**PLANT NUTRITION/PLANT
BIOCHEMISTRY**

Applications are invited for the above studentship from graduates in Science or Agriculture. Preference will be given to applicants of Northern Ireland parentage. The holder will undertake research work into biochemical aspects of plant growth and nutrition and will be required to register for a higher degree of the University. The Department of Agricultural and Food Chemistry is housed in new, well-equipped laboratories on the southern outskirts of Belfast.

The studentship is tenable up to three years and its value will be £750 in the first year and £800 in the final two years. An allowance of £250 to £300 per annum will be available for approved expenses.

Candidates should apply in writing, including a curriculum vitae, to the Dean of the Faculty of Agriculture and Food Science, Queen's University, Newforge Lane, Belfast BT9 5PX. (913)

Imperial College Postgraduate Studentship and Postdoctoral Assistantship in Cancer Chemistry

Applications are invited for a Postgraduate Studentship and a Doctoral Assistantship for a new project sponsored by the Ministry of Agriculture. The work concerns Physical and Organic Chemical aspects of the Interaction of Nitrites and Nitrates with Foodstuffs, particularly in the Digestive Tract.

The postgraduate studentship provides equivalent remuneration to an S.R.C. award (£695 per annum—at present under review), although students who are normally ineligible for an S.R.C. or M.R.C. grant will be considered. The candidate is expected to register for a higher degree.

The postdoctoral assistantship (Salary £2,118 per annum and £213 per annum London Allowance (under review) plus F.S.S.U.) is tenable for up to three years with the usual salary increments. Threshold payments at the authorised rate apply to this post.

Applications with curriculum vitae and the names of two referees should be sent as soon as possible to: Dr B. C. Challis, Department of Chemistry, Imperial College, London SW7 2AY. (902)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES PROFESSORIAL FELLOWSHIP DEPARTMENT OF NEUROBIOLOGY

The Department (Head: Professor G. A. Horridge) has an active programme of research in the neural basis of behaviour and perception in lower animals, mainly insects and crustacea. Applicants should be capable of a strong research programme in an aspect of neurobiology which is either complementary or supplementary to the present interests, which include: (a) mechanisms of arthropod vision; (b) growth and regeneration of insect nerves; (c) control of movement in crustaceans; (d) biochemistry of insect neurons; (e) insect sound production and hearing; (f) establishment of connections between nerve cells.

The University is looking for a man with an ambitious project who finds himself restricted by lack of assistance, research time or equipment. Vertebrate neurobiology is not excluded. The position is tenured, with opportunity to train graduate students.

Closing date: September 21, 1974.

Salary: Salary for a Professorial Fellow is \$A18,131 p.a. Current exchange rates are approximately \$A1: 67np; \$US1:49.

Other Condition: Tenure: Professorial Fellow to retiring age (65 years).

Reasonable travel expenses are paid and assistance with housing is provided for an appointee from outside Canberra. Superannuation is on the F.S.S.U. pattern with supplementary benefits.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should apply to the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further particulars before applying. (899)

UNIVERSITY OF ADELAIDE

Applications are invited for the following appointment:

SENIOR TEACHING FELLOW IN PHYSICAL AND INORGANIC CHEMISTRY

The Senior Teaching Fellow, who should have completed a Ph.D. degree, will devote about half his time to teaching and the other half to research. He will be responsible for the supervising arrangements for some undergraduate practical courses and will initiate new course developments; he will be encouraged to undertake research with one of the existing research groups in the Department, which has good modern facilities. A document detailing research interests within the Department and describing undergraduate class arrangements is available on request (September 13, 1974).

Salary Scale: Senior Teaching Fellow \$A7,545 by \$A292(2) by \$A291(3) to \$A9,002, with superannuation provision.

Further particulars about this post and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications should be sent in duplicate and giving the information listed in the Statement that will be supplied, to the Registrar, The University of Adelaide, North Terrace, Adelaide, South Australia, 5001. (894)

THE POLYTECHNIC OF CENTRAL LONDON SCHOOL OF ENGINEERING AND SCIENCE BIO-ORGANIC RESEARCH GROUP

RESEARCH FELLOW

£2,151 to £2,331

Applications are invited immediately for the above post which is sponsored by EURATOM for work on mutation in *Aspergillus nidulans* and its relationship in microdosimetric studies. Candidates should have or expect to obtain a Ph.D. in Genetics or Radiobiology.

Application including a curriculum vitae and the names of two academic referees should be sent to The Establishment Officer, PCL, 309 Regent Street, London W1R 8AL. 01-580 2020 Ext 212. (956)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY S.R.C./C.A.S.E. RESEARCH STUDENTSHIP

Applications are invited from graduates with a first or upper second class Honours degree in Botany, Biochemistry or other relevant subject for one S.R.C./C.A.S.E. Research Studentship, tenable from October 1, 1974.

The research topic concerns the use of isoenzyme analysis in assessing the purity of commercially important *Brassica* seeds.

Applications, together with details of academic background and names of two referees should be received not later than September 20, 1974, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote ref. R/V/N/276195. (946)

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

DEPARTMENT OF BIOCHEMISTRY S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited from graduates who hold Honours Degrees in Chemistry, Biochemistry, Biology or related subjects at the First or Upper Second Level, and from those who hope to have such qualifications by October 1974, for a C.A.S.E. Studentship concerned with research into sterol ester metabolism in mammalian skin. This will be carried out mainly at the University of Nottingham and in part at the Environmental Safety Division, Unilever Research Laboratory, Colworth House, Bedford, and should lead to a higher Degree.

Research will entail a study of sterol ester metabolism in relation to other lipids in skin, with particular emphasis on prostaglandins, and will also involve fractionation of mammalian skin into its constituent components and cell types, subcellular fractionation of skin, and the effect of environmental change upon sterol ester metabolism in skin.

Further details and application forms may be obtained from Professor J. N. Hawthorne, Department of Biochemistry, The Medical School, The University of Nottingham, Nottingham NG7 2RD. (920)

UNIVERSITY OF MANCHESTER, MEDICAL SCHOOL

RESEARCH ASSOCIATE/ FELLOW

for two years to join team working on immunological changes in connective tissue disease and immune deficiency. The work will be concerned either with variations in lymphocyte function and the effect of treatment, including immunotherapy on this, or with the part played by immunoglobulins in inflammatory joint disease.

Excellent facilities in a newly established and equipped laboratory. Salary on usual scales according to age and experience.

Enquiries to: Dr L. Holt, Medical School, Stopford Building, Oxford Road, Manchester 13. (954)

QUEEN ELIZABETH COLLEGE (University of London)

CAMPDEN HILL ROAD W8 7AH CHEMISTRY DEPARTMENT

Applications are invited for an S.R.C. C.A.S.E. Studentship for research into the physical properties of natural gas mixtures. There will be close collaboration with the London Research Station of the British Gas Corporation.

Further details may be obtained from Dr M. Rigby. (951)

NEW ZEALAND UNIVERSITY OF CANTERBURY CHRISTCHURCH UNIVERSITY POSTDOCTORAL FELLOWSHIP IN CIVIL ENGINEERING

Applications are invited for the abovementioned Fellowship. Applicants should possess a Ph.D. or equivalent degree, preferably followed by some research experience.

Fields within which the Fellowship may be held are: Fluid Mechanics; Highway Engineering; Materials; Photogrammetry; Soil Mechanics; Structural Mechanics; Systems Engineering and Planning.

The emolument is NZ\$6,600 per annum. The Fellowship will be tenable normally for one year, but with the possibility of extension.

Further particulars, and Conditions of Appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF.

Applications close on October 31, 1974. (941)

UNIVERSITY OF DUNDEE

DEPARTMENT OF MEDICAL BIOPHYSICS

Research in Radiation Damage

Applications are invited from recent honours graduates (preferably in Physics or Physical Chemistry) for a RESEARCH STUDENTSHIP leading to Ph.D. The project envisaged concerns experimental and theoretical studies of energy deposition by heavy charged particles in solid, liquid and gaseous tissue-like media and will form an important part of the general departmental studies into radiation damage by neutrons. Interested individuals should apply, as soon as possible, giving the names of two referees, to Professor J. H. Martin, Department of Medical Biophysics, The University, Dundee DD1 4HN, from whom further details can be obtained.

(958)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL FELLOWSHIP

A Research Fellow with experience in protein and nucleic acid metabolism is required for a three-year project on the post-transcriptional control of protein synthesis in eukaryotic cells.

Salary in the range of £2,118 to £2,412 plus F.S.S.U. and threshold payment.

Enquiries and applications with the names of two referees to Dr H. G. Klemperer, Department of Biochemistry, University of Birmingham, Birmingham B15 2TT.

(891)

LECTURES AND COURSES

THE CITY UNIVERSITY

DEPARTMENT OF CHEMISTRY

LECTURE COURSE ON DUST EXPLOSIONS

A course of lectures of postgraduate status, arranged by Professor J. H. Burgoyne and dealing with the theory and practice of dust explosions and methods of protection against them, will be given from November 4-6, 1974.

Further details may be obtained from The Secretary of the Chemistry Department, The City University, St John Street, London EC1V 4PB.

(911)

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ECOPHYSICS: The Application of Physics to Ecology by James Paul Wesley, *Univ. of Missouri, Rolla*. This book contains original research on selected problems in ecological physics. The field of ecology is assumed here to cover a broad range of topics, particularly, any area of research dealing with life as it naturally occurs and which does not involve any detailed internal examination of individual organisms. The book presents a brief review of thermodynamics and considers the strategy of how to optimize the utilization of a source of energy realistically at a finite rate. The author defines life physically, thereby permitting an analysis of the thermodynamic role of life in the ecosphere, and surveys all possible sources of energy since life needs to degrade high utility energy to low utility energy to survive. He explores the stability of the carbon cycle in the ecosphere yielding damped periodic oscillations following any perturbation. He discusses the territorial origin of human conflict and defines motivation in terms of normal ecological roles as predicted by a theoretical structure based upon the energy needs of the individual. The application of the rigorous concepts of physics to ecology has entailed the introduction of certain special definitions and basic new ideas which are presented here for the first time. Written primarily for physicists and mathematicians interested in theoretical ecology, this text should also interest ecologists, biologists, environmentalists and exobiologists with some background in mathematics and physics. '74, 368 pp., 39 il., 8 tables, cloth-\$19.75, paper-\$13.75

HUMAN AND ECOLOGIC EFFECTS OF NUCLEAR POWER PLANTS. Edited by Leonard A. Sagan, *Palo Alto Medical Clinic, Palo Alto, California*. Introduction by Rolf Eliassen. (15 Contributors) There is a widespread need and public desire to better understand nuclear power and its consequences. Reactor technology and its ecologic effects often exceed the detailed understanding of many experts. Compounding this difficulty, the interested layman is also faced with inaccessible and fragmented literature. Although the book is directed toward an exposition of human and ecologic effects, the first section details reactor design and engineering. Such material is necessarily included since many readers will be unfamiliar with reactor design and should have available some understanding of reactors, their operation and methods of generating and releasing radioactivity. Public attention has been focused on nuclear power because of the concern for the environment which has suffered from past and present technology. Contamination of air and water with reactor-produced radioactivity has aroused fears of radiation hazards to both persons and the environment. The mood of the public has generated the desire for wider participation in decision making and the loss of faith in those institutions to which these decisions have traditionally been delegated. This book is meant to serve both as a comprehensive introduction for the interested layman and as a useful reference for the technical expert. '74, 560 pp. (7 x 10), 138 il., 86 tables, \$34.50

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THE BIG BANG IS A BUST

Physicists generally agree that one should not postulate a force in order to explain a phenomenon that would occur even without such a force. One of our physicists has now shown that the big bang theory is a case in point. Writing in *Foundations of Physics*, A. D. Allen demonstrates that the uniform velocity of galaxies in unbounded space will cause the cosmos to evolve naturally into an expanding universe. If you want to get a bang out of cosmology, drop us a line and we'll send you a copy of Allen's paper.

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Genetic Control of Insect Pests

G. Davidson

August 1974, x+158 pp., £4.00/\$10.25 0.12.200850.2

This is the first book to consider, together, all the genetic methods for controlling insect pests agricultural, veterinary and medical importance, which have so far been developed to the point of practical application. As such it will be of the greatest interest to entomologists working in these fields, to doctors, concerned with preventive medicine and to biologists and those with more general interest in entomology and genetics.

contents

Introduction. The principles and dynamics involved in the sterile insect technique. Sterilization by irradiation. Chemosterilants. Hybrid sterility. Cytoplasmic incompatibility. Translocations. Other methods of genetic control. Summary and conclusion. References. Subject index.

British Acoustical Society Special Volume No. 2

The Vibration Syndrome

Proceedings of a Conference on the Medical, Engineering and Legal Aspects of Hand-Arm Vibration, at the University of Dundee, July 1972

edited by W. Taylor

August 1974, xii+226 pp., £6.00/\$15.50 0.12.684760.6

The Vibration Syndrome contains papers, given at a conference at the University of Dundee, in 1972, which present recent clinical and vibration studies, related mainly to the prolonged use of hand-held vibratory tools. This conference was inter-disciplinary and involved physicists and engineers as well as members of the medical and legal professions.

One of the main aims of the conference was to correlate the physical parameters of the vibration with the resulting VWF. From this data it was hoped to develop a Vibration Standard which would limit the damaging effects of hand-held vibratory tools in the future.

Introduction to Particle Production in Hadron Physics

S. Humble

August 1974, viii+254 pp., £6.80/\$17.50 0.12.361450.3

This volume introduces the important and rapidly growing field of particle production physics ranging from low energy single particle production to high energy many particle reactions. The work, which will function jointly as an introductory text, reference guide and critical review of recent advances, is the first in a popular subject, as yet not adequately covered by other books. Its value will be appreciated by graduate students and research workers in theoretical and experimental physics, and also applied mathematicians and high energy physicists.

The Structure of Mitochondria

E. A. Munn

August 1974, xiv+466 pp., £9.80/\$25.50 0.12.510150.3

The aim of this book is to provide a general survey of the structure of mitochondria from the level of resolution achieved by light microscopists down to the 2.45Å, achieved by X-ray crystallographers. The greatest emphasis, however, is placed on the results of electron microscopy and on the correlation of the results of the structural studies made while using biochemical and biophysical techniques.

This is the first book to deal with a topic which, because of the fundamental importance of mitochondria, is of enormous interest to biologists and yet has not been reviewed in its entirety for three years.

A NATO Advanced Study Institute

Phenomenology of Particles at High Energies

Proceedings of the Fourteenth Session of the Scottish Universities Summer School in Physics edited by R. L. Crawford and R. Jennings

August 1974, xii+744 pp., £18.20/\$47.25 0.12.197150.3

This volume presents the latest trends in high energy physics in a didactic fashion. The subject of the School was the phenomenology of particles at high energies: this was chosen as being particularly timely in view of the new and exciting data flowing from the CERN Intersecting Storage Rings and NAL (Batavia, USA), and in view of the very considerable developments in phenomenological theory for high energy processes. The papers were given by innovators in the field and contain new material, as well as reviews of recent developments often difficult to gather from the literature.

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